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The potential use of a layer-by-layer strategy to develop LDPE antimicrobial films coated with silver nanoparticles for packaging applications

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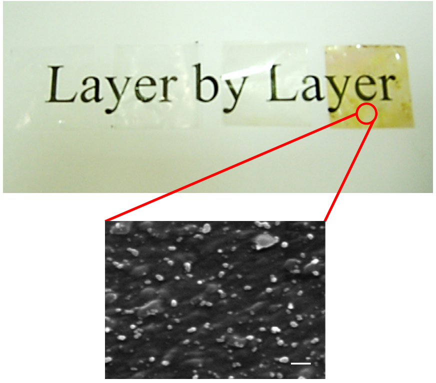
GRAPHICAL ABSTRACT



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ABSTRACT

Commercial low-density polyethylene (LDPE) films were UV/ozone treated and coated using a layer-by-layer (LbL) technique by alternating the deposition of polyethyleneimine (PEI) and poly(acrylic acid) (PAA) polymer solutions and antimicrobial silver (Ag). The effects of the initial pH of the PEI/PAA polymer solutions alternating layers (pH 10.5/4 or 9/6.5) on the antimicrobial activity of the developed LbL coatings combined with Ag against Gram-negative and Gram-positive bacteria were investigated. The results from fourier transform infrared spectroscopy and toluidine blue O assay showed that LDPE LbL coated using PEI/PAA polymer solutions with initial pH of 10.5/4 significantly increased the presence of carboxylic acid groups and after Ag attachment the coating had higher antimicrobial activity against both Gram-negative and Gram-positive bacteria compared to the LDPE LbL coated using PEI/PAA polymer solutions with initial pH of 9/6.5. The LDPE LbL coated films using non-modified pH PEI/PAA polymer solutions decreased the water contact-angle indicating an increased hydrophilicity of the film, also increased the tensile strength and roughness of LDPE LbL coated films compared to uncoated LbL samples. The LDPE LbL coated films attached with Ag^+ were UV/ozone treated for 20 min to oxidise Ag^+ to Ag^0. The presence of Ag^0 (Ag nanoparticles (NPs)) on the LDPE LbL coated films was confirmed by XRD, UV–vis spectrophotometer and colour changes. The overall results demonstrated that the LbL technique

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has the potential to be used as a coating method containing antimicrobial Ag NPs and that the manufactured films could potentially be applied as antimicrobial packaging.

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1. Introduction

The main approaches for the synthesis of nanoparticles (NPs) are 'top down' involving the size reduction of larger particles by some physical or chemical mechanism, such as mechanical milling, while the 'bottom up' approach involves the assembly of molecules and ions into NPs materials [1]. 'Top down' approaches are successful in fabricating inorganic materials, however, this solid state nanotechnology can encounter fabrication size limitations. Therefore, 'bottom up' approaches have attracted more attention as an alternative manufacturing strategy [2]. For film coatings, the most successful of the 'bottom up' approaches are self-assembled monolayers (SAM)s and Langmuir–Blodgett (LB) techniques. However, these techniques require expensive instruments and SAMs cannot readily be used for multilayer fabrication, while LB is generally unsuitable of non-amphiphilic materials [3]. Recently, an alternative 'bottom up' strategy layer-by-layer (LbL) technique was explored and developed. The LbL technique is simple, easy to use, low cost, adaptable to almost any kind of substrate and it is a flexible strategy of deposition method to create antimicrobial film surfaces [3,4], making this technique a versatile nanofabrication technique. This strategy can also control the physicochemical characteristics of the antimicrobial film surface such as thickness, chemistry, stability, gas permeability, mechanical, and bio-functionality [5–7].

Low-density polyethylene (LDPE) is a polymer that widely used in the food industry due to its low cost, good mechanical strength, excellent barrier properties to water, good sealing characteristics and transparency [8–10]. However, LDPE films are inert and hydrophobic in nature [11]. Thus, surface modification by physical or chemical means such as plasma, UV/ozone, piranha solution, etc. is needed to generate polar functionalities (such as those of carboxyl and aldehyde groups) or overall to yield negative charges which mainly will increase the hydrophilicity and wettability, consequently allowing better adhesion of the antimicrobial substances onto the surface of LDPE films [12].

Different types of metal like Cu, ZnO, TiO_2, MgO, and Ag have been used as antimicrobial substances against food pathogens and it have been inserted into polymer matrixes for the developing of antimicrobial food packaging [13–17]. It has been reported that Ag NPs based materials or Ag ion (Ag^+) are the most effective antimicrobial due to silver's strong toxicity against a wide variety of bacteria, viruses and other eukaryotic microorganisms [18,19]. In most cases of fresh or processed food products, microbial contamination occurs on the surface of the food due to the post-process handling, thus requiring an effective control of microbial growth on the surface of the food using antimicrobial active packaging films [20]. Coating of antimicrobial substances such as Ag NPs onto polymer surfaces have shown higher antimicrobial effects in inhibiting or delaying the growth of microorganisms on the surface of food products compared to antimicrobial substances embedded into polymer matrixes [21].

The polyelectrolyte multilayer systems fabricated using LbL technique by alternating the opposite charges of polyanion and polycation deposition have been used in many applications either under a laboratory scale or in real life application. LbL technique have been used to assemble a wide range of materials including DNA, enzymes, essential oils, proteins and polymers. It has been also applied in areas including biosensors, electronics, biomedical application, biocompatible coating (eye-lenses), cellulose microfiber coating and food packaging purposes [3,5,22–24]. This technique has also been used to assemble chitosan with alginate or gelatin, chitosan encapsulated cinnamaldehyde with pectin–$CaCl_2$, cellulose–alginate with lysozyme–chitosan–rectorite, followed by shelf life testing on food, which were fresh cut melon, fresh cut papaya, or meat products [11,21,25–27].

Many studies have applied LbL assembly on glass wafers or microscope slides and to the best of our knowledge, rarely applied to polymer surfaces such as LDPE despite having the potential to be used on a roll-to-roll system and easy scale up. Therefore, the objectives of this study were to study the effects of the initial pH of polycation polyethyleneimine (PEI)/polyanion poly(acrylic acid) (PAA) polymer solutions (pH (9/6.5) or (10.5/4)) on the antimicrobial activity of the LbL coating after attachment to the Ag^+ against Gram-negative and Gram-positive bacteria and the morphological, mechanical, and barrier properties of the antimicrobial Ag NPs on LDPE LbL coated were also assessed.

2. Materials and method

2.1. Materials

Commercial low-density polyethylene (LDPE) films were obtained from Fispak, Ireland. Poly(acrylic acid) (PAA, $M_w = 450000$), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), polyethyleneimine (PEI, branched, $M_w = 25{,}000$, $M_n = 10{,}000$), silver nitrate ($AgNO_3$) and toluidine blue O (TBO) were purchased from Sigma–Aldrich, Ireland and used without further purification. Milli-Q water (MQW) (18.2 MΩ cm) was obtained with a PURELAB Option-Q (Elga, UK) and used to prepare all aqueous solutions and for washing purposes. Glacial acetic acid was purchased from Fisher Scientific, Ireland.

2.2. Pretreatment and surface modification of LDPE films

Commercial LDPE films (6.25 cm^2) with a thickness of 0.04 mm were used as substrate. In order to remove any impurities, LDPE films were immersed in ethanol or acetone and sonicated (Cole-Palmer 8891) for 10 min, respectively. One side of the cleaned LDPE film was UV/ozone treated using a digital UV Ozone System (PSD Pro Series Digital UV Ozone System, USA, UV source: 180–254 nm) for 30 min under the flow of oxygen gas (rate 0.5 L min^{-1}) in order to increase ozone production. The UV/ozone treated LDPE films were used within 15 min or stored in sealed and clean glass petri dishes containing anhydrous calcium sulphate to avoid the potential to return to their original chemical state due to the reorganisation of the functional groups [28,29]. All LDPE films used in this study were treated with UV/ozone before use unless otherwise stated.

2.3. Functionalisation of LDPE films

The UV/ozone treated LDPE films were functionalised using two-step polymer reaction in polycationic and polyanionic solutions as described by Tian et al. [30] and Yang et al. [31]. The PEI and PAA polymer solutions were set at two different pH levels, modified pH (MpH; pH (9/6.5)) or a non-modified pH (NMpH;

pH (10.5/4)), respectively. For the modified PEI/PAA pH polymer, the UV/ozone treated side of the LDPE film surface was left in contact with the polycationic polymer solutions (PEI 30 mg mL^{-1} containing, 50 mM EDC and 5 mM NHS, pH 9 adjusted with 1 M HNO_3) for 15 min to attach the amine group. This step was followed by rinsing 3 times with MQW and dried under a stream of N_2 gas (Air Product, UK), and further dried in an oven at 60 °C for 15 min. The surface of the LDPE films attached with the amine group were dipped in the polyanionic polymer solutions (PAA 1 mg mL^{-1} containing 50 mM EDC and 5 mM NHS, pH 6.5 adjusted using 1 M NaOH) for 15 min to attach carboxylic acid group and followed by washing and drying steps as stated above. For the batches where the pH of the polycation and polyanion polymer solutions were not modified (NMpH), the LbL PEI/PAA polymer solutions were used as prepared (pH 10.5 and 4). Similar sequential processes of dipping in polycationic and polyanionic polymer solutions, rinsing and drying was carried out as outlined above in order to obtain the desired number of coating (1 or 3 coatings). Finally, the LDPE LbL coated films were then dried using an oven at 60 °C for 24 h before use. For the attachment of Ag^+ on the surface, the LDPE LbL coated films were immersed for 15 min in a water based solution containing different concentrations of $AgNO_3$ (0.5%, 2% or 5%, w/w) to allow the Ag^+ to be trapped onto the film by ion exchange [32]. After 15 min immersion in $AgNO_3$ solutions, the LDPE films were rinsed with MQW to remove unattached Ag^+ and kept in an environmental chamber ($T = 25$ °C, RH = 50%) until further analysis. For the antimicrobial activity of the LDPE LbL coated films with MpH or NMpH PEI/PAA polymer solutions and attached antimicrobial Ag^+ using $AgNO_3$ (2%, w/w) were used. In order to determine the time required to oxidise Ag^+ to Ag NPs LDPE LbL coated films containing Ag^+ were exposed to UV/ozone for 0, 10, 20, 40, or 60 min).

2.4. Surface characterisations

Structural changes on the LDPE films was performed using a FTIR (Fourier Transform Infrared spectroscopy) Varian 660 FTIR spectrometer using ATR Golden Gate (Specac). Spectra were taken with 32 scans at 4 cm^{-1} resolution. Three replicated spectra were obtained from three independent experiments ($n = 9$) and the average spectra was used for analysis. The resultant spectra were processed with OriginPro 8 SR3 (Origin Lab Inc.). Film thickness was measured using a handheld digital micrometer (51031 Käfer, Villingen-Schwenningen, Germany). The morphology and topography of the coated or uncoated LDPE film surface was imaged using Scanning electron microscopy (SEM, FEI Company, FEG Quanta 6700). The crystallinity of Ag NPs present on the LDPE film surface was examined by an X-ray diffractometer (XRD, PANalytical MPD, Netherlands) instrument using an Xcelerator detector and a Cu Kα radiation at a wavelength of 0.1541 nm and operating at 45 kV and 40 mA. Scans were performed with a step size of 0.02° per second from $2\theta = 20$–$80°$. The surface plasmon resonance (SPR) bands of Ag NPs formed on LDPE film surfaces were carried out using UV–vis spectrophotometry (Agilent technologies, Cary 60, Santa Clara, USA).

2.5. Mechanical properties

The effects of the surface treatment of the LDPE coated films using LbL technique and attachment of Ag^+ or Ag NPs on the mechanical properties of LDPE films were evaluated by measuring the tensile strength and elongation at break. These tests were determined using Imperial 2500 instruments, Mecmesin force and torque test solutions (Mecmesin Ltd., Slinfold, West Sussex, England) according to the ASTM-D882 [33]. A total of eight readings (two independent experiments × four samples) were conducted for each sample and the average values are reported.

2.6. TBO assay

The concentration of carboxylic acid (nmol cm^{-2}) on the surfaces of LbL coated or uncoated LDPE films were quantified using the method adapted from Kang et al. [34]. One side of the LDPE film was in contact with 0.5 mM TBO solution for 2 h (pH 10, adjusted using NaOH) and rinsed 3 times with NaOH solution (pH 10, 1 mM) to remove any non-complexed dye from the LDPE surfaces. These films were then dried in an oven at 80 °C for 1 h. Films attached with complexed dye was then desorbed by leaving the LDPE surface in contact for 15 min in a 50% glacial acetic acid solution. The absorbance of the acetic acid solutions were measured at 633 nm in a UV–visible spectrophotometer (UV Mini 1240, Shimadzu Instruments, Jiangsu, China) and compared to a standard curve generated from a stock solution of TBO in 50% glacial acetic acid ($n = 4$). The concentration of $—COO^-$ per unit area of the surface of the film was determined from a standard calibration curve prepared using TBO standard solutions. The TBO assay on each sample was carried out in triplicate from two independent experiments ($n = 6$). The total concentration of carboxylic acid generated on each sample was subtracted with value on the untreated LDPE films.

2.7. Water contact angle measurements

The water contact angle of the surface of the LDPE films before and after coating using the LbL technique was determined by static sessile drop method using a Dataphysics Contact Angle System OCA (Germany) provided with image analysis software. A 10 μL droplet of distilled water was deposited with a precision syringe (needle diameter = 0.5 mm) on the surface of films. The contact angle (θ), monitored at $t = 0$ s, was estimated from the first stable automatic image of the droplet. Values were successively calculated from the intersection between the baseline of the drop and the tangent at the drop boundary, the last determined through the use of sessile drop fitting method. The definition of the surface properties of films (hydrophobic or hydrophilic) was made considering $\theta = 90°$ as the discriminating value, with water contact angle $\theta > 90°$ representing hydrophobic surfaces, whereas water contact angle $\theta < 90°$ indicated hydrophilic surfaces [35]. All films were pre-conditioned in an environmental chamber under the same conditions ($T = 25$ °C, RH = 50%) to avoid interferences due to competing moisture exchange at the surface around the droplet. Results are an average of six replicates.

2.8. Antimicrobial test

The antimicrobial activity of the films was assessed against pure cultures of *Pseudomonas fluorescens* (NCIMB 9046) and *Staphylococcus aureus* (NCIMB 13062) as reported in Azlin-Hasim et al. [36]. The side of the LDPE films to be tested was sterilised by exposing the films to UV light for 15 min in a laminar flow (Airclean 600 PCR Workstation STAR LAB) before use. The LDPE films or LDPE LbL coated films containing Ag^+ or Ag NPs were then placed in the middle of the inoculated agar plates with *P. fluorescens* or *S. aureus* and incubated for 24 h at 30 °C or 37 °C, respectively. LDPE films (without the presence of Ag) were used as a negative control. The inhibition zone formed around the LDPE film substrates indicated antimicrobial activity against the bacteria tested and measured using an electronic caliper (Model ECA 015D Moore & Wright, Paintain tools Ltd., Birmingham, UK) in millimeters. Further optimisation process was carried out only for samples that has shown the best antimicrobial activity (LbL coated using either NMpH or MpH).

2.9. Colour

The surface colour of the LDPE films or LDPE LbL coated films (with or without the presence of Ag NPs) were measured using a Minolta chromameter (CR-300, Minolta Camera Co., Osaka, Japan). The films were placed on the white calibration plates and colour were measured. Five random films were measured three times ($n = 15$) and the average values of CIE L-value (lightness), a-value (redness) and b-value (yellowness) were reported. Total colour difference (ΔE^*) is a parameter that quantifies the overall colour difference of a given sample compared to a reference sample and ΔE^* between LDPE films and LDPE LbL coated films were calculated as follows:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

The smaller the value of ΔE^*, the closer the samples are in colour. Values of ΔE^* between 0–0.2 indicate undetectable colour difference, 0.2–0.5 for a very small difference, 0.5–1.5 for a small difference, 1.5–3.0 for distinct, 3.0–6.0 for very distinct, 6.0–12.0 for great and values >12 for a very great difference, as reported in Cruz-Romero et al. [37].

2.10. Statistical analysis

All data was analysed for means, standard deviations and analysis of variance. One-way analysis of variance of data was carried out using SPSS 22 for Windows (SPSS Statistical software, NY, USA) software package. A difference between pairs of means was resolved by means of confidence intervals using Tukey's test. The level of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Effects of pH of polycation/polyanion polymer solutions on the antimicrobial activity

FTIR spectra of UV/ozone treated LDPE films or LDPE LbL coated films using NMpH or MpH polyanion/polycation polymer solutions (1 coating) in the range of 1500–1900 cm^{-1} are shown in Fig. 1. The spectra showed that the LDPE films coated using NMpH polyanion/polycation polymer solutions had a higher absorption peak in the range of 1725–1700 cm^{-1} compared to films that were coated using MpH polyanion/polycation polymer solutions, indicating the LDPE LbL coated films using NMpH had higher concentration of carboxyl groups. Durán et al. [38] suggested that the availability of hydrophilic carboxyl groups can improve the attachment of hydrophilic Ag$^+$. However, active groups that are available for binding sites or adsorption of metal ions are not limited to carboxyl and amine groups, but also sulphonate functional groups [39].

The presence of carboxylic acid groups on the surface of LDPE LbL coated films using MpH or NMpH polyanion/polycation polymer solutions were confirmed by TBO assay (Fig. 2). The results showed that the carboxylic acid groups present on the surface of LDPE LbL coated films using NMpH polymer solutions was significantly ($p < 0.05$) higher than those LbL coated using MpH. In this study, the increases of hydrophilic sites may favoured the attachment of Ag onto the substrate. Karam et al. [40] reported that hydrophilic substrates favours attachment and adhesion with hydrophilic materials.

The antimicrobial activity of LDPE films and LDPE LbL coated films using MpH or NMpH polyanion PEI/polycation PAA polymer solutions, followed by immersed in 2% $AgNO_3$ solution were tested against *S. aureus* and *P. fluorescens* are presented in Table 1. The results showed that LDPE LbL coated films using NMpH polyanion/polycation polymer solutions had significantly ($p < 0.05$) higher antimicrobial activity against both *S. aureus* and *P. fluorescens* compared to LDPE LbL coated films using MpH polymer solutions. This finding may be supported by data obtained by the FTIR and TBO tests, where higher carboxylic acid group were found in LDPE LbL coated films using NMpH polymer solutions promoting a better attachment of Ag$^+$ and therefore obtained better antimicrobial activity.

Mak and Chen [41] reported that when the pH of PAA solutions were lower than the p*K*a value (~5), the PAA polymers were less protonated and deflated. Similar results were observed when the pH of the PEI solutions were higher than the p*K*a value (~9) [42]. These conditions may have reduced the solubility of the polymers and at the same time reduced the swelling effects and protonation of the polymers. However, it was noticed that the antimicrobial activity of the LDPE LbL coated using NMpH polyanion/polycation polymer solutions were higher compared to those LDPE LbL coated films using MpH polyanion/polycation polymer solutions. It was also found that the peak of carboxylic acid groups were lower when polyanion/polycation polymer solutions were made using buffered solutions of 4-(2-hyddroxyethyl)-1-piperazineethanesulphonic acid (HEPES) pH 9 and 2-(*N*-morpholino) ethanesulphonic acid (MES) pH 6.5 compared to the non-buffered solution (data not shown). Similar results were found by Mahdavinia et al. [43], which reported that the swelling of the polymer that can be further reduced in buffer solutions than those in the non-buffered media. Overall, it was clearly showed that the LDPE LbL coated films using NMpH polyanion/polycation polymer solutions had higher antimicrobial activity against both microorganisms tested and it is believed that may be due to the presence of higher concentrations of carboxylic acid groups in the LbL coated films

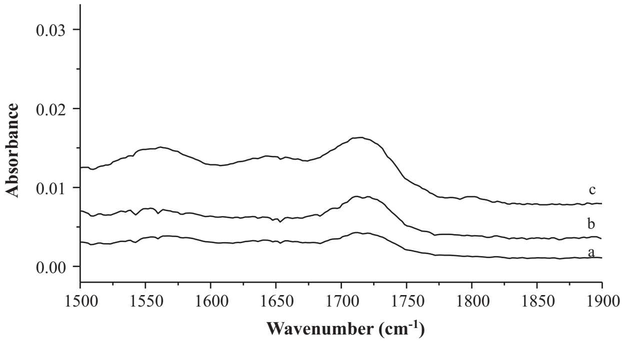

Fig. 1. The FTIR spectra of UV/ozone treated (a) LDPE films or (b) LDPE LbL coated films using modified pH, or (c) LDPE LbL coated films using non-modified pH polyanion/polycation polymer solutions.

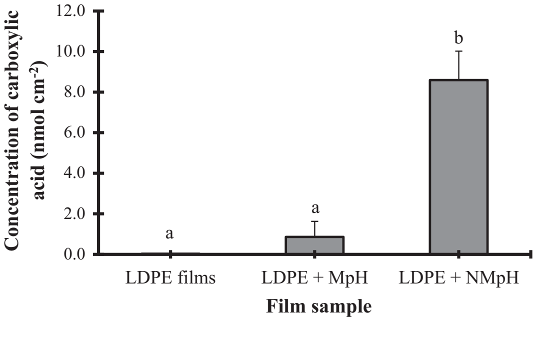

Fig. 2. The concentration of carboxylic acid on LDPE films (control), and LDPE LbL coated films using modified pH (MpH) or non-modified pH (NMpH) of polyanion/polycation polymer solutions (1 coating). Error bars represent standard deviation of analysis from quadruplicate independent films.

Table 1
Antimicrobial activity of LDPE films or LDPE LbL coated films using modified pH (MpH) or non-modified pH (NMpH) polymer solutions followed by immersed in 2% (w/w) $AgNO_3$ and tested against *S. aureus* and *P. fluorescens*.*

Films	Inhibition zone (mm^2)	
	S. aureus	*P. fluorescens*
LDPE films	0.00^a	0.00^a
LDPE + PEI/PAA (MpH)	350.4 ± 13.30^b	694.8 ± 19.15^b
LDPE + PEI/PAA (NMpH)	460.0 ± 25.41^c	737.0 ± 15.08^c

* All values are means ± standard deviation. The area of each inhibition zone was obtained by measuring the total area of inhibition.
a,b,c: Different letters in the same column indicate significant differences ($p < 0.05$).

before attachment of Ag^+. Therefore, the LbL coating of LDPE films using MpH polyanion/polycation polymer solutions was not considered for further characterisation and optimisation processes.

3.2. Characterisation of LDPE LbL films coated using non-modified pH polyanion/polycation solutions

3.2.1. FTIR

The use of UV/ozone has been widely used to initiate the radical graft polymerisation of polymer films. Under UV irradiation system at wavelength of 254 nm, hydrocarbons at the surface of the LDPE are excited and reacted with photons or atomic oxygen radicals generated in the system, until the hydrocarbon have sufficient energy to break the C—C bonds of LDPE polymer and inducing the chain scission, which also increased the adhesion of other functional groups on the surface on the LDPE films due to the presence of aldehyde or carboxylic acid groups [12,44]. FTIR spectra of untreated and UV/ozone treated LDPE film surfaces are shown in Fig. 3a and b. The FTIR spectra showed that UV/ozone treatment of LDPE film surfaces increased the absorption peak in the range of 1725–1700 cm^{-1} indicating C=O stretching vibration in the carboxylic groups (Fig. 3b).

The FTIR spectra of the UV/ozone treated LDPE films coated using polycation PEI polymer solutions showed a new absorption band in the range of 1570–1515 cm^{-1} (Fig. 3c), indicating the successful bonding of C—N and N—H band of amide group and also a C—O band of amine group at around 1680–1630 cm^{-1}. The surfaces of the LDPE film containing amine group coated with polyanion PAA polymer solutions (Fig. 3d) showed an increase of C—O absorption band of carboxylic acid groups at around 1725–1700 cm^{-1} due to the presence of carboxylic groups in the PAA polymer solution. It was also observed that the coating with both PEI and PAA (one LbL coating) of UV/ozone treated LDPE film surfaces significantly ($p < 0.05$) increased the carboxylic acid peak and no amide band was noticed than when the UV/ozone treated LDPE films was only coated with PAA polymer solutions (Fig. 3e). This effect may be due to the successful bonding of the carboxylic acid group of PAA to the amide group of PEI. The C—O absorption band in the range of 1570–1515 cm^{-1} also increased when the number of LbL coatings increased (Fig. 3f).

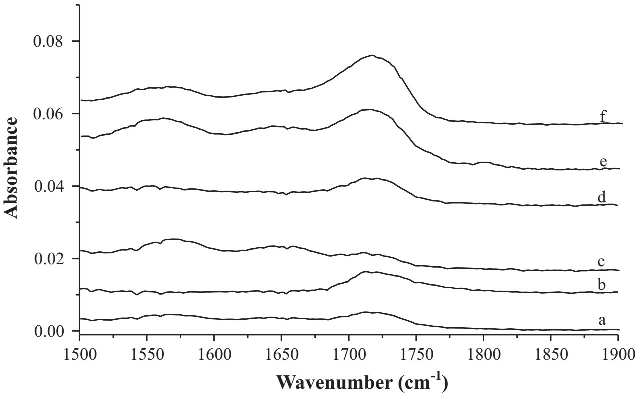

Fig. 3. The spectra FTIR of LDPE (a) untreated film or UV/ozone treated of (b) LDPE films, (c) LDPE LbL coated with 1 layer of PEI polymer solutions, (d) LDPE LbL coated with 1 layer of PAA polymer solutions, (e) LDPE LbL coated with 1 coating of PEI/PAA polymer solution or (f) LDPE LbL coated with 3 coatings of PEI/PAA polymer solutions in the range of 1500–1900 cm^{-1}.

3.2.2. TBO assay

The TBO assay confirmed the concentration of carboxylic acid (nmol cm^{-2}) on untreated or UV/ozone treated LDPE films and UV/ozone treated LDPE LbL coated films with different numbers of coating (Fig. 4). The UV/ozone treatment significantly ($p < 0.05$) increased the concentration of carboxyl groups on the surface of the LDPE films. Similar results were obtained by the FTIR and these results are in agreement with the results found by Tian et al. [30]. However, the presence of C=O on the UV/ozone treated LDPE films decreased after the conjugation of the carboxylic acids groups with polycation PEI polymer solution. This may indicate the successful attachment of an amine group with carboxyl groups forming C—N bonding, resulting in less available carboxylic acid groups. LDPE films containing amine group were further coated with PAA (one LbL coating), and the results indicated that the amount of carboxylic acid concentration was significantly increased, which may confirm the immobilisation of PAA. It was also observed that the amount of carboxylic acid groups increased significantly ($p < 0.05$) when the number of LbL coatings on the surface of the LDPE films was increased. The results suggested successful bonding of PAA with PEI, resulting in more available carboxylic acid groups after 3 LbL coatings compared to one LbL coating on the surface of the LDPE films.

3.2.3. Water contact angle

The water contact angle results obtained for untreated LDPE films was 89.32° indicating the hydrophobic nature of the LDPE surface. This results are in agreement with data previously reported where the contact angle of LDPE without surface modification ranged from about 87–95° [45,46]. The UV/ozone treatment of the surface of the LDPE films significantly ($p < 0.05$) reduced the water contact angle to 70.23°, indicating an increased wettability of the surface of the LDPE films and this is consistent with the introduction of polar groups such as carboxylic acids to the surface of the films. The UV/ozone treated LDPE films coated with polycation PEI polymer solutions had a higher contact angle value (77.58°) compared to the LDPE films. The increased value of water

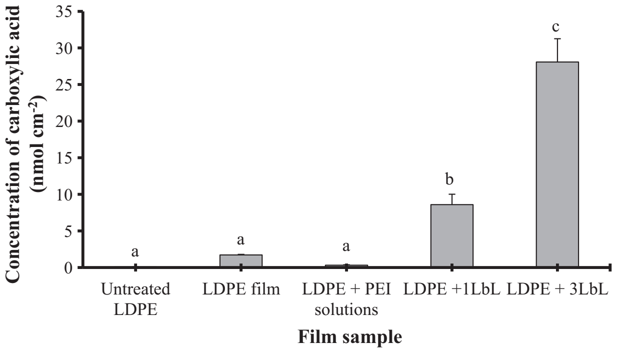

Fig. 4. The concentration of carboxylic acid on untreated or UV/ozone treated LDPE films, UV/ozone treated LDPE film coated with polycation PEI polymer solutions, or LbL coated with 1 coating or 3 coatings of PEI/PAA polymer solutions. Error bars represent standard deviation of analysis from quadruplicate independent films.

contact angle may due to the presence of carboxylic acid sites grafted to the amine groups present in the PEI and resulted in reduction of the hydrophilicity. Similar results were found in the FTIR spectra where a reduction peak of carboxylic acid groups was observed (Fig. 3b). The LDPE LbL films coated using 1 coating PEI/PAA polymer solutions significantly ($p < 0.05$) decreased water contact angle to 53.74°, indicating that the hydrophilicity of the LDPE films has increased. The increased numbers of LbL coatings decreased further the value of water contact angle to 50.83°. Similar results were noticed in the FTIR spectra where the carboxyl groups present increased when the number of LbL coatings increased. This is probably due to the synergistic effect between two layers of polyelectrolytes, which when deposited in conjunction have stronger effects [30].

3.3. Characterisation of LDPE LbL coated films containing Ag NPs

3.3.1. XRD

The crystalline nature of the Ag NPs on the surface of the LDPE LbL coated films were measured by X-ray diffraction (XRD) analysis and the results are shown in Fig. 5. The LDPE LbL coated films containing antimicrobial Ag NPs exhibited small diffraction peaks at 2θ value of 38.39° and 44.49° consistent with the (1 0 0) and (2 0 0) reflections from the face-centred cubic (fcc) structure of Ag respectively (JCPDS file No. 04-0783). When an increased concentration of $AgNO_3$ was used to attach the antimicrobial Ag^+, the two diffraction peaks also increased accordingly. Independent of the number of LbL coatings or concentration of $AgNO_3$ used, no (2 2 0) reflection was observed in the spectrum, this may be due to the low concentration of Ag NPs present or due to orientation effects of the particles on the surface. As well as the silver derived features, three high intensity peaks at 21.51°, 28.73°, 36.18° and a small intensity peak at 23.76° were observed which are assignable to diffraction from the substrate LDPE films [47].

3.3.2. UV–vis spectroscopy

Silver NPs are known to exhibit a maximum UV–visible absorption within the range of 400–500 nm, while the position of the absorbance and its width depends on the size or shape of the NPs due to the surface plasmon resonance phenomena [48]. The UV–vis absorption spectra of the LDPE LbL coated film surfaces containing or not containing Ag NPs are shown in Fig. 6. The spectra showed that no absorption band was observed for LDPE films and LDPE LbL coated (1 coating) films immersed in 2% of $AgNO_3$ solutions (Fig. 6a and b). This results are in agreement with the results reported by Ahmad et al. [49], in which no absorption peak was found in chitosan/PEG nanocomposite materials containing Ag^+. LDPE film surfaces containing Ag^+ can be oxidised by exposure to UV/ozone and forming Ag NPs [36]. When LDPE LbL coated containing Ag^+ were UV/ozone treated for 10 min, no significant differences on the absorption was observed when compared to LDPE films (Fig. 6c). However, after 20 min of UV/ozone exposure, a maximum absorbance peak around 430 nm was observed indicating the presence of Ag NPs (Fig. 6d). This results are in agreement with the results reported by Wani et al. [50], in which a maximum absorbance peak for Ag NPs in microemulsion solutions was observed after a reduction process from Ag^+ to Ag NPs.

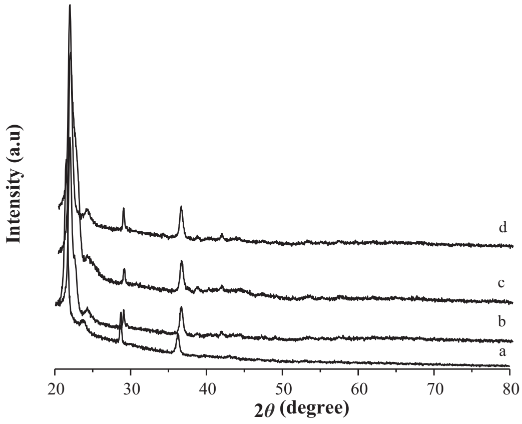

Fig. 5. XRD patterns UV/ozone treated of (a) LDPE films or LDPE LbL coated with 3 coatings immersed in different concentrations of $AgNO_3$ at (b) 0.5%, (c) 2%, and (d) 5% and exposed to UV/ozone for 20 min.

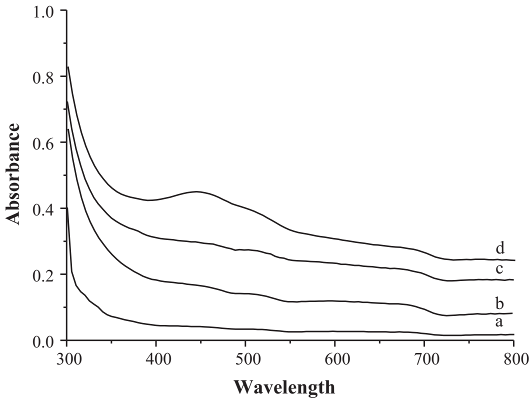

Fig. 6. The UV–vis spectra of (a) LDPE films and (b) LDPE LbL coated films with 3 coatings after immersed in 2% of $AgNO_3$, followed by exposure to UV/ozone for (b) 0 min, (c) 10 min, and (d) 20 min in the range of 300–800 nm.

Independent of the concentrations of $AgNO_3$ used to attach antimicrobial Ag^+ on LDPE LbL coated (1 coating), no significant increase on the absorbance peak was observed (data not shown) and these may be due to the lower concentration of Ag NPs attached on the surface of the LDPE films. However, an absorbance peak at the range of 430–470 nm was observed for LDPE LbL coated (3 coatings) films containing Ag NPs. Wani et al. [50] reported that the increased concentration of Ag NPs in a microemulsion was directly correlated to the absorption surface plasmon resonance bands. Due to the fact that no significant difference in the absorbance peak was observed in the range of 430–470 nm with longer exposure to UV/ozone (40 and 60 min) (data not shown), treatment of 20 min was selected as the maximum exposure time in order to obtain Ag NPs. Longer exposure time of the LDPE films to UV/ozone may be unfavourable as prolonged exposure to UV/ozone may damage the LDPE polymer, thus affecting the mechanical properties of the LDPE films.

3.3.3. Colour

The colour changes of LDPE films or LbL coated containing Ag NPs are shown in Table 2. The LDPE films were colourless and no differences were observed on the colour of LDPE LbL coated films (1 coating) without the presence of Ag NPs or with the presence of AgNPs after immersed in 0.5% or 2% $AgNO_3$ and followed by 20 min UV/ozone treatment. Compared to LDPE films, the *L*-values (lightness) decreased ($p < 0.05$) and the redness (*a*-values) and yellowness (*b*-values) significantly ($p < 0.05$) increased when number of LbL coating increased or when a concentration >2% $AgNO_3$ (for 3 coating LbL) was used to obtain LDPE films containing Ag NPs. Conversely, Rhim et al. [51] found that the *a*- and *b*-values of agar/Ag NPs composite films decreased significantly when the concentration of Ag NPs increased. The attachment of $AgNO_3$ on the surface of LDPE LbL coated films followed by UV/ozone treatment changed the colour of the films to yellowish-brown and the

Table 2

Changes in the *L*-, *a*- and *b*-values and the total colour differences of UV/ozone treated LDPE films coated with different number of LbL coating followed by immersed in different concentrations of $AgNO_3$ and then UV/ozone treated for 20 min.*

Sample	*L*-Value	*a*-Value	*b*-Value	ΔE
LDPE films	93.06 ± 0.45^{a}	−0.65 ± 0.03^{a}	4.06 ± 0.05^{a}	0.00
LDPE + 1LbL coating	94.20 ± 0.11^{a}	−0.72 ± 0.02^{a}	4.06 ± 0.04^{a}	1.13 ± 0.10^{a}
LDPE + 1LbL coating + Ag 0.5%§	93.02 ± 0.50^{a}	−0.67 ± 0.98^{a}	4.50 ± 0.44^{a}	0.63 ± 0.58^{a}
LDPE + 1LbL coating + Ag 2%§	91.97 ± 0.80^{ab}	−0.39 ± 0.18^{ab}	6.26 ± 1.09^{a}	2.50 ± 1.28^{a}
LDPE + 1LbL coating + Ag 5%§	87.41 ± 2.22^{cd}	−0.38 ± 0.24^{ab}	20.54 ± 3.45^{bc}	17.49 ± 3.94^{bc}
LDPE + 3LbL coating + Ag 0.5%§	89.51 ± 0.44^{bc}	0.13 ± 0.53^{b}	15.12 ± 2.36^{b}	11.66 ± 2.23^{b}
LDPE + 3LbL coating + Ag 2%§	85.87 ± 3.15^{d}	0.85 ± 0.51^{c}	23.05 ± 8.14^{c}	20.37 ± 8.65^{c}
LDPE + 3LbL coating + Ag 5%§	77.52 ± 1.59^{e}	1.16 ± 0.38^{c}	20.97 ± 6.06^{bc}	23.23 ± 5.42^{c}

* All values are means ± standard deviation.

^{a,b,c,d,e}: Different letters in the same column indicate significant differences ($p < 0.05$).

§ LDPE films immersed in $AgNO_3$ solution for 15 min before exposed to UV/ozone for 20 min.

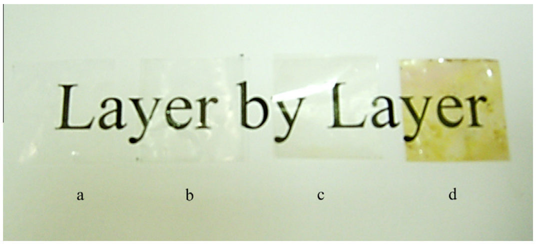

Fig. 7. The colour changes of (a) LDPE films and (b) LDPE LbL coated (3 coatings) films without the presence of Ag and (c) Ag NPs presence on LDPE LbL coated (3 coatings) films immersed in 0.5% $AgNO_3$, or (d) Ag NPs presence on LDPE LbL coated with (3 coatings) films immersed in 5% $AgNO_3$ and UV/ozone treated for 20 min.

Table 3

Tensile strength and elongation at break of LDPE films, LDPE LbL coated films containing Ag$^+$ or Ag NPs.*

Sample	Tensile strength (MPa)	Elongation at break (%)
LDPE films	15.37 ± 2.61^{a}	208.81 ± 22.47^{ab}
LDPE + 1LbL coating	28.50 ± 6.25^{bc}	302.93 ± 60.34^{c}
LDPE + 1LbL coating + Ag$^+$ 0.5%	34.29 ± 5.19^{c}	225.78 ± 33.32^{bc}
LDPE + 3LbL coating + Ag$^+$ 0.5%	32.85 ± 6.37^{c}	213.48 ± 43.48^{b}
LDPE + 1LbL coating + Ag 0.5%§	21.28 ± 2.64^{ab}	148.60 ± 22.26^{ab}
LDPE + 3LbL coating + Ag 0.5%§	20.25 ± 4.82^{ab}	131.21 ± 86.31^{a}

* All values are means ± standard deviation.

^{a,b,c}: Different letters in the same column indicate significant differences ($p < 0.05$).

§ LDPE films immersed in $AgNO_3$ solution for 15 min before exposed to UV/ozone for 20 min.

intensity of these colour changes correlated to the concentration of $AgNO_3$ used, especially for the higher number of LbL coating. The example of colour changes on the LDPE film LbL coated surfaces with or without the presence of Ag NPs are shown in Fig. 7. Vimala et al. [52] reported similar results in where the formation of Ag NPs can be observed from the colour changes to light brown. Using the ΔE classification scale, it can be concluded that the small differences (no significant difference) in colour were obtained between LDPE LbL coated films without the presence of Ag NPs, LDPE LbL coated films after immersion in 0.5% or 2% of $AgNO_3$ solutions. With the increased numbers of LbL coatings and the concentration of $AgNO_3$ solutions (5%) used to attach Ag on the LDPE films resulted in significantly ($p < 0.05$) great or very great colour differences compared to the control films.

3.4. Mechanical properties

The tensile strength and elongation at break of LDPE films, LDPE LbL coated films only or containing either Ag$^+$ or Ag NPs (after UV/ozone treatment) are shown in Table 3. To assess the effects of the LbL coating on the mechanical properties, only one concentration of $AgNO_3$ solutions were used for illustration purposes. The results indicated that the assemble of polyanion/polycation polymer solutions using LbL technique and LDPE film coated with LbL containing Ag$^+$ significantly ($p < 0.05$) increased the tensile strength of the films compared to LDPE films. The highest increase (123.10%) was observed using LDPE LbL coated films followed by immersed in 0.5% $AgNO_3$ solution. No significant changes on the tensile strength values were observed on LDPE LbL coated films containing Ag NPs (which were exposed to the UV/ozone for 20 min) compared to LDPE films. Regarding the elongation at break, no significant increase on the EAB was observed when the number of LbL coatings was increased; However, a slight reduction was observed on LbL coated LDPE films containing Ag NPs compared to LDPE films or LbL coated LDPE films containing Ag$^+$. The reduction on the tensile strength and elongation at break of LbL coated LDPE films containing Ag NPs may be due to the additional exposure of the surfaces of the film to UV/ozone that may have led to further breakage of C—C not only on the LDPE films but also on the LbL coating.

3.5. Scanning Electron Microscopy (SEM)

The SEM images showed that an increase of the number of LbL coatings from 1 to 3 coatings increased the roughness of the LDPE film surfaces (Fig. 8). When LDPE LbL coated (1 coating) films were loaded with Ag after immersion in 0.5% $AgNO_3$ and UV/ozone treated for 20 min, lower number of Ag NPs attached on the LDPE LbL coated films with a particle size range from 19 to 27 nm were observed (Fig. 8c). However, when the number of LbL coatings increased (3 coatings), the presence of Ag NPs attached on the LDPE LbL coated films increased and the particle size ranged from 55 to 86 nm (Fig. 8d). When the concentration of the immersion solution of $AgNO_3$ increased from 2% to 5%, the Ag NPs attached to the LDPE LbL coated films (3 coatings) formed aggregates and the particle size ranged between 98–122 nm and 128–160 nm, respectively (Fig. 8e and f). The particle size of the Ag NPs attached to the LDPE LbL coated films (3 coatings) using 5% $AgNO_3$ were significantly ($p < 0.05$) larger than when 0.5% $AgNO_3$ solution was used. The results are in agreement with the result reported by Zhou et al. [53] who found that when the concentration of Ag$^+$ increased, a large number of Ag NPs were deposited on glass substrate after reduction by formaldehyde and these particles formed aggregates with a rough 3D structure.

The rougher surface of the LDPE LbL coated films suggest that this structure can provide more sites for further attachment of Ag$^+$ and that higher concentration of $AgNO_3$ used allowed to attach more Ag onto the surface of the films forming Ag NPs of bigger

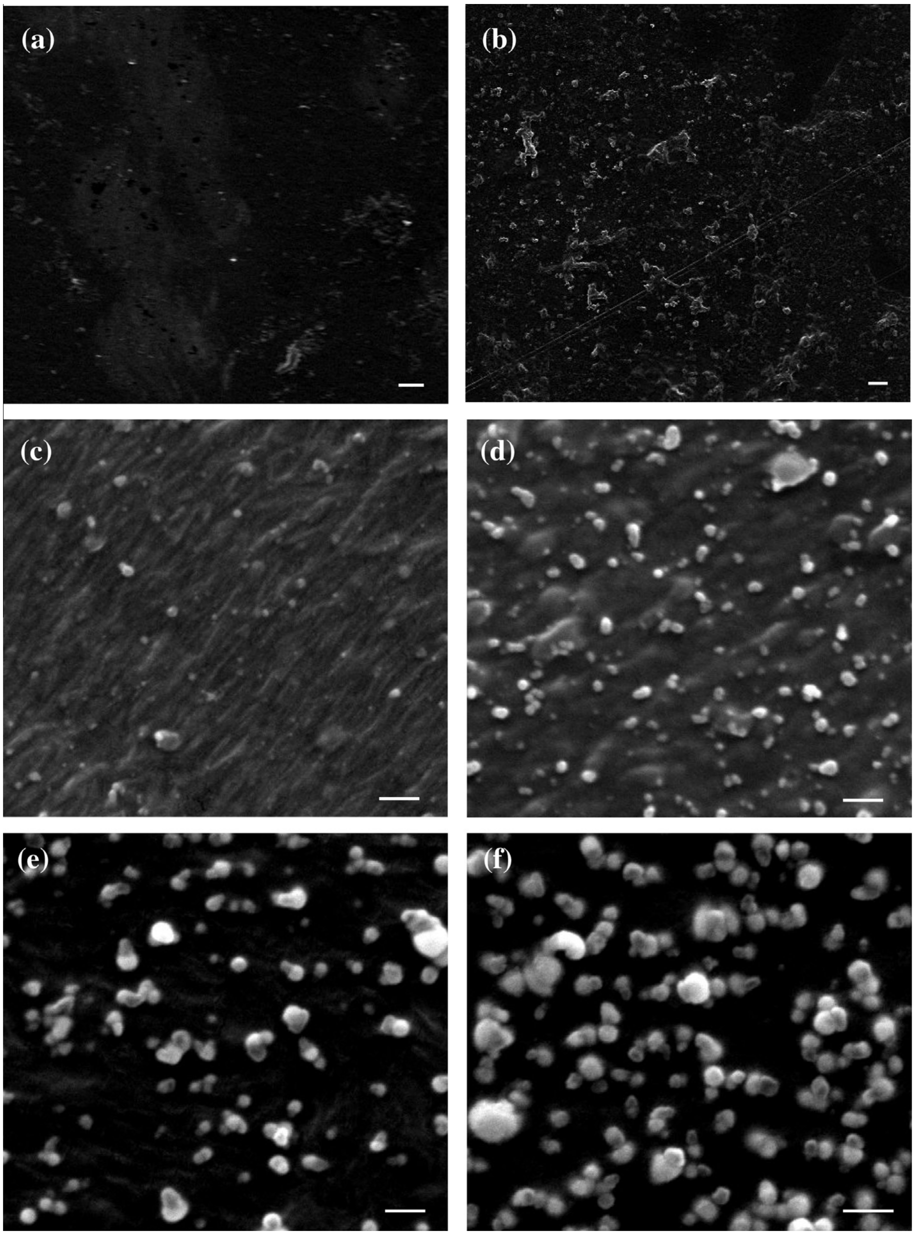

Fig. 8. The SEM images of UV/ozone treated LDPE films (a) 1 LbL coating, (b) 3 LbL coatings, (c) 1 LbL coating immersed in 0.5% $AgNO_3$, (d) 3 LbL coatings immersed in 0.5% $AgNO_3$, (e) 3 coatings LbL immersed in 2% $AgNO_3$, and (f) 3 LbL coatings immersed in 5% $AgNO_3$; Scale bar *a* and *b* = 500 nm; Scale bar *c*, *d*, *e* and *f* = 200 nm; All samples from *c*–*f* were exposed to UV/ozone in order to form Ag^+ to Ag^0.

particle size. Yang et al. [31] reported similar results when LDPE films were coated with polycation and polyanion polymer solutions at pH 10/4, the coating were the thickest and rougher compared to the films coated with polycation and polyanion polymer solutions at pH 8/6, 4/4 or 7/7, respectively.

3.6. Antimicrobial test

The antimicrobial activity of LDPE LbL coated films containing Ag NPs were assessed by agar diffusion method (Table 4). An example of LDPE films and the best inhibition zone using LDPE LbL coated films containing Ag NPs immersed in different concentrations of Ag solution tested against *P. fluorescens* are shown in Fig. 9. It was noticed that the antimicrobial activity against both tested microorganisms increased with the increasing number of LbL coatings and the higher concentrations of $AgNO_3$ used to attach the antimicrobial Ag. The most sensitive bacteria to the Ag NPs were *P. fluorescens* than *S. aureus.* Even though lower concentrations of $AgNO_3$ could form smaller particle size (Fig. 8c and d), it is clearly seen from data that the highest antimicrobial activity was obtained at higher concentrations of $AgNO_3$, which strongly suggests that the size effects in this regime are limited [36].

In order to assess if a direct addition of synthesized Ag NPs [20] to the PEI/PAA polymer solutions have any effect on the antimicrobial activity of LbL coated films and compared to the antimicrobial activity of LDPE LbL coated films containing Ag NPs, antimicrobial test was carried out on these films. The results showed that the antimicrobial activity of the LDPE LbL coated films immersed directly on $AgNO_3$ had better antimicrobial activity against the tested microorganisms compared to LDPE LbL coated films with the PEI/PAA polymer solutions directly added with the synthesized Ag NPs with a particle size of 10 nm at the same concentration level (data not shown). This results indicated that when the LDPE LbL coated films where immersed in the $AgNO_3$, the attached

Table 4
Antimicrobial effects of LDPE films containing Ag NPs immersed in different concentration of $AgNO_3$ and different numbers of LbL coating were tested against *S. aureus* and *P. fluorescens*.[*]

Sample	Inhibition zone (mm^2)			
	S. aureus		*P. fluorescens*	
	1LbL coating	3LbL coating	1LbL coating	3LbL coating
LDPE films	$0.00^{a,A}$	$0.00^{a,A}$	$0.00^{a,X}$	$0.00^{a,X}$
Ag 0.5%[§]	$66.45 \pm 15.63^{b,B}$	$107.06 \pm 21.59^{b,B}$	$81.83 \pm 18.1^{b,Y}$	$119.52 \pm 21.26^{b,YZ}$
Ag 2%[§]	$132.20 \pm 20.20^{c,B}$	$167.01 \pm 19.02^{c,B}$	$147.01 \pm 13.82^{bc,Y}$	$217.42 \pm 27.15^{c,Y}$
Ag 5%[§]	$182.65 \pm 26.28^{d,B}$	$226.70 \pm 23.42^{d,BC}$	$198.63 \pm 39.13^{c,Y}$	$396.34 \pm 38.15^{d,YZ}$

[*] All values are means ± standard deviation. The area of each clear zone was obtained by measuring the total area minus the area of the films (625 mm^2).

^{a,b,c,d}: Different letters in the same column indicate significant differences ($p < 0.05$).

^{A,B,C}: Different letters in the same row for *S. aureus* indicate significant differences ($p < 0.05$) compared to LDPE films.

^{X,Y,Z}: Different letters in the same row for *P. fluorescens* indicate significant differences ($p < 0.05$) compared to LDPE films.

[§] LDPE films immersed in $AgNO_3$ solution for 15 min before exposed to UV/ozone for 20 min.

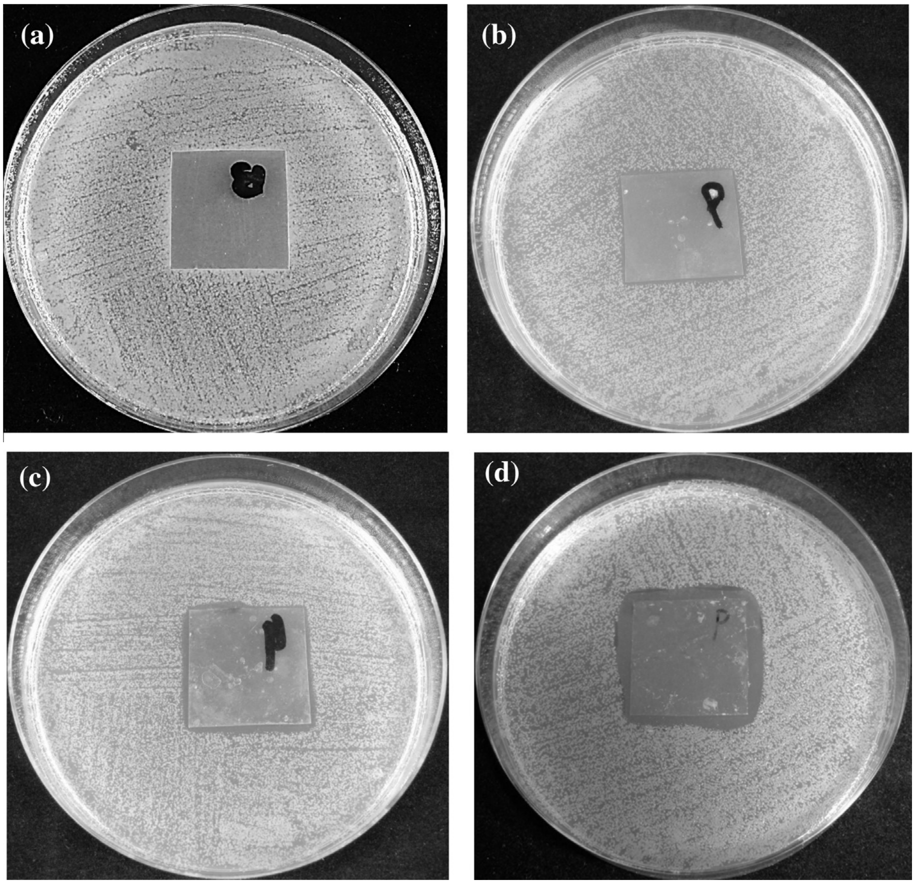

Fig. 9. Inhibition zones of (a) LDPE films and (b) LbL coated (1 coating) immersed in 2% $AgNO_3$, (c) LbL coated (3 coatings) immersed in 2% $AgNO_3$, and (d) LbL coated (3 coatings) immersed in 5% $AgNO_3$, followed by UV/ozone treated for 20 min against *P. fluorescens*.

antimicrobial Ag NPs were readily available at the surface of the coated films.

It was reported that the mechanism of antimicrobial activity of Ag^+ or AgNPs is the ability of Ag^+ formed complex with bacterial protein including enzyme, which can lead to a loss function and cell death in a number of ways; including increased membrane permeability, which can result in leakage of cell components [54] and interference with the bacterial respiratory chain [55]. Silver has high affinity and a higher tendency to react with phosphorus and sulphur compound and it was well known that membrane of the bacteria contains many sulphur containing protein, thus might be preferential site for the Ag^+ or Ag NPs [56]. Silver ions can also react directly with bacterial DNA, leading to a loss of protein expression and, therefore, cell death [56–58]. Silver nanoparticles can release Ag^+, but are also effective in additional ways as they can physically disrupt bacterial membranes and interact with cell components [56], leading to disruption of cell biochemical processes. There is also evidence that Ag NPs generate reactive oxygen species (ROS), which can have an antibacterial effect [55].

Different antimicrobial effects are observed on different types of bacteria due to the gram strain characteristics and differences thickness of the cell walls [59]. Gram-negative was reported to have very thin layer peptidoglycan with negative charge of the lipopolysaccharides outer layer. This probably caused the cell wall to be easily penetrated by Ag^+ and cause the cell death. On the other hand, Gram-positive bacteria have thicker 3D rigid structured peptidoglycan layer, which restricts the ability of Ag^+ to anchor and makes it difficult to penetrate the cells, thus, Gram-positive bacteria are more resistant to the antimicrobial Ag^+ [60].

4. Conclusions

The LDPE LbL coated films alternating the deposition of NMpH polyanion/polycation polymer solutions had significantly higher concentration of carboxylic acid groups promoting a better attachment of Ag^+, thus the LbL coated films attached with antimicrobial Ag^+ had significantly higher antimicrobial activity against both *S. aureus* and *P. fluorescens* compared to LDPE films that were coated using MpH polyanion/polycation polymer solutions. The results also suggested that LbL coating of LDPE films increased significantly the hydrophilicity and the mechanical properties of the films. The attached Ag^+ on the LDPE LbL coated films was successfully reduced to Ag NPs using UV/ozone treatment and the results indicated that when the LDPE LbL coated films where immersed in the $AgNO_3$, the attached antimicrobial Ag NPs had better antimicrobial that when the LDPE LbL coated films with the PEI/PAA polymer solutions directly added with the synthesized Ag NPs at the same concentration level. Overall, the results showed the potential of the LbL technique to attach antimicrobials such as Ag NPs to developed antimicrobial active packaging materials.

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