Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Time–kill kinetic of nano ZnO-loaded nanoliposomes against *Escherichia coli* and *Staphylococcus aureus*

Parvin Souri[1], Aryou Emamifar [2]*, Nafiseh Davati[3]

1- Graduated Student of Food Sciences and technology, Department of Food Science and Technology, Bu-Ali Sina University, Hamedan, Iran.
2- Associated professor, Department of Food Science and Technology, Bu-Ali Sina University, Hamedan, Iran.
3- Assistant professor, Department of Food Science and Technology, Bu-Ali Sina University, Hamedan, Iran.

ABSTRACT

The objective of this study was to in-vitro investigation of antimicrobial activity effect of nano-ZnO loaded nanoliposomes at different level of lecithin: nano-ZnO ratio (5:1, 15:1, and 25:1 w/w) against *Escherichia coli* (ATCC 2592) and *Staphylococcus aureus* (ATCC 25923). Nano-ZnO loaded nanoliposomes were prepared through thin layer hydration sonication and heat methods. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of nano-ZnO loaded nanoliposomes and free nano-ZnO against *Escherichia coli* and *Staphylococcus aureus* were determined and their antimicrobial activities were evaluated by time- kill curve analysis. Results showed that the encapsulation of nano-ZnO in nanoliposome systems significantly increased antimicrobial activities of them by increasing their penetration into the microbial cell. Nano-ZnO loaded nanoliposomes were prepared through thin layer hydration showed higher antimicrobial activity compared to those prepared by heat method. From the time- kill curves, the log phase growth of *Escherichia coli* (8 hours) and *Staphylococcus aureus* (7 hours) in the medium containing nano-ZnO loaded nanoliposomes prepared through the thin layer hydration sonication at the highest level of lecithin: nano-ZnO ratio (25:1 w/w) at MIC and MBC values decreased to 5 and 4 hours and to 2 and less than 1 hours, respectively.

ARTICLE INFO

Article History:

Received: 2023/7/4
Accepted: 2023/8/12

Keywords:

Time–kill kinetic,

Nanoliposome,

Nano-ZnO.

DOI: 10.22034/FSCT.20.141.36
DOR:20.1001.1.20088787.1402.20.141.3.1

*Corresponding Author E-Mail:
a.emamifar@basu.ac.ir

1. Introduction

Nanotechnology refers to the method of design, production and application of structures, tools and systems on a nano scale[1]. Among the important uses of thisTechnology in the food industry is the production of materials with new functions, facilitating the process of delivering the product to the customer, producing new food formulations and packaging, and developing material transfer processes in nanometer dimensions.[2] . Nanocarriers are systems for transporting nutrients, drugs or biomolecules by entrapping them in internal cavities or by adsorbing them on their external surface.[3]. Encapsulation is a process of entrapping active compounds in small capsules by releasing their contents at controlled rates and times. This technology is used in various fields of pharmaceuticals, making cosmetics and hygiene products and food industries[4] . Nanocarriers used in food industry are classified into two categories: biopolymer and lipid. Lipid nanocarriers include nanoemulsions, nanoliposomes and solid lipid nanoparticles. Liposomes have the ability to carry and absorb hydrophilic and hydrophobic compounds in oily and aqueous environments due to their polar structure consisting of two layers of phospholipid membrane.[5]. Among the advantages of liposomes, we can mention the possibility of production on an industrial scale, compatibility with the environment, biodegradability and the absence of toxic compounds in their structure.[6]. The size of the vesicle, the number of layers and the production method are the basis for the classification of liposomes[8 and 7]. Liposomes that contain only one bilayer membrane are called unilamellar vesicles. Single-layer vesicles are also divided into two types: small single-layer vesicles with a size of less than 100 nm and large single-layer vesicles with a size greater than 100 nm.[9]. Multilamellar vesicles are liposomes consisting of a number of concentric bilayer vesicles. Vesicles composed of several non-concentric vesicles surrounded by a bilayer membrane are called multivesicular vesicles.[10]. Among the methods of producing liposomes, we can refer to mechanical methods such as thin film coating and sonication, homogenization and microfluidization, and non-mechanical methods such as reverse phase evaporation, freeze drying, and thermal methods.[7]. Nanostructured materials with antimicrobial properties are divided into two groups: inorganic antimicrobial nanomaterials (silver, titanium dioxide, zinc oxide, copper, clays and carbon nanotubes) and organic antimicrobial materials.[11]. In the meantime, zinc oxide nanoparticles, which are in the group of safe compounds for the consumer, have attracted the attention of many industries, including the food industry, with many advantages such as easy production process, cheapness, high thermal conductivity, and suitable antimicrobial activity. . The antimicrobial activity of zinc oxide nanoparticles has been proven against many Gram-positive, Gram-negative bacteria and fungi.[13 and 12]. The composition and addition of zinc oxide nanoparticles to food formulations is very challenging due to the lack of uniform distribution in the components of the formula. Therefore, encapsulation of zinc oxide nanoparticles in nanoliposome structures with the aim of proper and stable distribution of zinc oxide nanoparticles in food formulations is one of the solutions to overcome the above challenge.[14]. Nanoliposome production by thin layer water coating method due to its ease of operation and cheaper price, and thermal or mozafari method due to the absence of organic solvent and the ability to be industrialized have received a lot of attention in the industry.[15]. According to

the mentioned cases, it is necessary to find a suitable method for uniform distribution and minimum amount of zinc oxide nanomaterials in food formulations with the aim of enriching or using the antimicrobial properties of this compound.

2- Materials and methods

2-1- Materials

Zinc oxide nanoparticles with a purity of 99.9% (Mina Tehiz company, Tehran), soy lecithin (L-alpha-phosphatidylcholine) (Sigma, Germany), ethanol 99.9%, phosphate buffer, Tween 80, Muller Hinton broth and Muller Hinton agar culture medium They were prepared from Merck, Germany. Microbial strains*Escherichia coli*(ATCC 2592) And*Staphylococcus aureus* (ATCC 25923(from the Scientific and Industrial Research Organization of Iran)IROST) were prepared.

2-2- Nanoliposome production by thermal method

Nanoliposomes were produced according to the method of Rasti et al. (2012) with a slight change and without using organic solvent.[16]. The aqueous phase contains a 3% (weight-volume) lecithin solution in phosphate buffer (2/7- 4/7 = pH) was prepared in a spa bath with a temperature of 50°C for 5 minutes. Certain amounts of zinc oxide nanoparticles were added to the aqueous phase with the aim of achieving different levels of lecithin to zinc oxide nanoparticles (at three levels of 5:1, 15:1 and 25:1 w/w) and heated in a hot water bath with a temperature of 50°C for 60 It was mixed on a magnetic stirrer at a speed of 600 rpm. In order to achieve a uniform distribution of nanoliposomes, emulsions with a homogenizer (Dragon-Lab, D-500, China) were stirred at a speed of 5000 rpm for 5 minutes.

2-3- Production of nano liposome by thin film coating method

Nanoliposomes were produced according to the method of Chow et al. (2020) with a slight modification[17]. First, a 3% (weight-volume) solution of lecithin in pure ethanol and then specified amounts of zinc oxide nanoparticles with the aim of achieving the ratios of lecithin to zinc oxide nanoparticles (at three levels of 5:1, 15:1 and 25:1 weight-weight) to the composition The above was added. This compound is slowly added to the phosphate buffer solution (2/7- 4/7 = pH) containing Tween 80 was added and stirred for 2 hours at 60°C. The resulting solution is sent to the rotary evaporator under vacuum (LabTech EV311H, China) was transferred to evaporate ethanol at a temperature of 60 °C. The thin layer formed with 10 ml of sterile deionized water washed with a homogenizer (Dragon-Lab, D-500, China) were stirred at a speed of 5000 rpm for 5 minutes. At the end, the liposomal mixture was mixed in an ice bath using a sonicator prop.Ultrasonic Homogenizer UH-600) was mixed with the power of 5 cycles of one minute with one minute rest.

4-2- Initial activation of microbial strains

Microbial strains were cultured linearly on Mueller Hinton agar medium at 37°C for 24 hours. To ensure the type of strain, a single colony was selected and microscopic examinations were performed after warm staining. The strains were stored in Mueller Hinton broth culture medium containing 15% glycerol at -18°C until use.[12].

5-2- Determining the minimum concentration of growth inhibition (Minimum inhibitory concentration (MIC)(and the minimum lethal concentration)Minimum Bactericidal Concentration (MBC))

In order to determine the minimum concentration of growth inhibitory (MIC) zinc oxide nanoparticles and nanoliposomes containing zinc oxide nanoparticles were used on the growth of the studied bacteria by dilution method using 96-well microplates. In the first well of each row, 100 microliters of nano-oxygen stock solutions with concentrations of 80, 100, 120 and 140 mg/ml were added separately. The concentration of nanostructured compounds was set in the range of 0.78 to 50 mg/ml. 100 microliters of fresh microbial culture suspension in Mueller Hinton Broth culture medium of each strain with half McFarland concentration (cfu/mL) 10^8 1.5 × was added to all studied wells. The last well of each row containing culture medium and bacteria (medium without nanoparticles) was considered as positive control and one row of microplate containing culture medium and different concentrations of nanoparticles (medium without bacteria) was considered as negative control. The microplates were kept in a greenhouse at 37°C for 24 hours. The lowest concentration of nano material suspension in the well that did not show turbidity as the minimum inhibitory concentration (MIC) Nanoparticles were determined. The amount of 100 microliters from each of the wells in which bacteria did not grow and no turbidity was observed was transferred to Mueller Hinton agar medium and cultured superficially. After 24 hours of incubation at 37°C, the plates were checked for bacterial growth. A concentration of nanomaterials at which the bacteria did not grow, as the minimum bactericidal concentration (MBC) Was considered[18].

6-2- Evaluation of the time curve–lethality (Time-kill curve analysis)

500 microliters suspension of each microbial strain that was at the end of the logarithmic phase with an approximate concentration of 49 ml of Mueller Hinton brothcfu/mL) 10^6- 10^5 added. 500 microliters of each of the concentrations (MIC AndMBC) the prepared nanoliposomes and uncoated nanooxydroid were added separately to the desired medium and placed in a greenhouse at a speed of 150 rpm and a temperature of 37 degrees Celsius. In order to evaluate the growth curve of bacteria without the presence of nanoparticles and in the presence of each of the nanoparticles and at specific time intervals (one hour) and within 8 hours, samples were taken from the environment, cultured on Mueller Hinton agar culture medium and kept at 37 degrees Centigrade were kept for 24 hours. The number of colonies as (Log cfu/mL(Report and growth curve of bacteria according to changes in the number of bacteria)Log cfu/mL) was drawn in the unit of time[19].

2-7- Statistical analysis

This evaluation was done based on the factorial method and completely randomized design with three replications. Data analysis with analysis of variance test (ANOVA(with software)SPSS) Done. Means based on least significant difference (LSD) were compared at the 5% probability level.

3. Results and Discussion

1-3- Examining the minimum concentration of growth inhibition (MIC(and the minimum lethal concentration)MBC)

Comparison of the averages of minimum growth inhibition and minimum lethal concentration of free zinc oxide

nanoparticles and nanoliposomes prepared by thermal method (Thin(and thin layer waterproofing)Therm) against bacteria*Staphylococcus aureus* And*Escherichia coli* It is given in Table 1. The results show a significant increase(05/0p<) The antimicrobial power of coated zinc oxide nanoparticles in nanoliposomal systems was compared to uncoated zinc oxide nanoparticles. This increase can be related to the increased flexibility and maneuverability of zinc oxide nanoparticles encapsulated in nanoliposomal carriers compared to free samples.[20]. The antimicrobial power of zinc oxide nanoparticles to create oxidative stress resulting from the production of free oxygen radicals (ROS) in the cell membrane, the release of zinc cations in the growth medium of microorganisms or the direct contact of zinc oxide nanoparticles with the cell wall of microorganisms is related[13]. The difference in the cell wall structure of Gram-positive and Gram-negative bacteria has an effect on the binding rate of zinc oxide nanoparticles to the surface of the cell wall. Despite the existence of a thicker peptidoglycan layer in the wall of gram positive bacteria which prevents the penetration of nanoparticles into the cell, the surface proteins of peptidoglycan as well as lipoteichoic acids act as binding sites for zinc oxide nanoparticles in the cell wall and therefore the sensitivity of gram positive bacteria is compared to Gram-negative bacteria increase zinc oxide compared to nanoparticles. The presence of lipopolysaccharide outer membrane around the cell wall of Gram-negative bacteria is a good protective factor against the penetration of nanoparticles.[21]. Li et al. (2009) in investigating the antimicrobial effect of zinc oxide nanoparticles coated on polyvinyl chloride film against bacteria*Staphylococcus aureus* And*Escherichia coli* They showed that the antimicrobial power of these nanoparticles against Gram-positive bacteria is more than Gram-negative, which was consistent with the results of this research.[22].

Table 1. The minimum inhibitory concentration (MIC) and the minimum bacteriocidal concentration (MBC) values of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by thin layer hydration sonication (Thin) and heat method (Therm) with the different ratio of lecithin: nano-ZnO (5:1, 15:1 and 25:1 w/w), against *Escherichia coli* (ATCC 2592) and *Staphylococcus aureus* (ATCC 25923). Different letters indicate statistically significant differences at ($p < 0.05$).

Antimicrobial agents	Microorganisms type			
	Escherichia coli (ATCC 2592)		*Staphylococcus aureus (ATCC 25923)*	
	MIC value	MBC value	MIC value	MBC value
Free nano-ZnO	15.27 ^{a} ± 0.07	20.00 ^{a} ± 0.07	7.59 ^{a} ± 0.07	15.15 ^{a} ± 0.07
Therm 5:1	6.66 ^{b} ± 0.07	13.34 ^{b} ± 0.07	6.66 ^{b} ± 0.07	6.65 ^{b} ± 0.14
Therm 15:1	5.33 ^{c} ± 0.14	10.65 ^{c} ± 0.14	2.65 ^{c} ± 0.14	5.32 ^{c} ± 0.08
Therm 25:1	2.65 ^{d} ± 0.08	5.31^{d} ± 0.08	1.32 ^{d} ± 0.08	2.65 ^{d} ± 0.02
Thin 5:1	6.66 ^{b} ± 0.07	13.34 ^{b} ± 0.07	6.66 ^{b} ± 0.07	6.65 ^{b} ± 0.03
Thin 15:1	5.33 ^{c} ± 0.14	10.65 ^{c} ± 0.14	2.65 ^{c} ± 0.14	5.32 ^{c} ± 0.15
Thin 25:1	2.65 ^{d} ± 0.08	5.31^{d} ± 0.08	1.32 ^{d} ± 0.08	1.32 $^{It\ is}$ ± 0.10

2-3- Examining the time curve–fatality*Escherichia coli*

According to Figure 1, the length of 8 hours of the logarithmic phase of bacteria*Escherichia coli* In the presence of uncoated zinc oxide nanoparticles, the minimum inhibitory concentration decreased to 5 hours and the minimum lethal concentration decreased to 3 hours. The length of the logarithmic phase of bacteria*Escherichia coli* In the presence of thermally coated zinc oxide nanoparticles at different levels of lecithin to nanoparticles

(5:1, 15:1 and 25:1 w/w) in the minimum inhibitory concentration to 5, 5 and 5 hours respectively and in the minimum lethal concentration It was reduced to 3, 2 and 2 hours. Also, in the presence of nanoliposomes containing zinc oxide nanoparticles prepared by thin layer coating method at different levels of lecithin to nanoparticles (5:1, 15:1 and 25:1 w/w) respectively at the minimum inhibitory concentration of 5, 4 and 4 hours and At the minimum lethal concentration, it was reduced to 3, 2 and 2 hours. Increasing the ratio of lecithin to zinc oxide nanoparticles decreases the minimum inhibitory concentration values (MIC(and the minimum lethal concentration)MBC) Nanoliposomes containing zinc oxide nanoparticles against the growth of bacteria*Escherichia coli* within 8 hours. The length of the logarithmic phase of bacteria*Escherichia coli* in equal amounts (MIC AndMBC) nanoliposomes containing zinc oxide nanoparticles produced by thermal and water coating method of thin layer in low ratios of lecithin to zinc oxide nanoparticles (5:1) were the same. Increasing the ratio of lecithin to zinc oxide nanoparticles in equal amounts (MBC) Nanoliposomes containing zinc oxide nanoparticles produced by thermal and water coating method of thin layer, the length of the logarithmic phase of bacteria*Escherichia coli* reduced Increasing the ratio of lecithin to zinc oxide nanoparticles in equal amounts (MIC) Nanoliposomes produced by thermal method change during the logarithmic phase of bacteria*Escherichia coli* did not create According to Figure 1, changing the number of (Log cfu/ml) bacteria*Escherichia coli* compared to the initial number during 8 hours of growth, it was equal to 2.44. The amount of these changes in equal amounts (MIC AndMBC) uncoated zinc oxide nanoparticles (+0.64 and -1.19) and nanoliposomes containing nanooxide with the ratio of lecithin to zinc oxide nanoparticles (weight-weight) at different levels (5:1), (15:1) and (25) 1) In the thermal method, respectively (0.34 and -1.36), (0.04 and -1.66), and (-0.24 and -2.66) and in the water covering method, respectively (34 0.0 and -1.66), (0.04 and -2.16) and (0.31 and -2.66) were measured. Examining the curves in Figure 1 showed that the nanoliposomes produced using the thin film coating method compared to the thermal method have greater antimicrobial power against*Escherichia coli* They had lecithin in the same concentrations. The logarithm of the number of bacteria *Escherichia coli*In the presence of nanoliposomes containing zinc nanooxide with a ratio (25:1) of lecithin to nanooxide (weight-weight), in the thin film coating method after 7 hours, and in the presence of the same nanoliposomes produced in the thermal method, it reached zero after 8 hours. .

Ware et al. (2004) investigated the antimicrobial power of nisin encapsulated in phospholipid liposomes against*Listeria monocytogenes* showed that the encapsulation of nisin in liposomes reduced the number of bacteria by 2 logarithmic cycles compared to free nisin.[23]. Jin et al. (2009) also showed that the use of zinc oxide nanoparticles coated with polyvinylpyrrolidone has a greater ability to reduce the number of bacteria compared to uncoated zinc oxide nanoparticles.*Escherichia coli* There is liquid in the egg white environment, which was consistent with the results of this research[24].

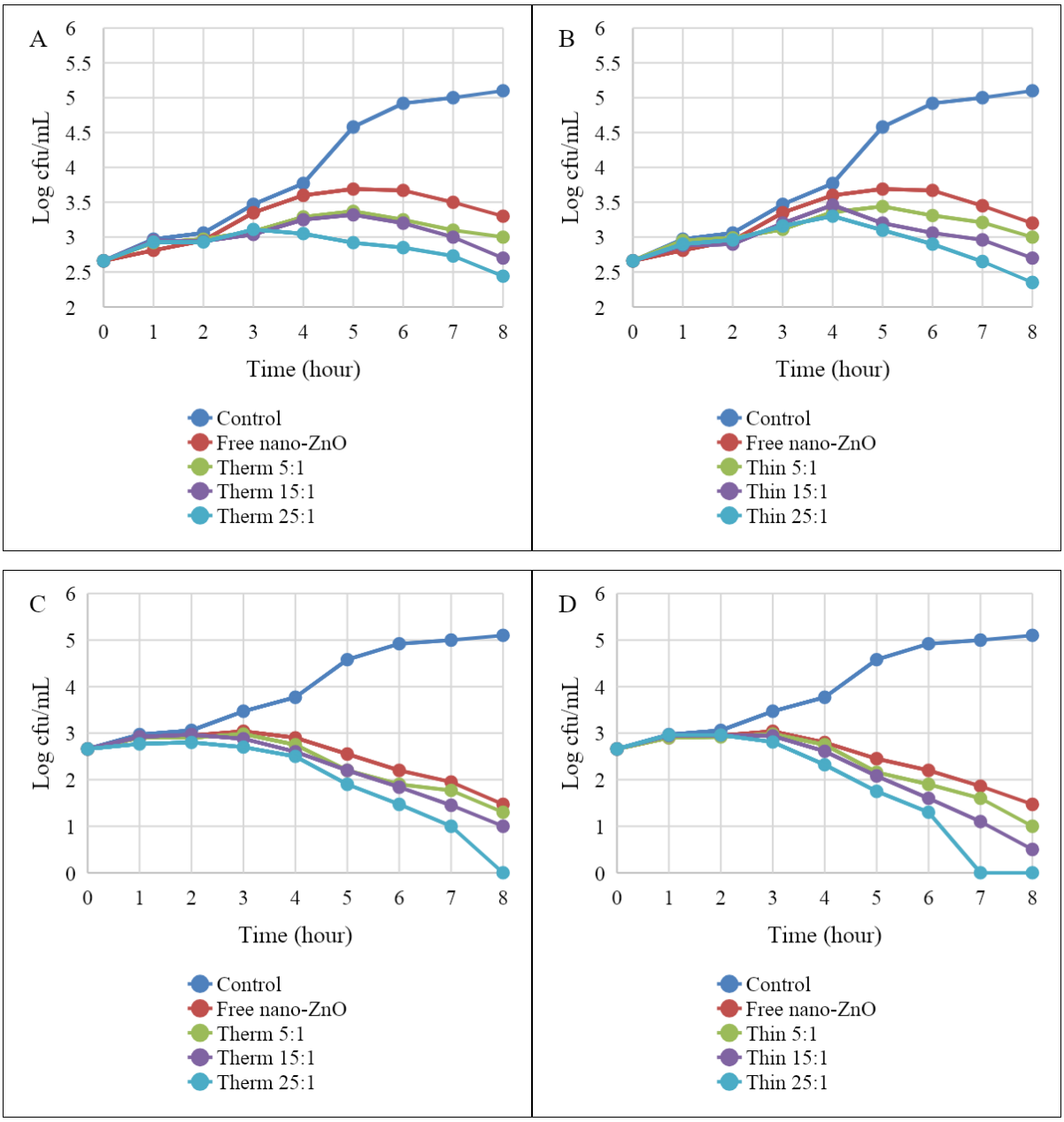

Fig. 1. Time-kill graph of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by thin layer hydration sonication (Thin) and heat method (Therm) with the different ratio of lecithin: nano-ZnO (5:1, 15:1 and 25:1 w/w) against *E. coli (*ATCC 2592*)* at MIC value of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by Therm (A) and Thin (B) and MBC value of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by Therm (C) and Thin (D). In all figures "Control" means growth without any antimicrobial agent.

3-3- Examining the time curve–fatality*Staphylococcus aureus*

According to Figure 2, the length of the logarithmic phase of bacteria*Staphylococcus aureus* It was about 7 hours in the presence of uncoated zinc oxide nanoparticles at the minimum inhibitory concentration (MIC(to 5 and at the minimum lethal concentration)MBC) was reduced to 4 hours. The logarithmic phase length of these bacteria in the presence of thermally coated

zinc oxide nanoparticles at different levels of lecithin to zinc oxide nanoparticles (5:1, 15:1 and 25:1 w/w) respectively at the minimum inhibitory concentration of 5, 5, and 4 hours and decreased to less than one hour in the minimum lethal concentration. On the other hand, the length of the logarithmic phase of bacteria*Staphylococcus aureus* In the presence of nanoliposomes containing zinc oxide nanoparticles prepared by thin layer coating method at different levels of lecithin to zinc oxide nanoparticles (5:1, 15:1 and 25:1 w/w) respectively at the minimum inhibitory concentration of 5, 4 and 4 hour and in the minimum lethal concentration reached less than one hour. The use of nanoliposomes in all ratios of lecithin to zinc oxide nanoparticles in reducing the length of the logarithmic period of bacteria*Staphylococcus aureus* in equal amounts (MIC AndMBC) was effective in both thermal and water coating thin film methods and increasing the ratio of lecithin to oxide nanoparticles on the length of the logarithmic phase of the bacteria *Staphylococcus aureus* reduced According to Figure 2, the number change (Log cfu/ml) bacteria*Staphylococcus aureus* compared to the initial number during 8 hours of growth, it was equal to 2.76. These changes in equal amounts (MIC AndMBC) uncoated zinc oxide nanoparticles (+0.21 and -1.54) and nanoliposomes containing nanooxide prepared with the ratio of lecithin to zinc oxide nanoparticles (weight-weight) at different levels (5:1), (15:1) and (25:1) in the thermal method respectively (-0.04 and -1.84), (-0.19 and -2.54) and (-0.39 and -3.04) and in the water covering method to The order of (-0.14 and -2.04), (-0.40 and -3.04) and (-0.54 and -3.04) were measured. The results of Figure 2 showed that increasing the ratio of lecithin to zinc oxide nanoparticles caused a decrease in the minimum inhibitory concentration (MIC(and the minimum lethal concentration)MBC) Nanoliposomes containing zinc oxide nanoparticles against bacteria*Staphylococcus aureus* It was over 8 hours. Examining the curves in Figure 2 showed that the nanoliposomes produced using the thin film coating method compared to the thermal method have greater antimicrobial power against*Staphylococcus aureus* They had lecithin in the same concentrations, so that in the presence of nanoliposomes produced with a ratio (25:1) of lecithin to zinc oxide nanoparticles (weight - weight), in the thin layer coating method after 7 hours and in the presence of the same nanoliposomes produced in the method The temperature reached zero after 8 hours. Elaghil et al.'s studies (2016) showed that the antimicrobial power of zinc oxide nanoparticles against*Staphylococcus aureus*Compared with Nanooxidation increased greatly[25]. Ziyai et al. (2017) by investigating the antimicrobial effect of nanoliposomes containing nisin and natamycin on growth*Staphylococcus aureus* reported that the use of encapsulated nisin and natamycin compared to the free form had a significant effect on reducing the number of bacteria*Staphylococcus aureus* had[26]. In this connection, Firouzabadi et al. (2016) showed in their studies that the use of a suspension of zinc oxide nanoparticles containing citric acid in the environment of mango fruit juice during storage at room temperature caused a decrease of 5 logarithmic cycles in the number of bacteria.*Staphylococcus aureus* which was consistent with the results of this research[27].

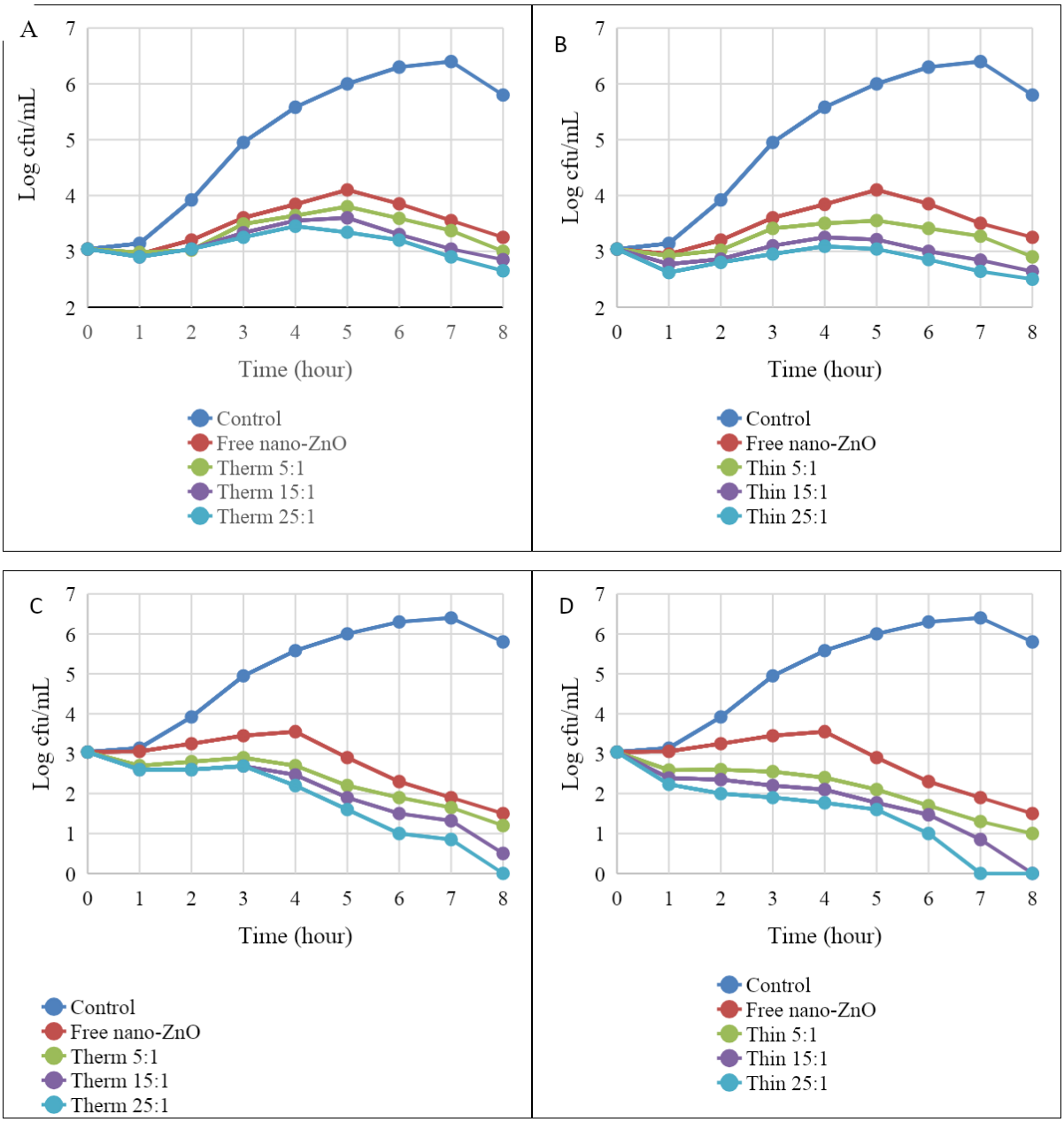

Fig. 2. Time-kill graph of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by thin layer hydration sonication (Thin) and heat method (Therm) with the different ratio of lecithin: nano-ZnO (5:1, 15:1 and 25:1 w/w) against *Staphylococcus aureus (*ATCC 25923*)* at MIC value of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by Therm (A) and Thin (B) and MBC value of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by Therm (C) and Thin (D). In all figures "Control" means growth without any antimicrobial agent.

4 - Conclusion

The use of antimicrobial compounds on the nanometer scale has created a huge transformation in various industries, especially the food industry. Meanwhile, metal nanoparticles, especially metal oxides, including zinc oxide nanoparticles, have attracted more attention among food industry researchers due to their favorable

antimicrobial power and on the other hand, the approval of the Food and Drug Organization for food use. The results of this research showed that by encapsulating zinc oxide nanoparticles inside nanoliposomes, their antimicrobial power increased significantly.(05/0p<). The type of method used in the production of this type of nanoparticles was also significantly effective in increasing their antimicrobial activity. Zinc oxide nanoparticles encapsulated in nanoliposomes produced by the thin layer water coating method had higher antimicrobial power compared to the thermal method.(05/0p<). Increasing the ratio of lecithin to zinc oxide nanoparticles has a significant effect on reducing the minimum inhibitory concentration values (MIC(and the minimum lethal concentration)MBC) had zinc oxide nanoparticles encapsulated in nanoliposomes. In general, the increase in the antimicrobial power of nanoparticles coated with nanoliposomes can be related to the increase in penetration into the microorganism cell and the better adhesion of nanoliposomes to the surface of the cell wall of microorganisms.

5- Resources

[1] Huang, Q., Yu, H., & Ru, Q. (2010). Bioavailability and delivery of nutraceuticals using nanotechnology. *Journal of Food Science*, 75(1), R50–R57.

[2] Nile, S. H., Baskar, V., Selvaraj, D. Nile, A., Xiao, J., & Kai, G. (2020). Nanotechnologies in food science: applications, recent trends, and future perspectives. *Nano-Micro Letters*, 12, 45.

[3] Acosta, E. (2009). Bioavailability of nanoparticles in nutrient and nutraceutical delivery. *Current opinion in colloid and interface science*, 14(1), 3-15.

[4] Sauvant, P., Cansell, M., Sassi, A. H., & Atgié, C. (2012). Vitamin A enrichment: Caution with encapsulation strategies used for food applications. *Food Research International*, 46(2), 469-479.

[5] Keller, B. C. (2001). Liposomes in nutrition. *Trends in Food Science and Technology*, 12(1), 25–31.

[6] Neshastegir, M. H., Mohebi, M., & Hadad Khodaparast, M. H. (2018). Preparation nanoliposome containing orange essential oil using heat method. *Journal of Innovation in Food Science and Technology*, 10 (2), 115-122 [in Persian].

[7] Mozafari, M.R., Flanagan, J., Matthias-Merino, L., Awati, A., Omri, A., Suntres, Z. E., & Singh, H. (2006). Recent trends in the lipid-based nanoencapsulation of antioxidants and their role in foods. *Journal of the Science of Food and Agriculture*, 86(13), 2038-2045.

[8] Mozafari, M. R. (2005). Liposomes: an overview of manufacturing techniques. *Cellular and molecular biology letters*, 10(4), 711–719.

[9] Taylor, T. M., Weiss, J., Davidson, P. M., & Bruce, B. D. (2005). Liposomal nano capsules in food science and agriculture. *Critical reviews in Food science and Nutrition*, 45(7-8), 587-605.

[10] Maherani, B., Arab-Tehrany, E., Mozafari, M.R., & Gaiani, C. and Linder, M. (2011). Liposomes: A Review of Manufacturing Techniques and Targeting Strategies. *Current Nanoscience*, 7(3), 436-452.

[11] Blanco-Padilla, A., Soto, K. M., Iturriaga, M. H., & Mendoza, S. (2014). Food antimicrobials nanocarriers. *The Scientific World Journal*, 2014, Article ID 837215.

[12] Mirhosseini, M. Kiany Harchegani, M., Kakai Dehkordi, S. Barzegary & Firouzabadi, F. (2013). Comparison of antibacterial effect of ZnO nanoparticles in apple juice at 25 and 4. *Quarterly Journal of Experimental Animal Biology*, 2 (3), 9-15 [in Persian].

[13] Emamifar, A., & Mohamadizadeh, M. (2020). Influence of sonication and antimicrobial packaging-based nano-ZnO on the quality of fresh strawberry juice during cold

storage. *Journal of Food Measurement and Characterization*, 14 (6), 3280-3290.

[14] Dimapilis, E. A. S., Hsu, C. S., Mendoza, R. M. O., Lu, M. C. (2018). Zinc oxide nanoparticles for water disinfection. *Sustainable Environment Research*, 28 (2), 47-56.

[15] Muthukrishnan, L. (2022). Nanonutraceuticals - challenges and novel nano-based carriers for effective delivery and enhanced bioavailability. *Food and Bioprocess Technology*, 15, 2155–2184.

[16] Rasti, B., Jinap, S., Mozafari, M. R., & Yazid, A. M. (2012). Comparative study of the oxidative and physical stability of liposomal and nanoliposomal polyunsaturated fatty acids prepared with conventional and Mozafari methods. *Food Chemistry*, 135, 2761-2770.

[17] Cao, D., Shu, X., Zhu, D., Liang, S., Hasan, M., & Gong, S. (2020). Lipid-coated ZnO nanoparticles synthesis, characterization and cytotoxicity studies in cancer cell. *Nano Convergence*, 7, 14.

[18] Momeni-Javid, Z, Hamishekar, H., Rahmati-Yamchi, M., Zarghami, N., Akbarzadeh, A. & Milani, M. (2017) Evaluation and study of antimicrobial activity of nanoliposomal meropenem against *Pseudomonas aeruginosa* isolates. *Artificial Cells, Nanomedicine, and Biotechnology, 45 (5)*, 975-980.

[19] Avila, J. G., de Liverant, J. G., Martınez, A., Martınez, G., Munoz, J. L., Arciniegas, A., & de Vivar, A. R. (1999). Mode of action of Buddleja cordata verbascoside against Staphylococcus aureus.*Journal of ethnopharmacology*, 66(1), 75-78.

[20] Gortzi, O., Lala, S, Chinou, I., & Tsaknis, J. (2007). Evaluation of the antimicrobial and antioxidant activities of Origanum dictamnus extracts before and after encapsulation in liposomes. *Molecules*. 12(5), 932–945.

[21] Tayle, A. A., El-tras, W. F., Moussa, Sh., El.bazi, A. F., Mahrous, H., Salem, M. F., and Brimer, L. (2011) Antibacterial action of zinc oxide nanoparticles against foodborne pathogens. *Journal of Food Safety*, 31(2), 211-218.

[22] Li, H., Li, F., Wang, L., Sheng, J., Xin, Z., Zhao, L., Xiao, H., Zheng, Y. & Hu, Q. (2009). Effect of nano-packing on preservation quality of Chinese jujube (*Ziziphus jujube Mill. var. unarmed (Bunge) Rehd*). *Journal of Food Chemistry*, 114, 547-552.

[23] Were, L. M., Bruce, B., Davidson, P. M., & Weiss, J. (2004). Encapsulation of nisin and lysozyme in liposomes enhances efficacy against Listeria monocytogenes. *Journal of food protection*, 67(5), 922-927.

[24] Jin, T., Sun, D., Su, J. Y., Zhang, H., & Sue, H. J. (2009). Antimicrobial efficacy of zinc oxide quantum dots against *Listeria monocytogenes, Salmonella enteritidis*, & *Escherichia coli* O157: H7.*Journal of food science*, 74(1), 46-52.

[25] Aleaghil, S. A., Fattahy, E., Baei, B., Saghali, M., Bagheri, H., Javid, N., & Ghaemi, E. A. (2016) Antibacterial activity of zinc oxide nanoparticles on *Staphylococcus aureus*. *International Journal of Advanced Biotechnology and Research*, 7(3), 1569-1575.

[26] Ziaee, M., Sowti Khiabani, M., Tizchang, S., Ghanbarzadeh, B., Hamishehkar, H., & Rezay Mokaram, R. (2017). Study of antibacterial effect of nano-encapsulated nisin and natamycin in liposomes against the growth of *Staphylococcus aureus* and *Aspergillus niger*. *Journal of food science and technology (Iran)*, 62 (14), 142-259 [in Persian].

[27] Firouzabadi, F. B., Marzban, Z., Khaleghizadeh, S., Daneshmand, F., & Mirhosseini, M. (2016). Combined effects of zinc oxide nanoparticle and malic acid to inhibit Escherichia coli and Staphylococcus aureus". *Iran Journal of Medical Microbiology*, 10(5), 52-9 259 [in Persian].

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

سینتیک زمان – کشندگی نانولیپوزوم‌های حاوی نانوذرات اکسیدروی علیه *اشرشیاکلی* و *استافیلوکوکوس اورئوس*

پروین سوری[۱]، آریو امامی‌فر[۲]*، نفیسه دعوتی[۳]

۱- دانش آموخته کارشناسی ارشد فناوری مواد غذایی، دانشگاه بوعلی سینا، همدان

۲- دانشیار گروه صنایع غذایی، دانشکده صنایع غذایی، دانشگاه بوعلی سینا، همدان

۳- استادیار گروه صنایع غذایی، دانشکده صنایع غذایی، دانشگاه بوعلی سینا، همدان

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۲/۴/۱۳

تاریخ پذیرش: ۱۴۰۲/۵/۲۱

کلمات کلیدی:

سینتیک زمان – کشندگی،

نانولیپوزوم،

نانواکسیدروی.

DOI: **10.22034/FSCT.20.141.36**

DOR:**20.1001.1.20088787.1402.20.141.3.1**

* مسئول مکاتبات:

a.emamifar@basu.ac.ir

چکیده

هدف این مطالعه بررسی اثر ضدمیکروبی نانولیپوزوم‌های حاوی نانوذرات اکسیدروی در نسبت‌های مختلف لسیتین به نانو ذرات اکسیدروی (۱:۵، ۱:۱۵ و ۱:۲۵ وزنی- وزنی) بر علیه سویه‌های *اشرشیا کلی*(ATCC 2592) و *استافیلوکوکوس اورئوس* (ATCC 25923) در محیط کشت آزمایشگاهی بود. نانولیپوزوم‌های حاوی نانوذرات اکسیدروی به دو روش آب پوشانی لایه نازک و حرارتی تهیه شدند. مقادیر حداقل غلظت بازدارندگی (MIC) و حداقل غلظت کشندگی (MBC) نانولیپووزم‌های حاوی نانوذرات اکسید روی تولید شده در مقایسه با نانوذرات اکسید روی بدون پوشش بر علیه سویه‌های *اشرشیا کلی* و *استافیلوکوکوس اورئوس* تعیین و فعالیت ضد میکروبی آن ها با استفاده از منحنی زمان- کشندگی ارزیابی گردید. نتایج نشان داد که درون پوشانی نانو ذرات اکسید روی با استفاده از سامانه های نانولیپوزومی با افزایش میزان نفوذ به داخل سلول میکروارگانیسم به صورت معنی داری سبب افزایش قدرت ضد میکروبی آن ها گردید ($p<۰/۰۵$). نانولیپوزوم حاوی نانوذرات اکسیدروی تولید شده به روش آب پوشانی لایه نازک در مقایسه با روش حرارتی قدرت ضد میکروبی بالاتری داشتند. بر طبق منحنی های زمان- کشندگی، طول فاز لگاریتمی باکتری‌های *اشرشیاکلی* (۸ ساعت) و *استافیلوکوکوس اورئوس*(۷ ساعت) در محیط کشت شامل نانولیپوزوم‌های حاوی نانو ذرات اکسیدروی تولید شده به روش آب پوشانی لایه نازک با بالاترین نسبت لسیتین به نانوذرات اکسید (۱:۲۵ وزنی- وزنی) به ترتیب در مقادیر حداقل غلظت بازدارندگی به ۵ و ۴ ساعت و حداقل غلظت کشندگی به ۲ و کمتر از یک ساعت کاهش یافت.