International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 12, Issue 2, 2020

Short Communication

PREPARATION AND CHARACTERIZATION OF ANCHOVY (*STOLEPHORUS SP*) PROTEIN CONCENTRATE NANOPARTICLE USING IONIC GELATION METHOD

ZULFIAYU SAPIUN[1*], ARLAN K. IMRAN[1], PRISCA SAFRIANI WICITA[1], VYANI KAMBA[1], NANGSIH SULASTRI SLAMET[1]

[1]Department of Pharmacy, Health Polytechnic, Ministry of Health, Gorontalo, Indonesia
Email: zulfiayu@poltekkesgorontalo.ac.id

Received: 08 Dec 2019, Revised and Accepted: 03 Feb 2020

ABSTRACT

Objective: Nanoparticle preparation can increase the bioavailability of anchovy protein. This study aims to optimize and characterize anchovy protein concentrate nanoparticle based on volume comparison of chitosan-TPP (Tripolyphosphate) matrix in forming the size of anchovy protein concentrate nanoparticle with ionic gelation method.

Methods: Fresh anchovy was extracted by an acid-base method using solvents of NaOH and HCl to get protein concentrate. After that, it was conducted by formulating anchovy protein concentrate into a mixture of chitosan-TPP matrix with volume ratio which was divided into 5 series of variations, namely F1 without matrix, F2 1:1, F3 1:3, F4 1:5 and F5 1:7 ratio of chitosan to TPP, then formed matrix was determined the value of the encapsulation efficiency and characterized using a particle size analysis tool.

Results: The results showed that the optimum volume comparison of the chitosan-TPP matrix in forming nanoparticle of anchovy protein concentrate was ratio 1:7 because this has a matrix encapsulation efficiency value of 99.11%, a small particle concentrate size of 687.26 nm, a good polydispersity index value of 0.406 and a value of zeta potential of+12.9 mV.

Conclusion: The optimum formula of the chitosan-TPP matrix volume ratio in forming anchovy protein concentrate nanoparticle is F5 with the ratio of chitosan-TPP matrix mixture 1:7.

Keywords: Anchovy, Protein, Nanoparticle, Ioninc Gelation, Chitosan-TPP

DOI: http://dx.doi.org/10.22159/ijap.2020v12i2.36570. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

Anchovy (*Stolephorus sp*) is one of the fish that has a very high protein content which is around 60% w/w from its body [1]. Anchovy protein consists of several types of essential amino acids such as isoleucine, leucine, lysine, and non-essential amino acids such as methionine and glutamic acid which play a role in the process of cell regeneration [2-5]. Protein is a complex compound of macromolecule size that can cause absorption in the body so poorly that it can reduce the value of its bioavailability in the body [6]. This cause's anchovy protein which is required a special strategy in the formulation.

The development of formulations of several decades has focused on developing nanoparticle technology. Reducing the particle size can increase the surface area of the particle, the greater the surface area the higher the absorption, so it can increase its bioavailability in providing a therapeutic effect [7-9].

The carrier polymer of nanoparticle drugs use in this study is chitosan because it is biodegradable, non-toxic and increases the solubility of medicinal ingredients [10-11]. Chitosan can form the particle size of active ingredients into nanoparticles by utilizing the positive charge of ionic reaction with other negatively charged compounds so that the drug ingredients can be trapped between ionic matrix mixtures interacting with each other [12, 13]. Preparation of Chitosan nanoparticle uses the ionic gelation method using TPP (Tripolyphosphate) as a crosslinker. This method has some advantageous which is not harmful organic solvent and without a heating process that can damage medicinal ingredients [14].

Nanoparticles can only be formed at a certain ratio of mixing volume between chitosan and TPP. The ratio of the volume of mixing between chitosan-TPP in which the volume of added chitosan has increased has been done by Mackay, 2003. Research with the volume of added TPP has never been done before. The aim of this study was to obtain the optimum formula of chitosan-concentrate of anchovy protein nanoparticles with an inverse volume mixing ratio, where the added TPP volume to the mixture was increasing.

The formed nanoparticle formula subsequently is characterized including testing the percentage of encapsulation efficiency, particle size, polydispersity index, pH and zeta potential. Overall this research is expected to obtain anchovy chitosan nanoparticle protein concentrate which can be used in the future as a formulation strategy in increasing the bioavailability of anchovy protein concentrate.

The research conducted is an experimental laboratory study. The material used in this study was chitosan (Zhejiang Golden-Shell Ltd. China), anchovy protein concentrate obtained from acid-base extraction methods that had been carried out by Oktasari *et al.*, 2013, TPP (Tripolyphosphate) (Aditya Birla Chemicals Ltd. Thailand), tween 80, potassium dihydrogen phosphate, disodium dihydrogen phosphate, Comassive Brilliant Blue, aquadestilates, glacial acetic acid were purchased in CV Chem-Mix, Solo.

Fresh anchovy was extracted by an acid-base method using solvents of sodium hydrochloride and chloride acid to get protein concentrate. Chitosan-concentrated nanoparticles were prepared using the ionic gelation method, which is the complexation of polyectrolites between positively charged chitosan and negatively charged. Chitosan was first dissolved into 1% w/v acetic acid solution to obtain chitosan with a concentration of 0.1% w/v as much as 10 ml, then a phosphate buffer solution was made at pH 7.4 by dissolving 1.18 g of potassium dihydrogen phosphate and 4.3 g disodium dihydrogen phosphate into 1L distilled water. Phosphate buffer that has been made and was used to make anchovy protein concentrate 0.01% w/v, to be diluted to a degree by taking as much as a certain volume to make anchovy protein concentrate to 0.004% w/v, the volume of anchovy protein concentrate used was adjusted to the total volume of the matrix-chitosan-TPP then was made a TPP solution 0.0125% w/v in distilled water, the amount of TPP was adjusted to the volume of TPP 0.0125% w/v in distilled water, the amount of TPP used was adjusted to the volume TPP varied as much as 10 ml, 30 ml, 50 ml and 70 ml as shown in table 1.

Table 1: Chitosan-TPP nanoparticle formula of anchovy protein concentrate

Materials	Formula				
	F1	F2	F3	F4	F5
Anchovy protein concentrate (ml)	0.4	0.8	1.6	2.4	3.2
Chitosan (ml)	-	10	10	10	10
TPP (ml)	-	10	30	50	70
Volume Total (ml)	10	20	40	60	80

Concentrate protein solution of anchovies and 0.5 ml tween were mixed into chitosan solution, homogenized with a stirrer, then TPP solution was dripped on a mixture of chitosan solution with a dropping speed of 1 ml/min while stirring using a magnetic stirrer (Scholab) at a speed of 1000 rpm for 1 h.

The nanoparticle matrix obtained from each formula was measured by the percentage value of the encapsulation efficiency which was each 5 ml matrix taken and placed in a dialysis membrane immersed in phosphate buffering pH 7.4 of 50 ml for 1 h 30 min. The phosphate buffer medium used each taken 4 ml was added 200 µl CBB (Comassive Brillian Blue) solution in 10 min incubation, absorbed readily using a UV-Vis (Genesis) spectrophotometer (Genesis) at a maximum wavelength of 595 nm to determine the amount of free protein that was not bound in the matrix, so it can be used to determine the efficiency value of matrix encapsulation. The percentage value of encapsulation efficiency was obtained from the ratio of free protein content and initial protein content that is bounded to the matrix. The formed matrix from each formula was characterized by particle size, polydispersity index, and potential zeta using PSA (Particle Size Analysis) (Horiba) tool.

An evaluation of the encapsulation efficiency was carried out to find out how successfully the nanoparticle matrix could encapsulate the anchovy protein concentrate in the matrix mixture. The matrix encapsulation efficiency test results obtained, as shown in table 2.

Table 2: The result of the entrapment efficiency of matrix ratio each formula

Formula	Percentage of entrapment efficiency
F1 no matrix	-
F2 matrix 1: 1	99.23±0.433%
F3 matrix 1: 3	99.17±0.096%
F4 matrix 1: 5	99.12±0.087%
F5 matrix 1: 7	99.11±0.100%

mean±SD, n=3, SD: Standard deviation

The results showed that ionic cross-interaction between chitosan positive charge and TPP negative charge occurred perfectly in trapping anchovy protein concentrate. This was indicated by the value of the encapsulation efficiency obtained was more 95% [16]. Otherwise, F1 anchovy protein concentrate without the chitosan-TPP matrix does not show the encapsulation efficiency testing process, because the concentrate without matrix can be assessed as a concentrate with free protein without any coating so that the testing of the encapsulation efficiency can be valued at 0%.

One Way ANOVA statistical test showed an increase in the volume of TPP added did not have a significant effect on the decrease in the value of the encapsulation efficiency of each nanoparticle formula. This can be interpreted that all formula of mix ratio matrix volume can bind anchovy protein concentrate well. The determination of the optimum formula for nanoparticles is also based on the characterization of the particles formed. The results of the particle size characterization and potential zeta values of the matrix, as shown in table 3.

Table 3: The characterization result of matrix ratio each formula

Formula	Size (nm)	Potential zeta (mV)
F1 no matrix	4732.6±1.496	-10.90±0.666
F2 matrix 1: 1	1166.7±35.3*	+37.2±0.529*
F3 matrix 1: 3	808.33±132.9*	+36.93±1.290*
F4 matrix 1: 5	753.03±263.1*	+29.03±0.416*
F5 matrix 1: 7	687.26±242.4*	+12.0±0.889*

*mean±SD, n=3, SD: Standard deviation (significantly different with F1).

Based on table 3, it can be seen that there is a significant effect of increasing the volume of TPP in the formula of anchovy protein concentrate nanoparticles in reducing the size of the matrix particles and decreasing its potential zeta value. This is caused by chitosan which is not protected at high pH. TPP is a negatively charged compound and has alkaline properties so that the more TPP volume added to the matrix mixture, the higher the pH of the matrix mixture that is formed, consequently the ionic crosslinking between chitosan and TPP does not occur perfectly. Bonds that occur are weaker and the matrix tends to dissolve in the solvent used [17]. Otherwise, F1 showed the obtained particle size is very large and low potential zeta. This result is affected by the low solubility of the concentrate in the phosphate buffer solvent on pH 7.4.

According to Wu Yan *et al*., 2005, the particle-matrix was said to have nano-size if the particle size is 50-1000 nm. On the other hand, Mohanraj and Chen, 2006 stated that stable nanoparticles were nanoparticles which have a potential zeta value of+/-30 mV, the smaller potential zeta is easier for particles to aggregate due to reduced repulsion between particles. The positive and negative value of zeta potential is related to the binding of anionic groups by the long amine groups of chitosan [18, 19].

The results of the characterization of other particles, which were carried out using the particle size analysis tool, are the polydispersity index values of the formed nanoparticles, as shown in table 4.

The results of the characterization of the polydispersity index showed that the increase of the TPP volume can reduce the value of the polydispersity index compared to the F1 anchovy protein concentrate without matrix but the decrease that occurred was not statistically significant using One Way Anova test. According to Laili

et al., 2014 referred to in Nidhin *et al.*, 2008 stated that the nanoparticle matrix with a polydispersity index below 7 showed particles formed homogeneously but those above 7 heterogeneity, as occurs in F1 anchovy protein concentrate without matrix [19, 20].

Table 4: The characterization of matrix particle using particle size analysis

Formula	Polydispersity index
F1 no matrix	1.216±0.266
F2 matrix 1: 1	0.547±0.008
F3 matrix 1: 3	0.448±0.044
F4 matrix 1: 5	0.488±0.062
F5 matrix 1: 7	0.406±0.020

mean±SD, n=3, SD: Standard deviation

The optimum formula for the volume ratio of the chitosan-TPP matrix in forming anchovy protein concentrate nanoparticles is F5 with a mixture of chitosan-TPP matrix mixture 1:7 because it has a good matrix encapsulation efficiency of 99.11%, small size 687.26 nm, good polydispersity index 0.406 and potential zeta+12.9 mV.

ACKNOWLEDGMENT

The authors would like thank to all participants who assist this research.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All of the authors listed in this manuscript have contributed equally.

CONFLICT OF INTERESTS

The author declares that there is no conflict of interest related to this report.

REFERENCES

1. Sankar TV, Anandan R, Mathew K, *et al.* Chemical composition and nutritional value of anchovy (*Stolephorus commersonii*) caught from Kerala coast, India. Eur J Exp Biol 2013;3:85-9.
2. Oktasari T, Suparmi, Karnila R. Isolation of gurame fish (*Osphronemus gurammy*) protein using different pH method. JOM 2015;2:1-12.
3. Anggo AD, Maruf WF, Swastawati F, Rianingsih L. Changes of amino and fatty acids in anchovy (*Stolephorus sp*) fermented fish paste with different fermentation periods. Procedia Environ Sci 2015;23:58-63.
4. Katili AS. Structure and function of collagen protein. Rainbow Sci J 2009;2:19-29.
5. Mackay D, Miller AL. Nutritional support for wound healing. J Clin Ther 2003;8:359-77.
6. Tungadi R, Susanty W, Wicita P, Pido E. Transdermal delivery of snakehead fish (*Ophiocephalus striatus*) nanoemulgel containing hydrophobic powder for burn wound. Pharm Sci 2018;24:313-23.
7. Tungadi R, Imran Ak. Formulation development and characterization of snakehead fish powder in an oral double emulsion. Int J Appl Pharm 2018;10:70-5.
8. Sinha VR, Kaur MP. Permeation enhancer for transdermal drug delivery. Drug Dev Ind Pharm 2000;26:1131-40.
9. Tungadi R, Abdulkadir W, Ischak N, Rahim B. Liposomal formulation of snakehead fish (*Ophiocephalus striatus*) powder and toxicity study in zebrafish (*Danio rerio*) model. Pharm Sci 2019;25:145-53.
10. Nakorn PNA. Chitin nanowhisker and chitosan nanoparticles in protein immobilization for biosensor applications. J Metals Materials Minerals 2008;18:73.
11. Erdawati Chitosan and Nanomagnetic Chitosan adsorption capacity for Ni (II) ions. proceedings of the national seminar on science and technology II. Lampung University; 2008. p. 248-56.
12. Baroli B, Ennas MG, Loffredo F, Isola M, Pinna R, Quintela MAL. Penetration of metalic nanoparticles in human full-thickness skin. J Investig Dermatol 2007;127:1701-12.
13. Singh A, Deep A. Formulation and evaluation of nanoparticles containing losartan potassium. Int J Pharm Res Technol 2011;1:17-20.
14. Agnihotri SA, Mallikarjuna NN, Aminabhavi T M. Recent advances on chitosan-based micro and nanoparticles in drug delivery. J Controlled Release 2004;100:5–28.
15. Mardliyati E, El Muttaqien S, Setyawati DR. Synthesis of chitosan-TPP nanoparticles by ionic gelation method: effect of concentration and volume ratio on characteristics of particles. Proceedings of the Scientific and Technological Meeting; 2012. p. 90-3.
16. Mohanraj VJ, Chen Y. Nanoparticles a review. J Pharm 2006;5:561-73.
17. Shu XZ, Zhu KJ. Controlled drug release properties of ionically cross-linked chitosan beads: the influence of anion structure. Int J Pharm 2002;2:217-25.
18. Wu YW, Changchum H, Jianhua F, Shoukuan. Chitosan nanoparticles as a novel delivery system form ammonium glycyrhizinate. Int J Pharm 2005; 295:224-35.
19. Laili HN, Winarti L, Sari LORK. Preparation and characterization of chitosan naringenin nanoparticle using a variation ratio of chitosan-TPP. J Health Library 2014;2:308-13.
20. Nidhin M, Indumathy R, Sreeram KJ, Nairm BU. Synthesis of Iron oxide nanoparticles of narrow size distribution on polysaccharide templates. Bull Mater Sci 2008;31:93-6.