

Development and Evaluation of Antibacterial Microsphere using Ionotropic Gelation Method

Mr. Saurabh Jawahar Sanghavi¹, Tanya Sharma², Dr. Mohammad Saleh Al. Ansari³, Mohammad Khalid⁴,
Sujata Bhati⁵, Prerna Verma⁶

¹Research Scholar, Mansarovar Global University, Sehor, Madhya Pradesh, India

²Choudhary Bansi Lal University Bhiwani, India

³College of engineering University of Bahrain, Bahrain

⁴Krishna Pharmacy college, Bijnor, India

^{5, 6}Sharda University Greater Noida, Uttar Pradesh, India

ABSTRACT

Nisin is a bacteriocin produced through Group N streptococci consisting of *Lactobacillus lactis* [1], Nisin is lively most effective in opposition to Gram-positive micro organism and is considered as a bacteriocin due to the fact it is a polypeptide with inhibitory action against intently associated species.

Its action has not been completely elucidated. The Gram negative organisms are insusceptible through distinctive feature of the outer membrane performing as a permeability barrier and it seems that cytoplasmic membrane disruption is the main goal in non-sporulating Gram-positive micro organism [1] even though peptidoglycan synthesis is inhibited [121] Nisin does not activate bacterial spores however, it enhances the thermal sensitivity of spores & in outgrowth of surviving no with heat . It does not prevent germination however germinated spores are sensitive to the bacteriocin [2].

Other bacteriocins are known, which includes subtilin [3]. This also inhibits outgrowth and enhances heat methods against a few kinds of spores [4]. Its mechanism of action and also the premise for differing responses of spores, and of germinating and outgrowing spores, stay speculative. Tylosin may be a macrolide made through actinomycete fradiae [5]. it's not sporicidal; it will increase the sensitivity of spores to heat and notably to ionising .

Keywords - IR- Spectrum, Nisin, Maltodextrin, Sodium Alginate, FTIR and Microspheres

Article Info

Volume 9, Issue 4

Page Number : 260-265

Publication Issue

July-August 2022

Article History

Accepted : 05 July 2022

Published : 20 July 2022

I. METHODS AND MATERIAL

This studies required a few Product including Sodium alginate pharmaceutical grade CaCl₂ pharmaceutical

grade, like B gelatin pharmaceutical grade, maltodextrin food grade, nisin and aqua dm (aquademineralisata). On the alternative hand, the

equipment used on this studies have been pH-meter SCHOTT Mainz glasstype CG 842, differential Thermal Analysis infrared also known as Mettler Toledo FP-65 DTA P-900 Thermal, plate stirrer (Dragon Lab MS pro), Mettler Toledo HB43 S Moisture Analyzer, analytical balance, Buchner funnel, aerosolization sprayer, Scanning Electron Microscopy (check out S50 Type FP 2017/12), optical microscope, and different laboratory glassware.

Research Method:

The studies began out with a qualitative evaluation of substances used, which consist of sodium alginate, nisin, gelatin, maltodextrin and calcium chloride. The next step become generating nisin microspheres primarily based totally at the method layout and classifying them into the feature.

Research Variable:

The other variable on this studies become the aggregate of the polymer microspheres, particularly alginate-gelation aggregate (2.25:0.25)% with the alginate of 2.5% for the comparison. The compound variable become the feature produced through the microspheres. Control variables on this studies had been the attention of a crosslinking solution, crosslinking time, stirring velocity, and distance.

Microsphere Formula Design:

Microspheres had been created through an ionic gelation technique with aerosolization method primarily based totally on the subsequent method with the crosslinking time of 90 mins and the stirring velocity of 1000rpm. The energetic ingredient-polymers solution become made in a extent of 100ml, and the crosslinking solution become in a extent of 200ml. During the freeze-dry process, microspheres had been dispersed in a 5% maltodextrin solution of 10 instances the load of the microsphere [7].

Table 1 - Materials for Formula 1 and Formula 2

Material	Ingredient	Formula 1	Formula 2
Nisin	API	0.02%	0.02%
Gelatin	Polymer	-	0.25%
Maltodextrin	Lyoprotectant	5%	5%
Sodium alginate	Polymer	2.25%	2.5%
CaCl ₂ .2H ₂ O	Cross-linking solution	1.75M	1.75M

Technique of Data Analysis:

The writers of the studies used FTIR spectroscopy because the method of statistics evaluation. This method become finished through evaluating the IR-Spectra statistics of nisin, sodium alginate, gelatin, and maltodextrin. The subsequent step become reading the DG- statistics by an analysis among diffractogram of nisin and x-ray spectra, gelatin, sodium alginate and medicinal X-ray diffraction and sodium ingredient.

This research required some material including Sodium alginate pharmaceutical grade, type B gelatin

II. RESULTS AND DISCUSSION

Result of Microspheres Characterization

Organoleptic:

The result of the organoleptic examination towards microspheres is presented in Table 1 below:

Based on the organoleptic examination, the table shows that both Formula I and Formula II produce white powdery, odorless and tasteless microspheres.

Formula	Formula 1	Formula 2
Color	White powder	White powder
Odor	Odorless	Odorless
Taste	Tasteless	Tasteless

Table 2 - Organoleptic Characteristic of Formulation

FTIR Spectroscopy:

The result of the infrared spectroscopy examination towards the microspheres is presented in Table 1 and Table 2. The infrared spectra data of nisin, sodium alginate, and gelatin are provided for recognizing the difference between infrared spectra microspheres and their ingredients.

Table 3- The infrared spectroscopy microsphere of Formula 1

Wavenumber (cm ⁻¹)	Analysis	Nisin	Sodium alginate	Gelatin	Maltodextrin
3281.52	Hydroxyl Group (O-H) Peptide Bond (NH-Stretching)	3288	3500-3200	3400 - 3200	3400
2924.46	C-H Stretching	2960		3100 - 2800	
1597.14	Carbonyl Group (C=O)	1645	1680-1600	1660 - 1600	
1412.42	C-H bending			1450 - 1300	
1147.00 1076.65 995.76	C-O stretching	1232	1300-1000		1200-980

Based on Table 1 above, guluronate fingerprint (900 – 890 cm⁻¹) and manuronate fingerprint (850 – 810 cm⁻¹) of sodium alginate do not provide absorption on the infrared spectroscopy toward the microspheres produced. Besides, C-N-H bending in gelatin (1565-

1500 cm⁻¹) and nisin (1527 cm⁻¹) do not provide absorption as well on the infrared spectroscopy toward the microspheres produced[9].

Table 4 - The infrared spectroscopy microsphere of Formula 2

Wavenumber (cm ⁻¹)	Analysis	Nisin	Sodium alginate	Gelatin	Maltodextrin
3277.15	Hydroxyl Group (O-H)	3288	3500-3200	3400 - 3200	3400
2927.40	C-H Stretching	2960		3100 - 2800	
1597.07	Carbonyl Group (C=O)	1645	1680-1600	1660 - 1600	
1146.91 1076.80 994.38	C-O stretching	1232	1300-1000		1200-980

Based on Table 2 above, guluronate fingerprint (900 – 890 cm⁻¹) and manuronate fingerprint (850 – 810 cm⁻¹) of sodium alginate do not provide absorption on the infrared spectroscopy toward the microspheres produced. Besides, C-N-H bending in nisin (1527 cm⁻¹) does not provide absorption as well on the infrared spectroscopy toward the microspheres produced.

X-ray Diffraction:

The result of X-ray diffraction examination towards microspheres is provided in Figure 1.

Particle Surface Morphology:

During the observation using an optical microscope, the shape of microspheres particle is spherical. In the following Figure 2 provides the examination result of

particle surface morphology with a Scanning Electron Microscope (SEM).

Discussion

The study end result of the infrared organoleptic indicates that nisin has a carbonyl group (1651.87 cm^{-1}), hydroxyl group (3270.49 cm^{-1}), carboxyl group (1274.22 cm^{-1}) and number one amine group (1575.06 cm^{-1}). This end result is much like previously observe study that nisin has a carbonyl group (1645 cm^{-1}), hydroxyl group (3288 cm^{-1}), number one amine group (1527 cm^{-1}), Crystal lattice and carboxyl group (1232 cm^{-1}) with the X-ray diffraction indicates nisin has crystalline properties visible from the pointy uptake peaks at 2θ 27.33° ; 31.65° ; 45.40° . This end result is just like other study that nisin has sharp uptake peaks at 2θ 32° . The identity end result of the Calcium chloride organoleptic indicates that the CaCl_2 is white crystalline powder, the hygroscopic and odorless [10].

The writers of the studies use a crosslinking solution of CaCl_2 due to the fact the Ca^{2+} ion is generally used & non-toxic. Sodium alginate includes 2 monomer units, specifically guluronate acid and manuronate acid and it is also a polymer. However, only the closed guluronate block that has a function withinside the crosslinking technique with a divalent cation (Ca^{2+} ion) to form an egg container structure.

The topical transport system is a drug transport system that has a nearby effect. The first direction of the topical transport device is by the skin. The example of a traditional topical transport system is through supplying creams, gels, and ointments. In this preparation, there's no changes in structures to the effectiveness of the drug which provide an extra controlled drug release [11].

Microsphere transport system is an strive for growing a topical transport system that is capable of provide a slower medicinal substances release [13]. Microspheres

are systems which include polymers as their constituent material and whether or not a molecular or bodily dispersed medicinal element. The microspheres substances are round with a diameter of $1\text{--}1000\text{ }\mu\text{m}$. As a end result, microsphere is frequently called microparticle [12].

This studies is similar to the preceding studies that maltodextrin has a a carboxyl group of the polysaccharide ($1200\text{--}980\text{ cm}^{-1}$) [13] and hydroxyl group (3400 cm^{-1}). Thermal analysis carried out with the DTA additionally indicates maltodextrin has an alteration at the temp. of 185.2°C . This situation is similar to the preceding studies that the alteration of maltodextrin takes place at the temp. of $40\text{--}185^\circ\text{C}$ [14].

The end result of the IR- spectroscopy in Formula 1 indicates a extensive absorption withinside the wavenumber of 3281.52 cm^{-1} , that is expected to be resulting from the absorption mixture of the maltodextrin and hydroxyl group in sodium alginate & the amine group of gelatin. The sodium alginate (1592.88 cm^{-1}), carbonyl group in nisin (1651.87 cm^{-1}), and gelatin (1636.96 cm^{-1}) experience a wavelength transferring into 1597.14 cm^{-1} . Furthermore, the ones 3 carbonyl groups only provide one microsphere absorption in Formula 1. The interaction happened causes no absorption withinside the manuronate fingerprint region ($850\text{--}810\text{ cm}^{-1}$), lack of C-N-H bending uptake from nisin (1527 cm^{-1}) and gelatin ($1565\text{--}1500\text{ cm}^{-1}$) [15].

The end result of the IR- spectroscopy in Formula 2 indicates comparable situations to Formula 1. The extensive absorption withinside the wavenumber of 3277.15 cm^{-1} is resulting from the absorption mixture of the hydroxyl group in sodium alginate and maltodextrin. The carbonyl group of nisin (1651.87 cm^{-1}) and sodium alginate (1592.88 cm^{-1}) experience a wavelength transferring into 1597.07 cm^{-1} , and each carbonyl groups provide one microsphere

absorption in Formula 2. Gelatin can't be determined in Formula 2. As a end result of this interaction, there's no absorption withinside the region of manuronate fingertint ($850 - 810 \text{ cm}^{-1}$) and the lack of nisinC-N-H bending uptake (1527 cm^{-1})[14].

The study end result of particle surface morphology with the Scanning Electron Microscope on each substances exhibits that microspheres produced are round with a clean surface. The use of nozzle in a particular length and spraying technique with strain purpose the polymer's solution are sprayed out into round. Furthermore, the microspheres produced also are round. The morphology of the particle surface produced are comparable although there's a distinction withinside the microsphere composing formula. The round form and a clean particle surface in microsphere substances are proper withinside the topical use of the microspheres. Spherical microsphere substances are capable of cover the utilization region more tightly and perfectly, consequently the content material of the drug substances of the utilization region is greater homogenous.

The common particle length primarily based totally on the exam indicates that Formula 1 has $4.048 \pm 0.069 \mu\text{m}$ and Formula 2 has $5.656 \pm 0.130 \mu\text{m}$. The particle length produced through Formula 2 is extra than Formula 1. This circumstance is similar to the preceding studies which exhibits the microspheres with greater sodium alginate produce larger particle length (withinside the comparable attention of the crosslinking solution and crosslinking time)[16]. This condition is resulting from an growth in viscosity in conjunction with an growth in the quantity of sodium alginate. Higher viscosity can purpose the droplet shaped will become large and influences the particle length produced. In addition, the extra quantity of sodium alginate reasons an growth of guluronate block quantity ensuing in numerous egg container systems that may be enlarged. The particle

length produced continues to be in the standards of microsphere length for the topical use of $5 - 300 \mu\text{m}$ [17].

III. CONCLUSION

Nisin microspheres which can be composed of the ionic gelation technique and aerosolization method with polymers of sodium alginate-gelatin (2.25:0.25)% have a few traits. Those traits are white powdery, odorless, tasteless, round with a easy and flat floor, the particle length of $2.861 - 7.439 \mu\text{m}$, a mean particle length of $4.048 \pm 0.069 \mu\text{m}$. The distinction among nisin microspheres with polymers of sodium alginate-gelatin (2.25:0.25)% and sodium alginate 2.5% lies withinside the common particle size range. The common particle length variety of nisin microspheres with the aggregate matrix is smaller than the sodium alginate matrix.

IV. ACKNOWLEDGEMENT

The authors are thankful to Mr. Sanjeev Chauhan for his valuable suggestions, timely support and guidance.

V. REFERENCES

- [1]. Vaishnavi (2017). "Release Kinetics of Nisin from Chitosan-Alginate Complex Films". Journal of Food Science. 81 (10): E2503-E2510. doi:10.1111/1750-3841.13443. PMID 27635864.
- [2]. Rizky HE, Mukono J. Levels of Chromium in Air with Chromium in the Blood of Workers Electroplating in Purbalingga. Journal Kesehatan Lingkungan. 2018;9(2):172-80.
- [3]. Taglietti M, Hawkins CN, Rao J. Novel topical drug delivery systems and their potential use in acne vulgaris. Skin Therapy Lett. 2008;13(5):6-8.
- [4]. Hariyadi DM, Hendradi E, Purwanti T, Fadil F, Ramadani CN. Effect of cross linking agent and polymer on the characteristics of ovalbumin

- loaded alginate microspheres. *Int J Pharm Pharm Sci.* 2014;6(4):469–74.
- [5]. Roy A, Bajpai J, Bajpai AK. Development of calcium alginate–gelatin based microspheres for controlled release of endosulfan as a model pesticide. 2009;
- [6]. Manjanna KM, Kumar TMP, Shivakumar B. Calcium alginate cross-linked polymeric microbeads for oral sustained drug delivery in arthritis. *Drug Discov Ther.* 2010;4(2):109–22.
- [7]. DM, Ma Y, Wang Y, Bostrom T, Malouf J, Turner MS, et al. The potential for production of freeze-dried oral vaccines using alginate hydrogel microspheres as protein carriers. *Journal of Drug Delivery Science and Technology.* 2014;24(2):178–84.
- [8]. Bernela M, Kaur P, Chopra M, Thakur R. Synthesis, characterization of nisin loaded alginate–chitosan–pluronic composite nanoparticles and evaluation against microbes. *LWT-Food Science and Technology.* 2014;59(2):1093–9.
- [9]. de Abreu LCL, Todaro V, Sathler PC, da Silva LCRP, do Carmo FA, Costa CM, et al. Development and characterization of nisin nanoparticles as potential alternative for the recurrent vaginal candidiasis treatment. *Aaps Pharmscitech.* 2016;17(6):1421–7.
- [10]. Rowe RC, Sheskey P, Quinn M. *Handbook of pharmaceutical excipients.* Libros Digitales-Pharmaceutical Press; 2009.
- [11]. Hariyadi DM. In Vivo Neuroprotective Activity of Erythropoietin-Alginate Microspheres at Different Polymer Concentrations. *Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm.* 2018;12(04).
- [12]. Hendrijantini N. Comparative in vitro study of the cytotoxicity of gelatine and alginate to human umbilical cord mesenchymal stem cells. *Dental Journal (Majalah Kedokteran Gigi).* 2019;52(1):36–40.
- [13]. Sun P, Yang H-J, Wang Y-Q, Liu K-Z, Xu Y-W. Lipase-catalyzed synthesis and characterization of stearic acid dextrin ester. *Research in Health and Nutrition.* 2013;1(1):7–11.
- [14]. Garnero C, Aloisio C, Longhi MR. Ibuprofen-maltodextrin interaction: study of enantiomeric recognition and complex characterization. 2013;
- [15]. Hosseini SM, Hosseini H, Mohammadifar MA, German JB, Mortazavian AM, Mohammadi A, et al. Preparation and characterization of alginate and alginate-resistant starch microparticles containing nisin. *Carbohydrate polymers.* 2014;103:573–80.
- [16]. Hariyadi DM, Purwanti T, Wardani D. Stability of freeze-dried ovalbumin-alginate microspheres with different lyoprotectants. *Research Journal of Pharmacy and Technology.* 2016;9(1):20.
- [17]. Nafisah S. Aktivitas Antibakteri Mikrosfer Probiotik *Lactobacillus acidophilus* FNCC-0051 Dengan Matriks Natrium Alginat Terhadap *Propionibacterium acnes* ATCC 11827 PADA pH 4, 5 DAN 6, 0. Universitas Airlangga; 2019.

Cite this article as :

Mr. Saurabh Jawahar Sanghavi, Tanya Sharma, Dr. Mohammad Saleh Al-Ansari, Mohammad Khalid, Sujata Bhati, Prerna Verma, " Development and Evaluation of Antibacterial Microsphere using Ionotropic Gelation Method", *International Journal of Scientific Research in Science and Technology(IJSRST)*, Print ISSN : 2395-6011, Online ISSN : 2395-602X, Volume 9, Issue 4, pp.260-265, July-August-2022. Available at doi : <https://doi.org/10.32628/IJSRST229431> Journal URL : <https://ijsrst.com/IJSRST229431>