

Characterization of Nisin Microspheres with Combination Matrix Sodium alginate - Gelatin

by Widji Soeratri

Submission date: 02-Jan-2020 02:24PM (UTC+0800)

Submission ID: 1238984583

File name: 5.vol6-issue5-2018-MS-15695.pdf (374.13K)

Word count: 3639

Character count: 18693



Original Article

Characterization of Nisin Microspheres with Combination Matrix Sodium alginate – Gelatin

Tutiek Purwanti*, Widji Soeratri, Muhamad Zainudin

Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.

ARTICLE INFO

ABSTRACT

Received:30 Nov 2018

Accepted:12 Dec 2018

The aim of this research is to characterize nisin microsphere were made by ionotropic gelation method aerosolization technique with sodium alginate-gelatin combination as matrix with total polymer concentration 2,5% and calcium chloride 1.5 M as the crosslinker solution. The composition of the nisin microsphere formulas are F1 (10:0), F2 (9:1), F3 (8:2), F4 (7:3), F5(6:4), and F6 (5:5). The interaction between polymer and nisin was confirmed by the changes in the density and wave number on the FT-IR spectra. X-Ray Diffraction of nisin microsphere showed amorf characteristic. Characterization of nisin microsphere include organoleptic,yield value, moisture content, morfology, particle size, and swelling index. The organoleptic of all nisin microspheres are white fine powder,tasteless and odorless.Nisin microspheres are spherical particles with smooth surface as displayed by Scanning Electron Microscope.Yield value of nisin microsphere were F1(67,85%), F2(46,97%), F3(57,84%), F4(41,34%), F5(49,95%), F6(31,61%), Moisture content of nisin microsphere are F1(12,37%), F2(10,72%), F3(11,67%), F4(10,26%), F5(11,90%), and F6(9,24%).Nisin microspheres have mean particle diameter F1(5,66µm), F2 (4,05µm), F3 (3,75µm), F4(3,60µm), F5(3,53µm), F6(3,35µm).The presence of gelatin as a matrix resulted in changes of swelling patterns from the microspheres, which were sloping increasingly and occurred at longer time intervals when compared to those without gelatin. This phenomenon increased by the time of the increasing gelatin concentration and the decreasing of the concentration of sodium alginate in the matrix of nisin microspheres

Keywords: Nisin, Microsphere, Ionotropic Gelation, Sodium Alginate, Gelatin, Characterization

1. INTRODUCTION

Nisin is a bacteriocin produced by the strain *Lactococcus lactis*, consisting of 34 amino acids, with a molecular weight 3354, and having antibacterial activity against gram positive and gram negative bacteria¹. Nisin has activities that can inhibit pathogenic bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis*². Since 1969, nisin has been recommended as a safe food preservative by the World

Corresponding author *

Tutiek Purwanti

Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

E mail: tutiek_purwanti@yahoo.com

Health Organization (WHO), because it does not cause resistance effect³. Based on this activity, nisin has predicted potential to be developed for the treatment of bacterial infections of the skin and formulated in an appropriate drug delivery system.

Drug delivery system (DDS) is defined as a formulation technique that can enhance the therapeutic and safety effects of active ingredients by controlling the rate, time and place of drug release in the body⁴.

Drug delivery systems can be applied for oral, topical and parenteral use. Topical delivery systems can be intended for local or systemic effects. The use of topical drug delivery systems aimed at local effects is usually used for antibacterial, antifungal, anti-inflammatory drugs⁵. The aim of topical delivery system are to minimize fluctuation drug level, extend drug action, reduce side effects, improve patient compliance, and improve the safety and stability of active ingredients.⁶

Microsphere is modern delivery systems that are quite widely applied as topical delivery systems. Microsphere is a spherical solid particles, which has particle size range of 1-1000 μm , consisting of active ingredients trapped inside the matrix that formed of natural or synthetic polymers

One of the advantages of the microsphere is can be release the drug constantly and has long lasting effect⁷. In topical use, which the active ingredients work up to the dermis layer, the recommended size of microspheres is 1-50 μm ⁶.

The method of microspheres preparation is divided into two classes, which are chemical processes and mechanical processes.

Methods that involve mechanical processes include spray drying and supercritical fluid precipitation, while methods involving chemical processes include ionotropic gelation, solvent evaporation, coacervation, and interfacial polymerization

Ionotropic gelation is a microencapsulation method based on the ability of polyelectrolytes to form a hydrogel as a result of reaction of polymers and crosslinking solutions. This is good method, because it does not require organic solvents or heating, which can damage the integrity of the active ingredients. The process is also easy, fast and inexpensive. The aerosolization techniques are used to obtain spherical microspheres with uniform size in range 1-50 μm . This technique is done by spraying the drug - polymer solution in a crosslinking solution to form a microsphere⁸.

Sodium alginate is a polymer derived from brown algae and is a natural polymer that is biocompatible, biodegradable, and non-toxic. Sodium alginate is widely used as a matrix of microspheres which made by ionotropic gelation method, because it can experience crosslinking reactions forming the eggbox structure. The molecular structure of sodium alginate consists of 1.4 monomer bonds β -D-mannuronic acid (M block) and α -L-guluronic acid (G block). Both monomers contain free carboxylic groups (COO^-)⁹. Crosslinking

solutions that are often used in making microspheres are calcium chloride solutions with at concentration range 0.05-1.5M¹⁰

Ionic gelation of sodium alginate and calcium chloride occur through the interaction of divalent cation (Ca^{2+}) with a free carboxylic group (COO^-) from guluronic acid blocks forming a three-dimensional structure called an egg-box⁹. This egg-box is the basic structure of microspheres. The free carboxylic group in M block is not involved in this processes and stay free, so the resulted microspheres porous and crumbly¹¹. This characteristic results in low entrapment efficiency and drug loading and short-term drug release. In order to obtain an extended drug release pattern, sodium alginate needs to be combined with other polymers which can slow down the release of drug. One of the polymers that can be used is gelatin. Gelatin is a polymer that is biodegradable, biocompatible, non-toxic. It has an isoelectric point at pH 4.8-5, and has good swelling capacity¹². Gelatin contains amine groups (NH_2) in this polymer chain. When at pH below the isoelectric point, the amine group becomes NH_3^+ group and will interact electrostatically with the free carboxylic group in the mannuronic block of the sodium alginate chain, which is not involved in the crosslinking process.

Microspheres with a matrix of sodium alginate and gelatin combination are expected to be more compact and have lower porosity. Good gelatin swelling character will affect the release of drug from the microsphere. The drug release is expected to be slowed down and last longer so resulted a sustained release pattern¹¹. The slowing down system aims to increase the duration of action of the drug, minimize fluctuations in drug levels, reduce the frequency of doses and reduce the risk of drug side effects⁴.

One of the factors that influence the release of drug is the characteristic of the microsphere, which is also influenced by several factors including the polymer matrix composition¹³.

Based on the results of the optimization of the technical implementation and adjustments to the conditions of the equipment used, the total polymer content of 3% was chosen, because with this level the process of spraying and stirring was still running smoothly and obtained relatively large yields.

The microspheres are preparation by various compositions of sodium alginate - gelatin as a matrix, 1.5 M calcium chloride solution as crosslinking agent, and using the ionotropic gelation method aerosolization technique. The factors that must be controlled during the process of preparation microspheres are the ratio of polymer, concentration of crosslinking solution, time and speed of stirring when crosslinking process, spraying distance, and spray pump pressure. Microspheres characterization included organoleptic, yield, morphology, moisture content, particles size, and swelling index.

2. MATERIALS AND METHOD

Materials

Natriumalginate (low viscosity) pharmaceutical grade, gelatine B pharmaceutical grade, CaCl₂·2H₂O pro analysis, Nisin Lactococcus lactis N5764, aquade-mineralisata, natrium citric pro analysis, citric acid pro analysis, HCl 0.2 N.

Methods

Preparation of Nisin Microspheres

Tabel 1: Nisin Alginat-Gelatin Microsphere Formula

Bahan	F1	F2	F3	F4	F5	F6
Nisin (mg)	20	20	20	20	20	20
Na-Ag (g)	2.5	2.25	2 g	1.75	1.5	1.25
Gelatin	-	0.25	0.5	0.75	1 g	1.25
Aquadem ad (mL)	100	100	100	100	100	100
CaCl ₂ 1.5 M ad (mL)	200	200	200	200	200	200

Weighed ingredients according to the formula. Sodium alginate, gelatin dissolved in aquadem. Nisin is dissolved in 0.2 ml HCl 20 ml, then mixed with a polymer solution until a volume of 100 mL is obtained.

The pH of the solution was adjusted to 4. Then the polymer drug solution was sprayed through the aerosolization nozzle into 200 mL calcium chloride solution 1.5 M, while stirring at 1000 rpm for 90 minutes. The microspheres formed are separated by filtering using a Buchner funnel, and then washed with aquadem until free of calcium chloride.

The microspheres obtained were dispersed into a 5% maltodextrin solution of 10 times the microspheres weight. The microspheres were dried by freeze drying method for 24 hours, then stored in a desiccators

Tabel 2: Yield Value of Nisin Microspheres

Nisin Microsphere	R1 (%)	R2 (%)	R3 (%)	Average±SD (%)
F1	71.65	77.26	74.63	74.51 ± 1.91
F2	49.10	49.67	42.15	46.97 ± 4.19
F3	54.74	59.82	58.97	57.84 ± 2.72
F4	45.42	37.87	39.85	41.34 ± 3.58
F5	53.31	46.10	50.45	49.95 ± 3.63
F6	31.03	34.84	28.94	31.61 ± 2.99

Characterization of the Nisin Microspheres

Yield Value

Yield value is the efficiency parameter of the microspheres preparation, it can be determined by the formula:

$$\text{Yield Value} = \frac{\text{weight of microspheres produced}}{\text{weight of constituent microspheres}} \times 100\%$$

FTIR Spectroscopy

Evaluation of the occurrence of crosslinking reactions was carried out by infrared spectra examination using the KBr pellet technique. The results of the examination were

compared with the infrared spectrum of sodium alginate, gelatin, nisin, physical mixture, and nisin microspheres.

X-ray Diffraction

Changes in the crystal structure of nisin due to the formation of microspheres, can be seen in the diffractogram of nisin microsphere. The results of the examination using X-ray diffraction compared with diffractogram of sodium alginate, gelatin, nisin, physical mixture

Moisture content

The moisture content of microspheres has a certain limit because it is related to active ingredient stability. Moisture content is measured using Moisture Content Analyzer

Morphology microsphere

To see the shape and surface morphology of nisin microspheres, a scanning electronic microscope (SEM) was carried out.

Particle Size of Nisin Microsphere

Particle size measurements were carried out using the XSZ-107 Series Biological Microscope, with a sample of 300 particles for each formula.

Swelling Index Determination

Prepared citrate buffer solution with a pH of 4.5 ± 0.05 in the amount of 10 ml of the solution. Then weigh 100 mg of nisin microsphere and put it into a wire mesh containing the media. The swelling process was carried out at 37 ° C, observed a change in weight at a certain time period. Samples were drained each time using filter paper, then weighed as final weight. The price of the swelling index is calculated by :

$$\text{Swelling Index} = \frac{\text{Final weight} - \text{Initial weight}}{\text{initial weight}} \times 100\%$$

3. RESULT AND DISCUSSION

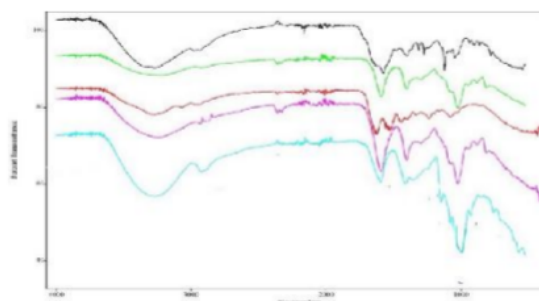


Fig 1: Infrared spectra of (A) nisin, (B) sodium alginate (C) gelatin, (D) physical mixture of nisin, sodium alginate, and gelatin, (E) nisin microspheres.

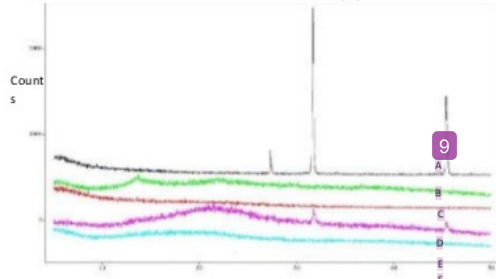


Fig 2: Diffractogram of (A) nisin, (B) sodium alginate, (C) gelatin, (D) physical mixture of nisin, sodium alginate, and gelatin, (E) nisin microspheres.

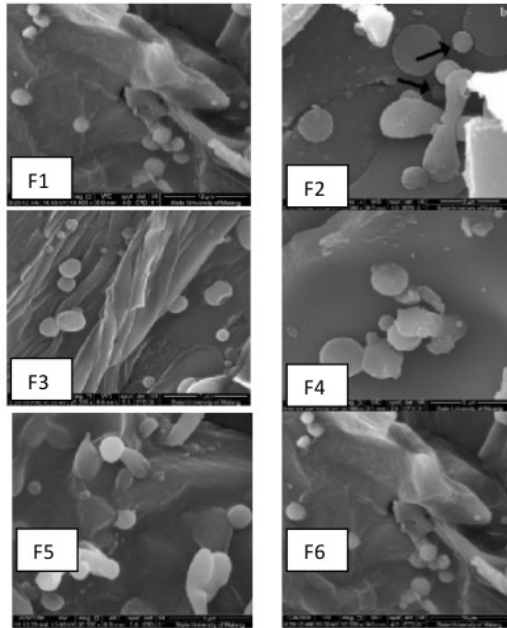


Fig 3: Morphology of nisin microspheres of various formulas, results of Scanning Electron Microscope (SEM) examination

Table 3: Moisture content of nisin microspheres in various formula

Formula	R 1 (%)	R 2 (%)	R 3 (%)	Average±SD (%)
F1	13.52	12.01	11.58	12.37 ±1.02
F2	11.07	10.17	10.93	10.72 ± 0.48
F3	12.58	11.03	11.40	11.67 ± 0.81
F4	10.87	10.16	9.76	10.26 ± 0.56
F5	11.87	11.73	12.15	11.90 ± 0.22
F6	9.20	9.60	8.93	9.24 ± 0.34

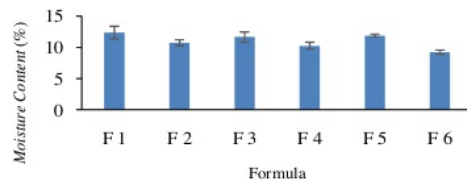


Fig 4: Histogram of average% moisture content of nisin microspheres in various formulas

Table 4: The average diameter of nisin microspheres in various formulas

Formula	Rep 1 (µm)	Rep 2 (µm)	Rep 3 (µm)	Average±SD (µm)
F1	5.581	5.807	5.581	5.66 ± 0.13
F2	3.982	4.043	4.120	4.05 ± 0.07
F3	3.710	3.801	3.751	3.75 ± 0.04
F4	3.481	3.700	3.621	3.60 ± 0.11
F5	3.359	3.641	3.590	3.53 ± 0.15
F6	3.189	3.480	3.381	3.35 ± 0.15

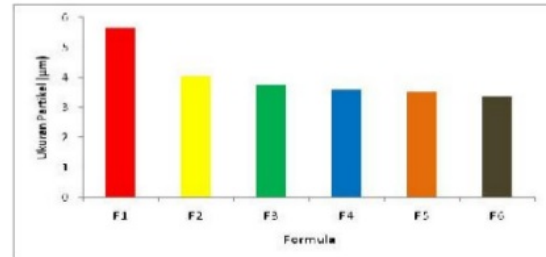


Fig 5: Histogram of average particle size diameter of nisin microspheres in various formulas

Table 5: Swelling Index of nisin microspheres in various formulas

Formula	Time (hour)	AverageSwelling Index ± SD (%)
F1	1	382.33 ± 37.36
	2	333.33 ± 32.33
	3	250.67 ± 19.22
	4	208.67 ± 28.02
	5	179.33 ± 18.90
	6	150.67 ± 20.13
F2	1	315.33 ± 30.08
	2	293.33 ± 36.35
	3	212.00 ± 48.04
	4	187.33 ± 44.02
	5	150.67 ± 18.15
	6	118.67 ± 16.29
F3	1	253.33 ± 08.08
	2	378.67 ± 16.20
	3	422.67 ± 09.29
	4	354.00 ± 30.80
	5	251.33 ± 08.62
	6	178.00 ± 21.07
F4	1	284.67 ± 09.86
	2	417.33 ± 20.03
	3	376.67 ± 13.32
	4	345.67 ± 13.50
	5	285.33 ± 12.22
	6	183.33 ± 15.01
F5	1	166.67 ± 10.07
	2	185.00 ± 08.00
	3	232.00 ± 35.53
	4	230.67 ± 34.20
	5	188.00 ± 23.06
	6	148.00 ± 20.78
F6	1	233.33 ± 08.00
	2	336.00 ± 24.58
	3	386.00 ± 34.18
	4	350.67 ± 24.19
	5	300.67 ± 18.90
	6	264.67 ± 18.04

The whole formula of nisin microspheres have the same organoleptic; white powder, odorless and tasteless.

The occurrence of crosslinking reactions in the nisin microspheres formation is characterized by the shifting of the length of the wave number from the carbonyl group to nisin (1651.87cm^{-1}), sodium alginate (1592.88cm^{-1}), and gelatin (1636.96cm^{-1}) to 1597.14cm^{-1} . The guluronate fingerprint area ($900 - 890\text{cm}^{-1}$) and manuronate ($850 - 810\text{cm}^{-1}$) from sodium alginate also did not provide absorption in infrared spectra. This is due to the interaction of the guluronate group with Ca^{2+} ions during the crosslinking process and the interaction of the manuronic group with NH_3^+ groups from nisin and gelatin during the ionotropic gelation process.

Nisin has an isoelectric point at pH 8.8; gelatin has an isoelectric point at pH 4.8 – 5.

At pH 4.00 + 0.05 the NH_2 group in nisin and gelatin will be in a protonated state forming NH_3^+ can electrostatically binding to the COO^- group in the mannuronic chain of alginate. Interactions that occur caused no absorption in the area of manuronate fingerprints ($850 - 810\text{cm}^{-1}$) and loss of CNH uptake bending from gelatin ($1565-1500\text{cm}^{-1}$) and nisin (1527cm^{-1})¹⁴.

The results of X-ray diffraction examination showed that the resulting microspheres were amorphous without the specific absorption peaks possessed by nisin (2θ 27.33°; 31.65°; 45.40°). When compared with the physical mixture between nisin, sodium alginate, and gelatin, nisin still gives absorption peak at 2θ 31.74°; 45.43°. This shows that nisin has been encapsulated in the microspheres system in an amorphous state so that it no longer shows specific absorption peak.

The results of examination of the shape and surface morphology of microspheres using Scanning Electron Microscope (SEM) showed that the nisin microspheres produced using spheres with a flat and smooth surface. The use of a nozzle spray with certain diameter produce a uniform spherical shape microsphere.

In the yield examination, it can be seen that the smallest yield (31.61%) is nisin microsphere F6 and the largest microspheres (67.85%) is nisin microsphere F1. Yield value is an illustration of the efficiency of the process of microspheres preparation. The yield value is related to the concentration of sodium alginate, because it is related to the formation of the egg-box structure. The yield value was linearly related to the concentration of sodium alginate¹⁵.

Moisture content examination results the range between 9.24 – 12.37 %. The ideal moisture content of probiotic microsphere is between 5%-10%. Moisture content that is too high can cause the growth of microorganisms to be faster, and for certain active ingredients that are not stable against moisture will be more susceptible to degradation.

The size of the microsphere is influenced by several factors, including the type and composition of the polymer forming

matrix. The higher concentration of sodium alginate in a formula causes the number of guluronic blocks are being greater, so that more egg-boxes are and increase the particle size of microsphere¹⁶.

The presence of gelatin in a formula can reduce particle size. The amine group of gelatin will electrostatically interaction with the free carboxylic group in the M block sodium alginate chain which is not involved in the crosslinking process, so can produce a more compact microsphere and will reduce the size of the microsphere.

Microspheres with a compact matrix structure because the penetration of water into the particles of the microsphere to be more difficult so the swelling occurs becomes lower. But a compact matrix structure will result greater entrapment efficiency and nisin loading. Therefore the addition of gelatin provides a dual function, which is to produce microspheres with a compact matrix and improve swelling of the microsphere.

4. CONCLUSION

The presence of gelatin combined with alginate as a matrix in the nisin alginate-gelatin microspheres influences the characteristics of the microsphere, which is smaller in size and has a larger and longer expanding power (swelling index better) compared to microspheres which only contain alginate as a matrix.

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Conflict of Interest: None

Source of Funding: Nil

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