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Self-assembly of ZnO nanoparticles on Low-Density Polyethylene Film with sol- gel and its Application for Milk Active Packaging

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Abstract

This study reports the antibacterial capability of low-density polyethylene (LDPE) modified with ZnO nanoparticles using a Sol-Gel technique. Antibacterial activity of prepared films against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) was examined. The operational conditions such as pH, time, amount of ZnO nanoparticles, and silanol concentration were optimized using the response surface methodology (RSM). The proposed film under optimum conditions was applied for the packaging of the milk sample. The highest antibacterial activity of ZnO/LPDE were pH (6.0), time (103 min), amount of ZnO nanoparticles (0.68 % w/v) and silanol agent concentration (4.81 % v/v). The antibacterial properties of ZnO/LDPE films were assessed based on the diameter of the inhibition zone in a disk diffusion test against E. coli and S. aureus. These films have significantly reduced the growth of mentioned bacteria. Overall, antimicrobial packaging shows promise as an effective method to inhibit the growth of certain bacteria like E. coli and S. aureus in milk. The resulting ZnO/LDPE package films containing milk samples exhibit superior and prolonged antibacterial activity against E. coli and S. aureus in 7 and 14 days.

Keywords: Antibacterial Package, Zinc Oxide, Sol-Gel, E. coli, S. aureus.

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1. Introduction

The demand for minimally processed, easily prepared and ready-to-eat 'fresh' food products, globalization of food trade, and distribution from centralized processing pose most important concern

s for food safety and quality (de Moura et al., 2012; Llorens et al., 2012). In recent years, interest has been growing in using smart or active packaging that meets producers' and consumers' demands for products with longer shelf lives (Panea et al., 2014). Food packaging systems are designed to act as passive barriers, protecting food from the surrounding environment. On the other hand, active food packaging systems are supposed to perform some role other than providing an inert barrier to external conditions (Rotem et al., 2015). Antimicrobial packaging is a kind of active packaging (Mangalassary, 2012). Antimicrobial food packaging materials have to extend the lag phase and reduce the growth rate of microorganisms to extend shelf life and maintain product quality and safety (Appendini and Hotchkiss, 2002). Nano food packaging with antimicrobial properties represents a new generation of active packaging based on nanocomposites (de Azeredo, 2013). Inorganic materials such as metals and metal oxides have been the focus of nanotechnology research (Dastjerdi and Montazer, 2010). Because of the high surface area-to-volume ratio and enhanced surface reactivity of nanosized antimicrobials, these systems can inactivate more microbial cells when compared to higher-scale counterparts. Nanoscale materials have been investigated for antimicrobial activity as growth inhibitors, killing agents, or antimicrobial (Duncan, 2011; Guo et al., 2013).

Nevertheless, of the variety of antimicrobial agents, the search for effective biocidal agents has focused on the development of nanostructures of certain metals such as Ag, Cu, Zn, and Au (Longano et al., 2012; Rai et al., 2012). Metal oxide materials such as titanium dioxide (TiO2), zinc oxide (ZnO), and magnesium oxide (MgO) have been shown antibacterial activity, which may be attributed to the generation of ROS(Barnes et al., 2013; Leung et al., 2016). The great advantage of metal oxides over organic antimicrobial agents is their higher stability. However, given their reduced cost, recent research efforts have focused on using ZnO nanoparticles (Tankhiwale and Bajpai, 2012). The Zinc Oxide particles are particularly interesting since they appear to cause no harm to either animals or humans (Moezzi et al., 2012) but have a strong antimicrobial effect on a broad spectrum of microorganisms (Al-Naamani et al., 2016; Jafari et al., 2016; Venkatasubbu et al., 2016). Nano-sized ZnO particles present biocidal activity and have some advantages compared to AgNPs, such as their lower cost, white appearance, and UV blocking properties (Dastjerdi and Montazer, 2010). They also present high versatility, and inorganic carriers, such as hydroxyapatite, can also be doped with zinc oxide providing novel structures with antimicrobial activity against E. coli, S. aureus, and Candida albicans (Al-Naamani et al., 2016; Selvam & Sundrarajan, 2012; Tankhiwale & Bajpai, 2012). The feasibility of ZnO incorporated in polymer nanocomposites intended for food packaging has been examined in many studies. For example, Li et al. (2010) reported antimicrobial activities of the poly (vinyl chloride) films coated with ZnO nanoparticles. In another study, Li et al. (2011) confirmed the potential of the nano-packaging modified ZnO nanoparticles during the storage of Fuji apple cuts, observing better preservation of quality indicators such as ascorbic acid and polyphenol content, and lower counts of typical altering microorganisms. Emamifar et al. (2010) evaluated the antimicrobial activity of nanocomposites of low-density polyethylene (LDPE) containing Ag, NPs, and ZnO showing a significant impact of the proposed nano-packaging on the shelf-life of orange juice. Additionally, jin and Gurtler (2011) reported that glass jars coated with allyl isothiocyanate, nisin, and ZnO nanocomposite could inactivate effectively Salmonella in liquid egg albumen.

In antimicrobial packaging, antibacterial particles may be deposited (Quadrini et al., 2017), incorporated (Shaaban et al., 2016), immobilized (Yuan et al., 2016), or surface modified into packaging materials (Ebrahimiasl and Rajabpour, 2015). There are only a few approaches describing the surface coatings of ZnO nanoparticles such as the "pad-dry-cure" method, radiation, and thermal treatments. (Li et al., 2015; Mirhosseini & Barzegari Firouzabadi, 2014).

The use of appropriate coatings can impart antimicrobial effectiveness to a substrate. A sol-gel-based solution coating would be the most appropriate method in terms of stability and adhesiveness of attaching an antibacterial agent to a polymeric film (Cotolan et al., 2016; Nasirizadeh et al., 2015). In this study, low-density polyethylene (LDPE) film was successfully coated with nano-sized ZnO using the Sol-Gel technique. Additionally, the antibacterial properties of modified films were evaluated versus gram-negative and gram-positive bacteria.

2. Experimental

2.1. Material *and apparatus*

Potassium trimethoxy silane (PTMS) (purity 95%) was supplied from Merck (Germany). ZnO nanoparticles (average diameter 10 -30 nm, surface area 20-60 m2 /g) were purchased from US Research Nanomaterial Co. (USA). Hydrochloric acid and methanol (with a purity grade 98%) were purchased from Sigma -Aldrich Co. (U.K). The pH measurement was made with a Metrohm model 827 pH/mV meter. The X-pert-Philips X-ray diffractometer (PW1930 generator, PW 1820 goniometer) with CuK radiation source (λ = 0.15418 nm) was used to compare the chemical structure of the blank and modified films with ZnO.

2.2. Preparation *of Antibacterial Film*

A response surface method (RSM) was employed to determine the optimal conditions for preparing the antibacterial film using Sol-Gel. The experimental results were analyzed using Design-Expert Version: 7.0.1, and the regression model was proposed. The pH solution, time (min), amount of ZnO nanoparticles (w/v %), and PTMS concentration (v/v %) were selected as four independent variables in the deactivation of E. coli and S. Aureus. The experimental ranges and the levels of the independent variables for antibacterial response are given in Table 1. It should be noted that initial experiments were accomplished to determine the extreme values of the variables. Accordingly, the CCD matrixes of 30 experiments covering the design of four process parameters were selected to study, names and results are shown in Table 2. Generally, the antibacterial film was prepared as follows: 0.48 mL PTMS was added to 10.0 mL methanol/de-ionized water solution with a ratio of 9:1. Before adding PTMS, the solution pH was adjusted by adding several drops of the 0.1 M HCl until solution pH equal to 6.5. Then, 0.068 g ZnO nanoparticles were added to this mixture. The mixture was placed on a magnetic stirrer and stirred with moderate speed for 95.0 min. Subsequently, a 2.0 cm × 2.0 cm LDPE film is immersed in the prepared solution and dried at ambient temperature for 60.0 min.

Table 1: Experimental range and levels of independent variables.

Factors	**+α**	**+1**	**0**	**-1**	**-α**
pH	1.0	3.5	6	8.5	11
Time (min)	1.0	30.75	60.5	90.25	120.0
ZnO (w/v %)	0.5	1.63	2.75	3.88	5
PTMS (v/v %)	0.25	1.44	2.63	3.81	5

2.3. Antibacterial *activity test*

The antibacterial response of the prepared films against E. coli and S. Aureus was studied utilizing the Kirby–Bauer technique. Accordingly, the bacterial inhibition zone around the circumference of a prepared film was employed to qualitatively assess whether or not the modified film possesses antibacterial properties (Mahendran et al., 2016; Shemesh et al., 2015). Square pieces (of 12 mm in diameter) were cut out of modified films and placed into the surface of a full concentration of Nutrient Broth (NB) agar in 9.0 cm Petri plates inoculated with 0.1 ml of 108 colony forming units (CFU)/mL of bacterial culture The plates were incubated at 37 °C for 24 h. Then, the antibacterial activity was recorded by observing the presence or absence of an inhibition zone diameter (IZD) around the prepared films. Unmodified films were assayed as a negative control. The inhibition zone tests were performed in triplicates and reported the average values. Schematic of antibacterial test stages of the prepared films are shown in Figure. 1.

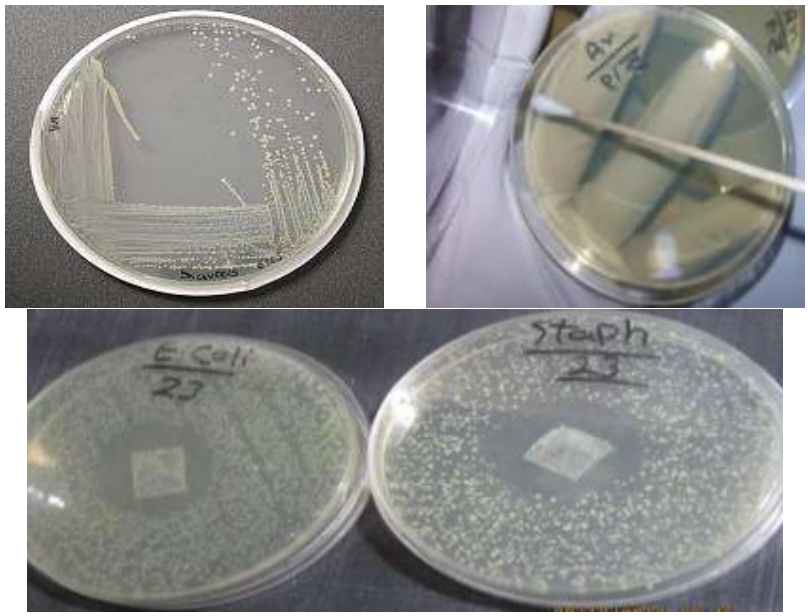

Fig 1. The antibacterial experiment processes of films

3. 3. Results and discussion

3.1. XRD

The structural characteristics of the nano-ZnO/LDPE film were determined by X-ray diffraction (XRD). Figure 2 shows the XRD patterns of the nano-ZnO/LDPE film, for which five main peaks were detected in the XRD spectrum, at 2θ of 26.5 °, 33.8 °, 37.9 °, 51.7 °, and 54.6 °, respectively, corresponding to the crystal structure of nano-ZnO. The peaks at 26.5 °, 33.8 °, 37.9 °, 51.7 °, and 54.6 °, belong to the (110), (101), (200), (211) and (220) planes of ZnO nanoparticles. Liang et al. (2012) also found similar results.

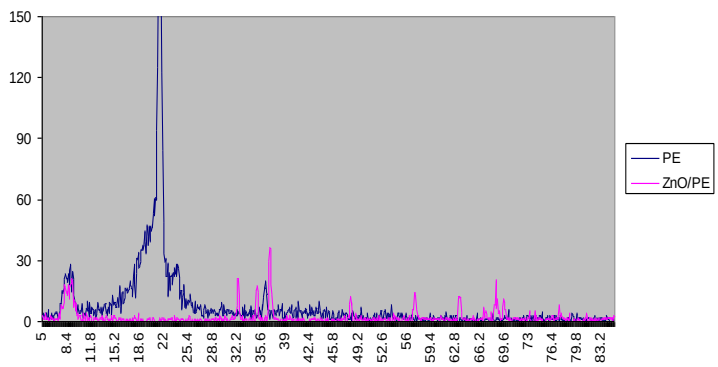

Fig 2. XRD patterns of LDPE and ZnO modified LDPE film

Table 2: Experimental design based on Central Composite design (CCD) used in present study

Order Test	pH	Time (min)	ZnO (w/v %)	PTMS (v/v %)	IZD (mm) for E coli	IZD (mm) for S. aureus
1	8.5	90.25	1.63	3.81	0	6
2	8.5	30.75	3.88	1.44	1.5	1.25
3	3.5	90.25	1.63	1.44	3	0
4	8.5	90.25	3.88	3.81	3.5	4
5	6	60.5	2.75	2.63	3.7	7.5
6	6	60.5	2.75	2.63	7	6
7	8.5	30.75	3.88	3.81	5	3
8	6	60.5	0.5	2.63	5	5
9	11	60.5	2.75	2.63	2	1
10	6	60.5	2.75	5	9	8
11	3.5	90.25	3.88	3.81	11	7.5
12	8.5	30.75	1.63	3.81	0.5	2
13	6	60.5	2.75	2.63	6	6.5
14	6	60.5	2.75	0.25	11	9
15	3.5	30.75	1.63	3.81	0	3.5
16	1	60.5	2.75	2.63	7	10
17	8.5	90.25	3.88	1.44	4	6
18	3.5	90.25	1.63	3.81	8.5	15
19	3.5	30.75	3.88	3.81	10	9
20	3.5	90.25	3.88	1.44	5	5
21	6	60.5	2.75	2.63	4.8	5.5
22	6	60.5	2.75	2.63	5.5	5.75
23	6	1	2.75	2.63	6.5	10
24	3.5	30.75	3.88	1.44	7	9
25	6	120	2.75	2.63	9	9
26	6	60.5	2.75	2.63	7	7
27	6	60.5	5	2.63	4	1.5
28	8.5	30.75	1.63	1.44	4	5
29	8.5	90.25	1.63	1.44	0	0
30	3.5	30.75	1.63	1.44	9	4

3.2. Effect *of Variables as Response Surface and Counter Plots*

In Figure. 3a, the effect of reaction time and pH on deactivation of E. Coli (as inhibition zone diameter (mm)) is shown at ZnO and PTMS concentration of 2.75 (w/v %) and 2.63 (v/v %), respectively. Figure 3a shows that as the PH of the solution increases from 5.5 to 11.0, the IZD value decreases. Figure 3a, also, illustrates that by increasing the reaction time to 95 min, the IZD value increases. The pH effect on IZD was significantly higher than the process variable of reaction time. On the other hand, when a lower pH was available, IZD increased with increasing the reaction time.

In Figure.3b, the change of IZD for E. Coli with ZnO and PTMS dosage is depicted at a time of 70 min and pH = 4.7. As the amount of nano-sized-ZnO increased, the IZD increased. Given the antibacterial properties of zinc oxide nanoparticles, it can be expected that as the amount of nanoparticles increases, more bacteria will be killed, and thus deactivation efficiency is higher. Several researchers (e.g., Li et al., 2011; Li et al., 2010; Li et al., 2015; Mirhosseini et al., 2014) also stated the effect of antimicrobial activity of ZnO nanoparticles on Listeria monocytogenes, Salmonella enteritidis, and E. coli. The results of their studies revealed that antibacterial efficiencies were related to the interaction or penetration of ZnO nanoparticles into microbial cells, which may be bactericidal or bacteriostatic, depending on the ZnO concentrations. The ZnO nanoparticles, which have positive zeta potential, easily disrupt the cell membrane of E. coli (gram negative) on contact and discharge Zn2+ ions, which cause lysosomal and mitochondrial destructions. In conclusion, this leads to the death of bacterial cells (Halder et al., 2015; Seil & Webster, 2012).

On the other hand, when the concentration of PTMS is increment, the IZD will be reduced gradually. Figure. 3c shows the response surface of the deactivation efficiency of S. Aureus (in IZD (mm)) as a function of pH and reaction time. As Figure 3 c shows, IZD increased with the increase of pH at 1.0 to 4.8 range, and then decreased. This can be related to the dehydration PTMS and formation of the 3D networks of SiO2. The PTMS is a silanol compound, which is hydrolyzed in the pH range of 4.5-5.0, and through its hydrolysis, a three-dimensional network of SiO2 will be formed. Only in this range PTMS was hydrolyzed and formed a three-dimensional network. It can be expected that nanoparticles are fixed on the surface of the film by the formation of the SiO2 layer. These findings are in agreement with those observed in our previous study (Nasirizadeh et al., 2015).

The effect of PTMS and ZnO values on deactivation efficiency of the S. Aureus are shown in Figure.3d. Figure 3d shows that IZD increased with the increase in nano-sized ZnO values from 0.1 to 4.0 w/v % and then plateaued. This observation implies that due to the higher concentration of nanoparticles on film, the inhibition zone surrounding the film will be larger (Jalal et al., 2010; Radheshkumar & Münstedt, 2006). These studies revealed that increasing the concentration of nanosized- ZnO resulted in higher antibacterial activity.

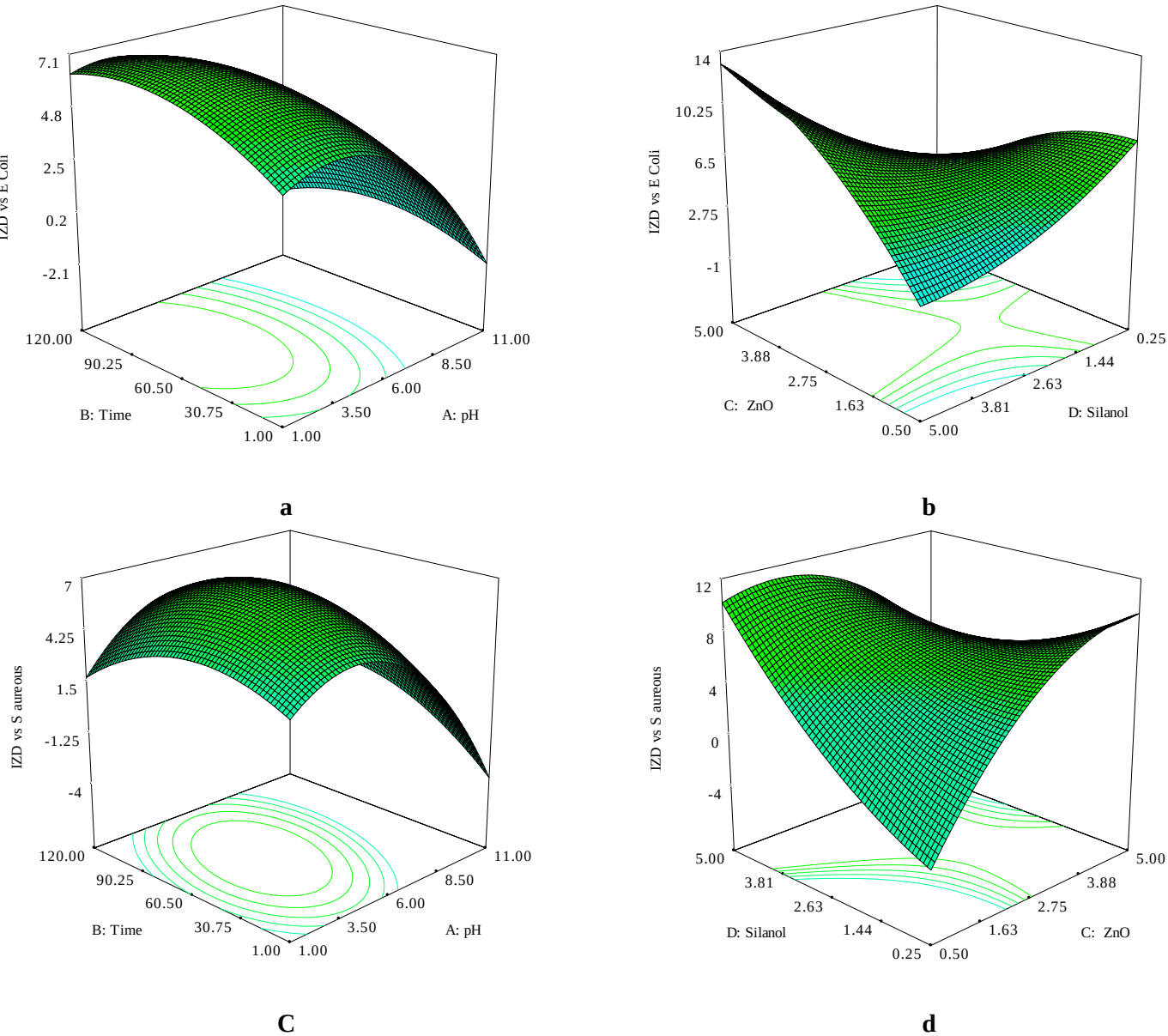

Fig. 3. The effect of operational parameters on inhibition growth E. Coli (a,b) and S. Aureus (c, d)

3.3. Statistical *analysis*

To find the optimum conditions for the preparation of the antibacterial packaging film, the experimental design as a function of the selected main factors has to be determined. The total number of experiments was 30 determined by the expression: 2n (24 = 16: factor points) +2n (2 *4=8: axial points) + 6 (center points: six

replications), as shown in Table 2. A full second-order polynomial model found by multiple regression method for four factors using the design of the expert 7.0.1 package was approved to describe the response surface.

The behavior of the system can be defined by the resulting quadratic equation:

$$Y=b_0+\sum b_i\ x_i+\sum b_{ii}\ x_i^2+\sum b_{ij}\ x_i\ x_j$$

where Y is the predicted response, b0 is the constant, bi is the linear effect of factor xi, bij is the linear interaction effect between the input factors xi and xj and bij is the quadratic effect of factor xi. Results attained for deactivation efficiencies of the E. coli and S. aureus are given in Table 2. Based on these results, an experiential model (for each bacterium) was generated in terms of coded factors (standardized equation) and stated by the following second-order polynomial equation (Eqs. (2) and (3)):

IZD vs E Coli =+5.38 - 2.41 A - 0.26 B + 0.44 C + 0.51 D - 0.51 AD + 0.56 BD + 1.24 CD eq. (2)

IZD vs S aureous =+ 5.61 - 1.64 A + 0.079 B + 0.27 C + 0.93 D + 0.32 AB - 0.094 AC - 1.22 AD - 0.26 BC + 1.29 BD - 1.27 CD eq. (3)

The achieved results were then examined by ANOVA to evaluate the "goodness of fit" and the significance and adequacy of the model (Esfandiyari et al., 2017; Etemadifar et al., 2014). As can be seen from Table 3 and 4, the model had a high value of coefficient of determination the R^2 = 88 % and R'^2= 91 % for E coli and S aureus, respectively. A desirable R^2 value is close to 1 and a reasonable agreement with adjusted R2 is essential. A high correlation coefficient (R2) provides a suitable adjustment of the quadratic model to the experimental data (Etemadifar et al., 2014). This high R^2 value implies that 88 and 91 % of the deviations for antibacterial efficiency are clarified by the independent variables which means that the model does not demonstrate only about 12 and 9 % of variation. The value of the adjusted R2 of 0.845 and 0.851 was also high to advocate the high significance of the models for the killing of E coli and S aureus. Therefore, the response surface model is precisely applied for forecasting variation of antibacterial efficiency.

The F values were used as a tool to check the significance of each of the interactions among the variables, which, in turn, may show the patterns of the mutual interactions between the experiment variables. Overall, the larger the magnitude of the F-value and the smaller the p-value, the more significant the corresponding coefficient term is pH. Here, the pH parameter was a more effective factor for two bacterium (F- value = 124.26 for E coli and 17.68 for S. aureus). Also, D (5.69), AD (6.53), BD (7.36), and CD (7.07) for S aureus, and D 2 (8.49) and BCD (5.17) for E coli were the next effective factors.

3.4. Determination *of optimal conditions*

The key objective of the optimization is to define the optimal values of variables for the preparation of antibacterial LDPE with ZnO nanoparticles from the model obtained using experimental data.

Concerning storage conditions of Milk in the range in neutral pH, best conditions in terms of pH of Milk storage i.e. pH= 6.0 were chosen. The optimization results which was selected included pH= 6.0, time= 103 min, 0.68 % w/v ZnO nanoparticles and 4.81 % v/v silanol agent. Then a specific experiment was performed under these optimum conditions to confirm the agreement of the result attained from the model and experiment. The results revealed that the killing efficiency of the E. coli and S. aureus for response parameter obtained from the experiment and as estimated by the model was satisfactory.

Table 3. ANOVA analysis of data for growth inhibition of E. Coli

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	186.257	10	18.62570542	14.49104684	< 0.0001	significant
A-pH	139.6355	1	139.6355042	108.6382817	< 0.0001	
B-Time	1.6485	1	1.648504167	1.282558194	0.2715	
C- ZnO	4.6025	1	4.602504167	3.58080953	0.0738	
D-Silanol	6.2935	1	6.293504167	4.896430048	0.0394	
AB	0.2232	1	0.22325625	0.173696335	0.6815	
AC	0.0770	1	0.07700625	0.059911888	0.8093	
AD	4.1107	1	4.11075625	3.198223103	0.0897	
BC	0.00075	1	0.00075625	0.000588373	0.9809	
BD	4.9395	1	4.93950625	3.843001639	0.0648	
CD	24.7257	1	24.72575625	19.23696762	0.0003	
Residual	24.4211	19	1.285325044			
Lack of Fit	16.1078	14	1.150560179	0.6919969	0.7312	not significant
Pure Error	8.3133	5	1.662666667			
Cor Total	210.67823	29				

R-Squared= 0.8841 and Adj R-Squared= 0.845

Table 4. ANOVA analysis of data for growth inhibition of S aureus

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	166.1038	10	16.61038	4.562322	0.0022	significant
A-pH	64.35375	1	64.35375	17.67585	0.0005	
B-Time	0.150417	1	0.150417	0.041314	0.8411	
C- ZnO	1.760417	1	1.760417	0.483528	0.4952	
D-Silanol	20.72042	1	20.72042	5.691215	0.0276	
AB	1.625625	1	1.625625	0.446506	0.5120	
AC	0.140625	1	0.140625	0.038625	0.8463	
AD	23.76563	1	23.76563	6.527634	0.0194	
BC	1.050625	1	1.050625	0.288572	0.5974	
BD	26.78063	1	26.78063	7.355755	0.0138	
CD	25.75563	1	25.75563	7.074221	0.0155	
Residual	69.17467	19	3.640772			
Lack of Fit	66.20592	14	4.728994	7.964622	0.0158	not significant
Pure Error	2.96875	5	0.59375			
Cor Total	235.2784	29				

R-Squared= 0.9104 and Adj R-Squared= 0.851

3.5. Application *of Antibacterial LDPE for packaging of Milk*

The application of such packaging materials is not meant to be a substitute for good sanitation practices, but it should enhance the safety of food as an additional hurdle for the growth of pathogenic and/or spoilage microorganisms. Milk is a good source of vitamins and minerals and a staple food for human beings. So it's no surprise that an appropriate medium for bacterial growth. Bacteria that are randomly entered into milk, can cause health problems for consumers. Therefore, the main objective of this research was established to provide antibacterial polyethylene films for milk packaging (Abo-Elnaga et al., 1985; Davoodi et al., 2013; Larson, 1974).

To prepare antibacterial packaging and study the anti-bacterial properties of the prepared packages on milk, a packaging film according to the size of milk packet available in the market was prepared based on optimum conditions. Some packets of milk using the sewing machine were prepared as antibacterial films. Then, a liter of milk (low-fat milk supplied from KALLEH dairy Co.) was transferred into each prepared packets, and 1.0 mL of a solution containing bacteria E Coli and s. aureus with a concentration of 1.50 × 10 8 CFU/mL was added to each packet.

The packets containing milk were gently shaken until a homogeneous solution was made. Packages were placed in an incubator for 48 h at 37 °C. Then, the number of surviving bacteria were counted after 7 and 14 days of culturing. A 1.0 mL sample of each dilution was plated on Count Skim Milk Agar and incubated at 37 °C for 48 h. The number of colony-forming units (CFU) per gram of sample was determined by counting typical colonies. All of these stages were repeated for unmodified polyethylene film. The results are shown in Figure 4. As Figure 4 illustrates, there is no antibacterial activity observed for unmodified LDPE. The ZnO nanoparticles coated LDPE result in better antibacterial activity than LDPE due to the presence of ZnO nanoparticles, which are having inhibition properties of bacteria. Moreover, the number of E. coli colonies in prepared milk packaging from nano-ZnO/LDPE decreased after 7 and 14 days. However, it is clear that the ZnO nanoparticles inhibit the growth of S. aureus in its medium.

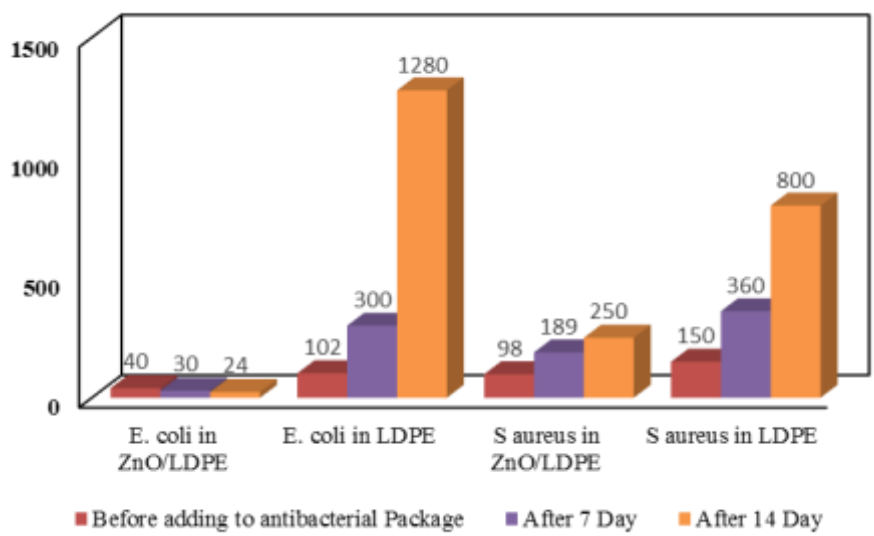

Fig 4. Antibacterial activity test of the unmodified and ZnO modified LDPE against S. aureus and E. coli after 7 and 14 day culturing in Milk.

Figure. 5 a and 5 b illustrate the visual growth of E. coli and S. aureus bacteria after 14 days incubation in prepared milk packaging. It can be seen that milk color in blank LDPE was changed (from white to brown) due to the duplication of bacteria in milk.

a b

Fig. 5. Milk sample contain a) E. Coli and b) S aureus packaged into LDPE and ZnO/LDPE films.

It was also important to evaluate the acidity value of milk samples after packaging in developed packaging film. For this, the acidity value (in terms of D: Dornic acidity degree) of milk samples in LDPE and ZnO/LDPE packages after 14 days was determined for E coli and S aureus inoculated, and its changes were depicted in Figure 6.

Figure 6. illustrates that the amount of milk acidity in samples containing S aureus remained relatively constant after 14 days of inoculation, and the amount of milk acidity in samples inoculated with E. coli bacteria was slightly increased than in sterilized milk. In the control samples, the samples acidity was much higher than those packaged in antibacterial films.

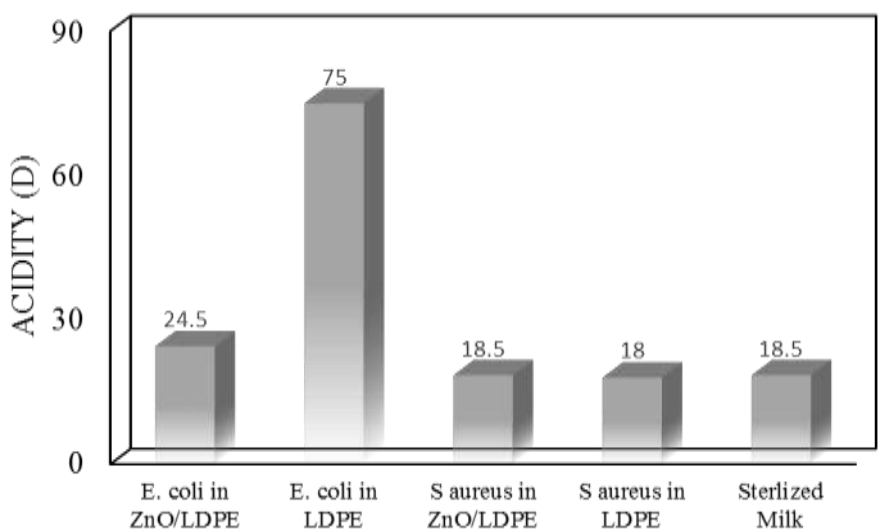

Fig. 6. Acidity changes of MILK packaged in LDPE and ZnO/LDPE films after 14 days inoculation of E coli and S aureus

4. Conclusion

This study has focused on presenting a method for coating LDPE film with ZnO nanoparticles using Sol-gel. The effect of pH solution, time (min), amount of ZnO nanoparticles (w/v %), and silanol agent concentration (v/v %) were investigated on the antibacterial activity of films using response surface methodology (RSM). Characteristics of the prepared films were investigated by FT-IR and XRD. The highest antibacterial activity of ZnO/LPDE were pH (6.0), time (103 min), amount of ZnO nanoparticles (0.68 % w/v) and silanol agent concentration (4.81 % v/v). The antibacterial properties of ZnO/LDPE films were evaluated based on the diameter of the inhibition zone in a disk diffusion test against E. coli and S. aureus. These films have significantly reduced the growth of mentioned bacteria. Overall, antimicrobial packaging shows promise as an effective method to inhibit the growth of certain bacteria like E. coli and S. aureus in milk.

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