Journal of
Food and Pharmaceutical Sciences

Original Article

SARS-CoV-2 Oral Vaccine Design Based on Nanoparticle Encapsulation with a Combination of Chitosan and Alginate

Ni Putu Fiona Cista Dewi[1]*, Ni Luh Dian Saptari Amelia Putri[1], I Wayan Ari Sudarma[1], Sang Ayu Putu Chika Iswari Anjani[1], Gusti Nyoman Oka Saputra[2], Sagung Chandra Yowani[1]

[1]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Udayana, Jimbaran, Indonesia

[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Udayana, Jimbaran, Indonesia

*Corresponding author: Ni Putu Fiona Cista Dewi | viona.kekeran@gmail.com; Tel.: +6285792344292

Received: 31 July 2024; Revised: 6 September 2024; Accepted: 25 September 2024; Published: 31 December 2024

Abstract: Vaccines are the most effective intervention in reducing COVID-19 mortality rates. Compared to parenteral vaccines, oral vaccines offer a more convenient process with dual immune responses (systemic and mucosal). Nanoparticle encapsulation is a strategic method used to enhance the efficiency of oral vaccines, antigen stability, and the effectiveness of immune induction. Combining chitosan and alginate as encapsulating polymers interacts through an ionic gelation process, which protects the vaccine from gastrointestinal disturbances. Our research aims to perform nanoencapsulation of the intravenous "Inavac" vaccine using chitosan and alginate polymers to create an oral vaccine. The optimal formulation was obtained using Design Expert 13, determined by the parameters of % transmittance and % encapsulation entrapment. The optimal formula consists of 1.75% chitosan, 0.1% NaTPP, and 0.05% alginate, with a stirring speed of 1150 rpm and a duration of 60 minutes. The PSA characterization results show that 94.45% of particles are sized at 83.81 nm with a zeta potential of (+32.86), indicating that the COVID-19 vaccine nanoencapsulation formula has a nanometer size with homogeneous distribution and system stability, correlates with good mucoadhesive strength. The good stability of the design is also indicated by the absence of significant changes in formula concentration (p-value = 0.69), the presence of appropriate functional groups as observed through FTIR, spherical surface morphology as seen through SEM, and the highest vaccine release in the intestines (Simulated Intestine Fluid medium). The oral SARS-CoV-2 vaccine using chitosan and alginate polymers with the optimal formula shows great potential for development as an alternative option for the public.

Keywords: Alginate, Chitosan, COVID-19, Nanoencapsulation, Oral Vaccine

1. INTRODUCTION

As of January 2024, the pandemic is far from over, with a cumulative total of 774,469,939 global COVID-19 cases. The WHO emphasized the pandemic has entered an endemic phase, meaning the virus continues to circulate indefinitely and may still pose a significant public health threat. Vaccines remain one of the most effective public health interventions in significantly reducing new cases and COVID-19-related mortality [1]. Indonesia's COVID-19 vaccine, such as Inavac, is administered through the parenteral route (needle-based), which has drawbacks in management, including pain/swelling, risk of infection, and discomfort. Unlike parenteral administration, oral immunization can stimulate both cellular and humoral immune responses at systemic and mucosal levels to induce broad and long-lasting immunity, especially since mucosal surfaces are the primary site of SARS-CoV-2 infection [2]. Moreover, the Omicron variant of COVID-19 has shown gastrointestinal manifestations [3]. Therefore, mucosal immunity is crucial for long-term protection against the virus. Efforts are underway to develop an oral COVID-19 vaccine, with probiotic-based oral vaccines already designed [1], [4], [5].

The current goal of oral vaccine development is to enhance antigen delivery to Gut Associated Lymphoid Tissue (GALT), triggering a strong immune response [1]. However, oral vaccines face significant challenges, such as acidic pH (especially in the stomach), poor epithelial cell absorption in the digestive tract, and low immunogenicity. Oral vaccination efficiency can be improved through nanoencapsulation [6]. Nanoencapsulation is a technique used to package active substances, like drugs or vaccines, into a nanoscale carrier (10-6 cm). Nanoencapsulation protects its payload from early degradation in biological environments, increases bioavailability, and prolongs its presence in the bloodstream and cell absorption [7],[8]. Biodegradable and biocompatible polymer particle systems can address oral vaccine challenges. The natural polymer chitosan has been used as an alternative material for oral vaccine delivery compared to synthetic polymers. Chitosan has been extensively studied for the delivery of therapeutic proteins and antigens, especially through the mucosal route due to its excellent mucoadhesive properties and enhanced absorption into M cells from the Follicle-associated epithelium (FAE). Research has shown dendritic cell, macrophage, and lymphocyte activation by chitosan-mediated oral vaccine delivery systems [2] . According to studies, immunizing mice with chitosan NPs alone provided 47% protection against parasitic infection, demonstrating chitosan's key role in inducing a protective immune response [9].

Chitosan particles coated with alginate can effectively protect acid-sensitive drugs from degradation at acidic pH compared to chitosan particles alone and enhance antigen absorption by mucosal lymphoid tissue [10]. Research has shown that oral administration of poultry typhoid vaccine in chitosan-alginate-coated microparticles can induce innate and adaptive immune responses comparable to the subcutaneous route and provide protection against virulent strains of *S. gallinarum* [11]. Additionally, the administration of alginate-chitosan-coated NPs significantly increased mucosal IgA responses and serum IgG antibodies compared to naked OVA [7]. Therefore, the development of an oral COVID-19 vaccine involves the direct nanoencapsulation of COVID-19 vaccine (Inavac) suspensions, which already contain adjuvants, using polysaccharides like chitosan and alginate to enhance mucosal penetration and oral antigen delivery. Our research aims to formulate the optimal nanoencapsulation of an oral SARS-CoV-2 vaccine using chitosan and alginate polymers and to evaluate the effectiveness and efficiency of the oral vaccine in the digestive tract.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in the research were COVID-19 Vaccine (Inavac) obtained from the Badung Regency Health Office, Chitosan (Shrimp SHELL extract), Sodium alginate (Sigma aldrich), Glacial acetic acid, Sodium tripolyphosphate (Xilong scientific), Bovine Serum Albumin (BSA) (Himedia MB083-25G), Sodium chloride, Potassium chloride, Sodium phosphate dibasic, Potassium phosphate monobasic, NaOH, DI Water (Cleo). Tools used include glassware, syringes, analytical balance (Radwag), magnetic stirrer (Thermo Scientific), pH meter (Mettler Toledo), sonicator (Branson), centrifugator (Eppendorf 5702), and freeze dryer (Lab Freeze). The instruments used were UV-Vis spectrophotometer (Shimadzu UV Mini-1240), Particle Size Analyzer/PSA, FTIR spectrophotometer (Shimadzu; IRPrestige-21), Scanning Electron Microscopy/SEM (JSM-6510LA), Design Expert software.

2.2. Preparation of Nano Encapsulation Process of Chi-Alg@Vaccine

2.2.1. Formula Development with Design Expert

Design Expert was used to determine the design and interpretation of multifactor experiments of COVID-19 vaccine nanoencapsulation. The multifactors used were stirring duration (minutes), stirring speed (rpm) and concentration (%) of chitosan, NaTPP and alginate.

2.2.2. Preparation of Chitosan, Na TPP and Alginate Solution

The formula for chitosan solution was made at a concentration of 1.75% and 0.2% dissolved in 1% glacial acetic acid solvent, with a volume of 10 mL. NaTPP was made at concentrations of 1%

and 0.1% in demineralized water solvent, with a volume of 5 mL and sodium alginate was made at concentrations of 0.6% and 0.05% in demineralized water solvent with a volume of 5 mL.

2.3. Nano-encapsulated Chi-Alg@Vaccine Formulation

The COVID-19 vaccine nanoencapsulation formulation design using chitosan and alginate has been made in 16 different formulas based on Design Expert analysis. Each formula has varying concentrations of chitosan, alginate and NaTPP. The Design Expert also determined different stirring speed and duration for each formula. Each formula contains 1.5 mL of vaccine. The stages of formulation work are as follows: (a) The vaccine was mixed with 10 mL of chitosan with stirring for 2 minutes; (b) The mixture was added with 5 mL of NaTPP and 5 mL of sodium alginate, stirred for 60 minutes or 15 minutes, at a speed of 600 rpm or 1150 rpm, according to the variation of formula from Design Expert.

2.4 % Transmittance and % Efficiency Encapsulation Capacity

2.4.1. Maximum Wavelength Determination

Standard series were made using Bovin Serum Albumin (BSA) in phosphate buffered saline solvent pH 7.4 with series concentrations of 50 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm. The maximum wavelength used was 277 nm. A standard curve of nanoencapsulated vaccine was made to obtain a linear equation.

2.4.2. % Transmittance Evaluation

Transmittance test was conducted to see the clarity of the preparation using UV-Vis spectrophotometry with distilled water as a blank. A total of 16 nanoencapsulated vaccine preparation formulas were homogenized and then put ± 3 mL into a cuvette and measured the percent transmittance at a wavelength of 650 nm.

2.4.3. Vaccine Encapsulation Entrapment Evaluation

A total of 16 nano encapsulated formulations of COVID-19 Chi-Alg vaccine were centrifuged for 30 min at 4000 rpm to separate the nanoparticles from the supernatant solution. The supernatant was analyzed by UV-Vis spectrophotometry at a wavelength of 277 nm to measure the level of unencapsulated vaccine in the supernatant with phosphate saline buffer as a blank. The total amount of vaccine added in the initial formulation was recorded. Encapsulation entrapment (%EE) was calculated using the following formula [12]:

$$\%EE = \frac{Total\ Drug\ Concentration - Supernatant\ Drug\ Concentration}{Total\ Drug\ Concentration} \times 100\%$$

2.5. Optimum Formula Determination

Determination of the optimum formula was carried out using Design Expert. The % transmittance and %EE values of 16 formulas were entered, then the best formula was determined based on the evaluation results.

2.6. Characterization of Nano Encapsulated Vaccines

2.6.1. Particle Size Analyzer

The samples were first homogenized and tested for particle size, poidispersity index, and zeta potential measured using a Malvern Instrument Zetasizer Advance conditioned at 25^{o}C.

2.6.2. Chemical and Physical Stability

Physical and chemical stability tests of the nanoencapsulated vaccine formula were carried out by organoleptic analysis and measuring the levels of the liquid form on days 0, 3, 5, 7. The samples were stored in a refrigerator at 40C. Measurement of encapsulation levels was carried out by UV-Vis spectrophotometry at a wavelength of 277 nm using ethanol as a blank. Then, the absorbance value until day 7 will be compared to determine the stability level of the formula.

2.6.3. Scanning Electron Microscopy (SEM)

The morphology of the encapsulated structure was characterized using Scanning Electron Microscopy (SEM) with magnifications of 35, 500, 3000, 5000 to 10,000 times after being coated with platinum under vacuum conditions, taken with an acceleration voltage of 20 kV. The samples were freeze dried for 24 hours before analysis.

2.6.4. Fourier transform Infrared Spectroscopy (FTIR)

The microscopic characterization performed was qualitative analysis with FTIR to characterize the functional groups of nanoencapsulated samples based on specific wave numbers. The sample ratio with KBr is 1:4.

2.6.5. In Vitro Release

Nanoencapsulation was weighed 10 mg each and then dissolved in SGF (Simulated Gastric Fluid) media pH 1.2 in 20 mL volume; SIF (Simulated Intestinal Fluid) media pH 6.8 in 20 mL volume; SCF (Simulated Colonic Fluid) media pH 7.4 in 20 mL volume. In SGF media, the nanoencapsulated vaccine was stirred at 75 rpm for 2 hours and at a temperature of 37±0.5°CAfter 2 hours, the sample was centrifuged at 3000 rpm for 15 minutes and the supernatant was measured for absorbance. Next, the SIF media was stirred at 50 rpm for 2 hours and 37±0.5°CIn SCF media, stirring was carried out at 50 rpm for 4 hours, temperature 37 ± 0.5 °CSamples from SIF and SCF media were centrifuged for 15 minutes at 3000 rpm and the absorbance was measured.

3. RESULTS AND DISCUSSION

Chitosan is a cationic polysaccharide derived from the N-deacetylation of chitin, which is a linear copolymer composed of 2-amino-2-deoxy-β-d-glucan repeating units with glycosidic bonds, where the amine group gives chitosan special properties, such as high charge density, readiness for chemical reactions, and salt formation. The solubility of chitosan depends on the position of the amino and N-acetyl groups and can be enhanced by aqueous acids such as formic acid and acetic acid. Chitosan has significant adsorption and mucoadhesive properties, as well as antifungal activity [13], [14]. These properties make chitosan very promising for applications in the food, environmental, and pharmaceutical industries [15].

Alginate is a linear biopolymer consisting of two uronic acids, namely 1,4-linked-β-d-mannuronic acid (M) and α-l-guluronic acid (G). The carboxylic group of the uronic acid is responsible for the negative charge of alginate. In drug carrier systems, polymers such as chitosan and alginate are often used because they are non-toxic, biocompatible, and biodegradable. Chitosan and alginate can react together because they have opposite charges, the ease of solubility of chitosan at low pH can be prevented by the alginate network because alginate is insoluble at low pH conditions. The possible disintegration of alginate at higher pH is prevented by chitosan, which is stable at higher pH ranges [15]. The structure of chitosan and alginate interacts ionically between the carboxyl residues of alginate and the amino terminals of chitosan. This complexation reduces the porosity of alginate and decreases the leakage of encapsulated substances [16]

3.1. Optimum Formulation Determination

In determining the optimum formulation, this research used the software, Design Expert with a factorial design which is the application of regression equations to model the relationship between the response variable and one or more independent variables. The independent numerical factors that affect the output are chitosan concentration (%), NaTPP (%), alginate (%), stirring speed (rpm) and stirring duration (minutes). The effects or response changes associated with these factors are transmittance (%) and EE (Encapsulation Entrapment) (%) which can be quantified. Based on this process, a total of 16 experiments were found to be conducted to obtain the response factors which can be seen in table 1. Based on the Design Expert analysis, there are 3 factors that affect transmittance, namely chitosan concentration, NaTPP/stirring speed, and NaTPP concentration. Figure 1 shows that chitosan affects transmittance positively so that an increase in chitosan concentration will be followed by a higher transmittance value. Meanwhile, the second and third

factors affect negatively so that the greater the NaTPP concentration and stirring speed will make the transmittance value smaller. For the second response, %EE, was not influenced by any factor because of the sixteen formulas used, all formulas succeeded in producing a large %EE (more than 85%). Through this data, the optimum formulation obtained was chitosan 1.75% volume 10 mL, NaTPP (crosslinked agent) 0.1% volume 5 mL and alginate 0.05% volume 5 mL. The Inavac vaccine used as much as 1.5 mL has a desirable value of 0.918 which can be interpreted as the high ability of the formulation to meet the optimal %transmittance and %EE criteria.

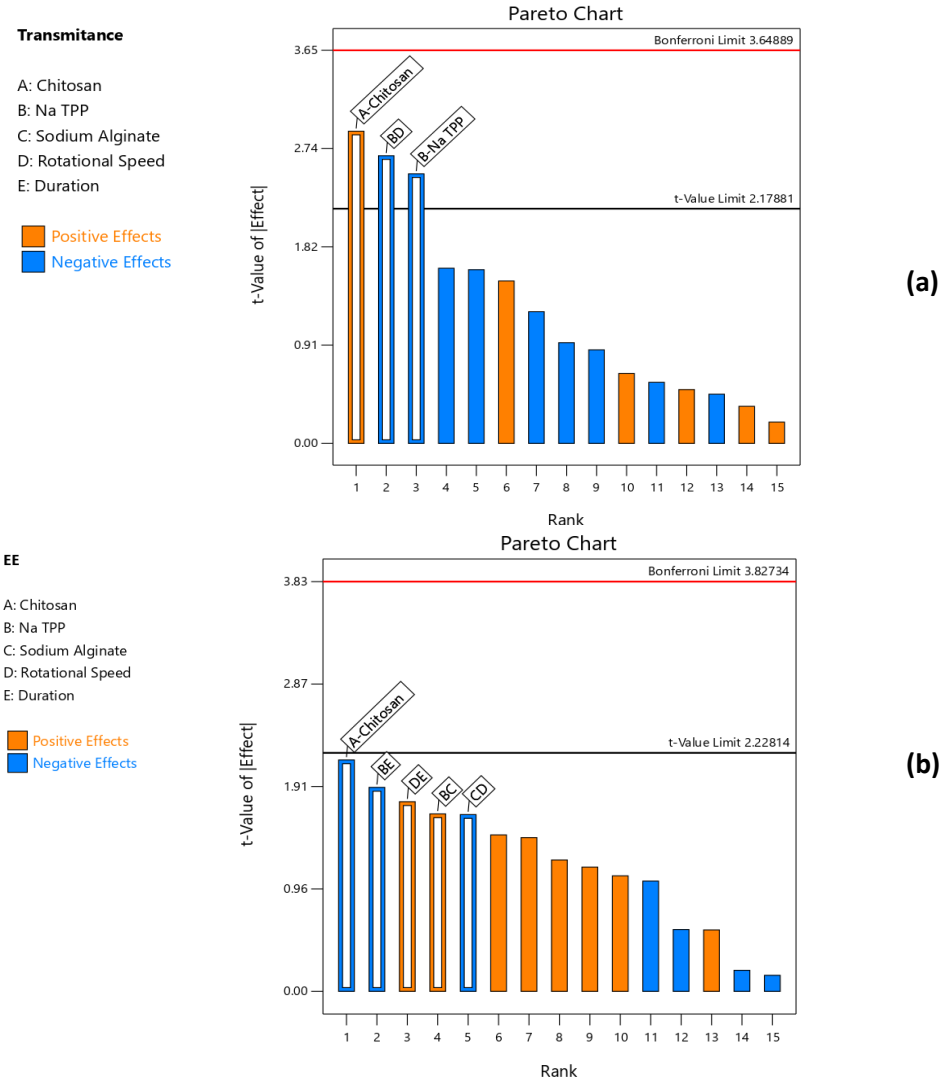


Figure 1. (a) Relationship of % Transmittance to Independent Factors; (b) Relationship of % EE to Independent Factors. Independent factors: Chitosan Concentration, NaTPP Concentration, Alginate Concentration, Stirring Speed, and Stirring Duration

Table 1. Vaccine Nanoencapsulation Formulation Design from Design Expert

Formula	Chitosan (%v/v)	NaTPP (%b/v)	Sodium alginate (%b/v)	Stirring Speed (rpm)	Stirring Duration (menit)
1	0.2	1	0.05	600	15
2	1.75	0.1	0.6	600	60
3	0.2	0.1	0.05	1150	15
4	1.75	0.1	0.6	1150	15
5	0.2	1	0.6	600	60

continued Table 1…

6	1.75	0.1	0.05	600	15
7	0.2	0.1	0.05	600	60
8	0.2	1	0.05	1150	60
9	0.2	0.1	0.6	1150	60
10	1.75	1	0.6	600	15
11	1.75	1	0.6	1150	60
12	1.75	1	0.05	600	60
13	0.2	0.1	0.6	600	15
14	1.75	1	0.05	1150	15
15	1.75	0.1	0.05	1150	60
16	0.2	1	0.6	1150	15

3.2. Particle Size Analyzer (PSA)

Particle size analyzer is a tool used to determine the size distribution of nanometer-sized particles with a measurement principle based on the scattering of laser light by particles in the sample. Light is emitted through a small needle that is sent towards the sample particles and scattered back by the particles towards the detector to be converted into a digital signal [17]. The resulting measurement results showed a particle size of 83.81 nm for 94.4%. The size and shape of nanoparticles can significantly influence their cellular uptake. Spherical nanoparticles with diameters between 50 and 100 nm are particularly effective in cellular internalization, with 50 nm being the most efficient size within this range [18] .The measured Polydispersity (PDI) value is 0.43 so that the particles can be said to be homogeneously dispersed and is an appropriate number for biological polymers. The zeta potential value obtained is +32.86 with a conductivity value of 2.79 with one peak, where the phase graph shows the electrophoresis process and the electro-osmosis process. The resulting zeta potential value illustrates the stability of the nanoparticle system and has a mucoadhesive effect on absorption [19]. The charge on the particle surface generates electrostatic forces that are positively correlated to the mucoadhesive strength. Although there is a lack of research on the correction of zeta potential with mucoadhesion so as to support the enhancement of oral absorption. In addition, a positive zeta potential may favor nanoparticle and mucosal attachment compared to a negative zeta potential [20] [21].

Table 2. Particle Size Distribution Statistics

No.	Name	Mean
1	Z-average (nm)	415.6
2	Polydispersity index (PI)	0.43
3	Intercept	0.97
4	Mean Count Rate (kcps)	229.5
5	Peak 1 Mean by Number orders by size (nm)	83.81
6	Peak 1 Area by Number ordered by size (%)	94.4
7	Peak 2 Mean by Number ordered by size (nm)	359.8
8	Peak 2 Area by Number ordered by size (%)	5.60

Table 3. Zeta Potential Statistical Data.

No.	Name	Mean
1	Zeta Potential (mV)	32.86
2	Zeta Deviation (mV)	4.55
3	Conductivity (mS/cm)	2.79
4	Quality Factor	6.38
5	Mean Count Rate (kcps)	309.3

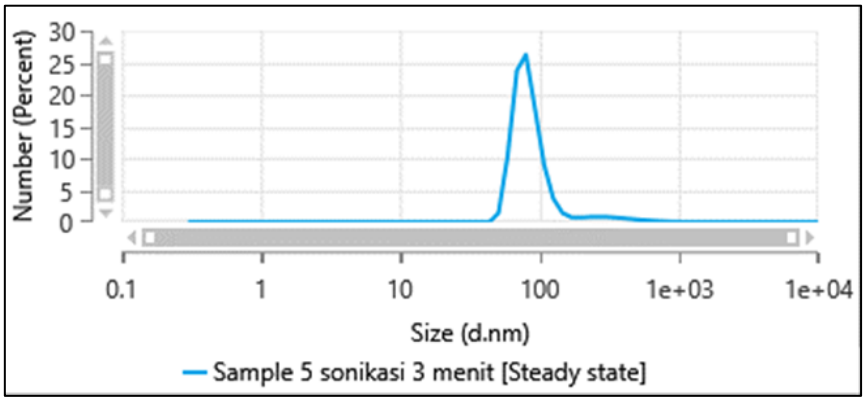


Figure 2. Distribution of Nanoencapsulation Vaccine Particle Chart

3.3. Physical and Chemical Stability

The nanoencapsulated preparation of the COVID-19 vaccine in liquid form with storage at 4°C for 7 days was physically evaluated based on its organoleptic state. Meanwhile, chemical stability was measured through vaccine nanoencapsulation levels. Organoleptically, the samples from day 0 to 7 were liquid, cloudy and contained fine particles as shown in table 4, so they had good stability in terms of organoleptics because they did not show changes during the test time. Figure 3 shows that the absorbance of the sample changed, although not significantly, which could be due to the sample being less homogenized and light fluctuations resulting in variations in the intensity of light reaching the instrument detector. The difference in levels obtained during the test showed a very low F-value (0.213) and a very high p-value (0.69) by one-way ANOVA test. This illustrates that the nanoencapsulated levels obtained were not significantly different from day 0 to 7. The figure 3 also shows an R square value of 0.0962, meaning that storage duration has very little effect on the nanoencapsulation content.

Table 4. Vaccine Nanoencapsulation Stability

Hari ke-	Amount of Vaccine	Organoleptics
0	223.8	Transparent cloudy, fine particles present
3	246.2	Transparent cloudy, fine particles present
5	181.0	Transparent cloudy, fine particles present
7	222.2	Transparent cloudy, fine particles present

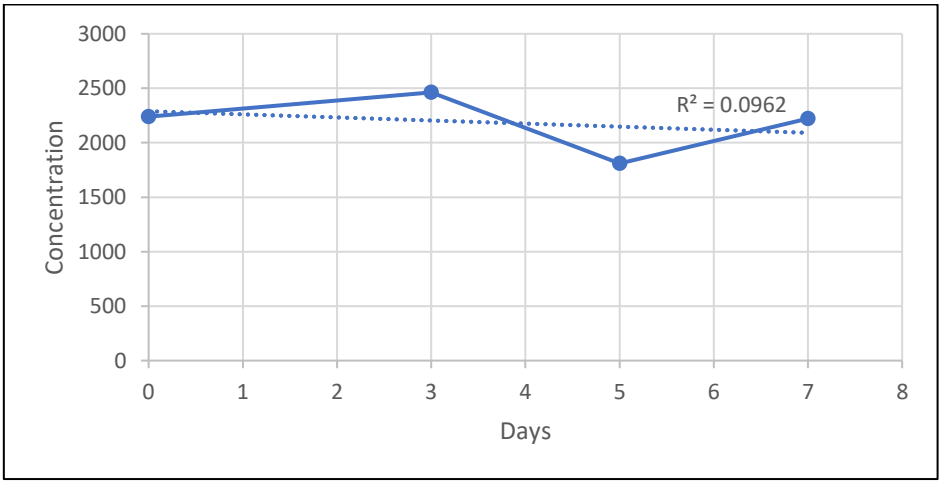


Figure 3. Vaccine Nanoencapsulation Level Chart for 7 Days

3.4. Morphological Characterization of Nanoencapsulation with SEM

The surface morphology of vaccine nanoencapsulated particles was evaluated using Scanning Electron Microscopy (SEM). SEM is capable of providing high resolution images of the surface of a sample. SEM works by utilizing the backscattering of electrons that appear on the surface of the object and taking pictures by detecting electrons that appear on the surface of the object [22]. Evaluation carried out at 5000x magnification shows a tendency to be spherical, less solid, and less uniform. SEM results are less uniform and less solid can be caused because the sample is less homogenized before drying and the sample drying time is less long. Spherical nanoparticles are often found to have higher cellular uptake rates compared to non-spherical shapes like rods or discs [23]. For example, in studies using Caco-2 cells, the uptake of rods and discs was significantly higher than spheres, but there was no difference between the uptake of rods and discs [24].

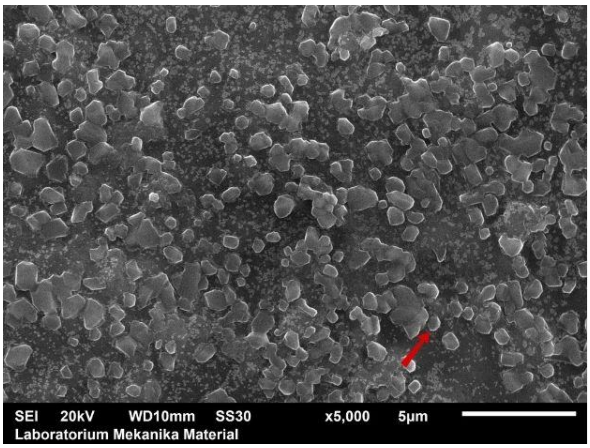
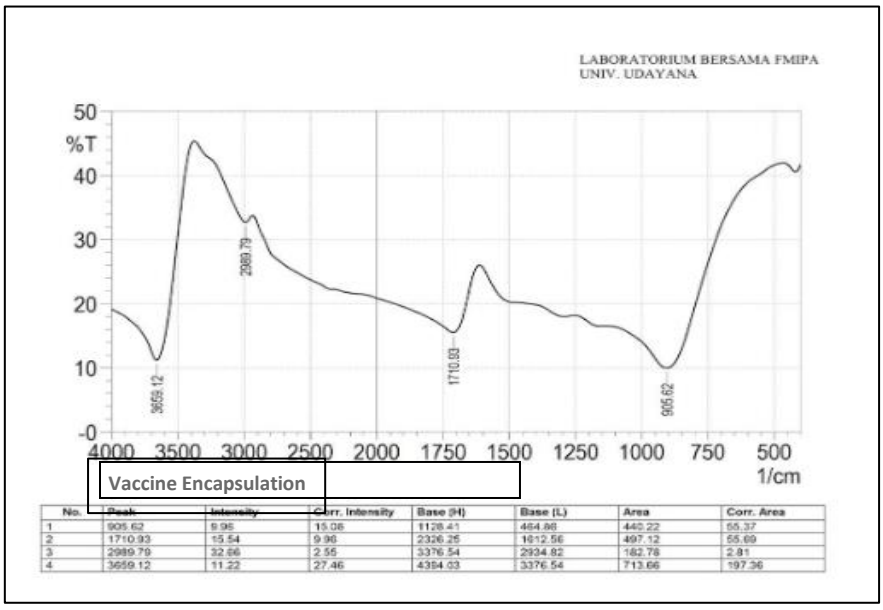


Figure 4. Scanning electron microscopy 5000x SEM results nanoencapsulation

3.5. Characterization of Nanoencapsulated Functional Groups with FTIR

Fourier Transform Infrared (FTIR) is one of the tools to represent molecular structures based on atomic vibrations. FTIR shows the specific nature of chemical bonds and molecular structures in a material, especially when analyzing specimens related to polymers and organic components [25]. FTIR peaks and spectra act as fingerprints of specific molecular structures and chemical bonds. Qualitatively, FTIR is used to identify chemical functional groups in vaccine nanoencapsulation, including components such as chitosan, and alginate.



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	905.62	9.96	15.08	1128.41	464.86	445.22	55.37
2	1710.93	15.54	9.96	2326.26	1612.56	497.12	55.69
3	2989.79	32.66	2.55	3376.54	2934.82	182.78	2.81
4	3659.12	11.22	27.46	4384.03	3376.54	713.66	197.36

Figure 5. FTIR Spectra of Nanoencapsulated Vaccine

Table 5. Infrared Data Interpretation

No.	Functional Group	Wave Number (cm^{-1})	Reference Wave Number (cm^{-1})	Interpretation
1	O-H *stretching*	3659.12	3700-3584	chitosan; alginate; *spike* protein vaccine
2	C-H *stretching*	2989.79	3000-2840	chitosan
3	C=O *stretching*	1710.93	1725-1705	chitosan; *spike* protein vaccine;
4	N-H *bending*	905.62	910-665	interaction of chi-NaTPP-alg

Based on Figure 5 and Table 5, the FTIR spectra results show the following: (a) functional groups of chitosan, alginate, namely O-H stretch; (b) functional groups of chitosan, namely aliphatic C-H stretch; (c) C=O stretch bond of acetamide group which is also present in the main chain of chitosan; (d) vaccine protein spike functional groups (amino acids) namely COOH (C=O and O-H); (e) secondary N-H groups with sharp absorption bands, where there is interaction between chitosan with alginate and NaTPP through N atoms in NH and O groups in OH and C=O groups [26].

3.6. In Vitro Release

In vitro release studies are an important evaluation to assess the safety, efficacy, and quality of nanoparticle-based drug delivery systems [27]. In this evaluation, release simulations were performed in three types of fluids that simulate the stomach, intestine, and colon. The release of COVID 19 vaccine nanoencapsulation is expected to be well absorbed in GALT (Associated Lymphoid Tissue) in the intestine. Simulation of the release of samples containing antigens through in vitro release tests in the digestive tract using SGF solution pH 1.2. The absorbance measured was 0.0639, indicating that the vaccine was able to pass through the extreme pH in the stomach because the low absorbance is proportional to the low level of vaccine released. Furthermore, the highest absorbance measured at SIF liquid pH 6.8 was 0.1266 and at SCF liquid pH 7.4 was 0.104. This shows that the release of nanoencapsulated vaccine occurs most in the intestine and decreases in the colon. Based on the graph in appendix 7, the release of nanoencapsulated vaccine is in the right location, namely in the intestine targeting GALT (Gut-Associated Lymphoid Tissues) which is sensitive and an important area for immune response.

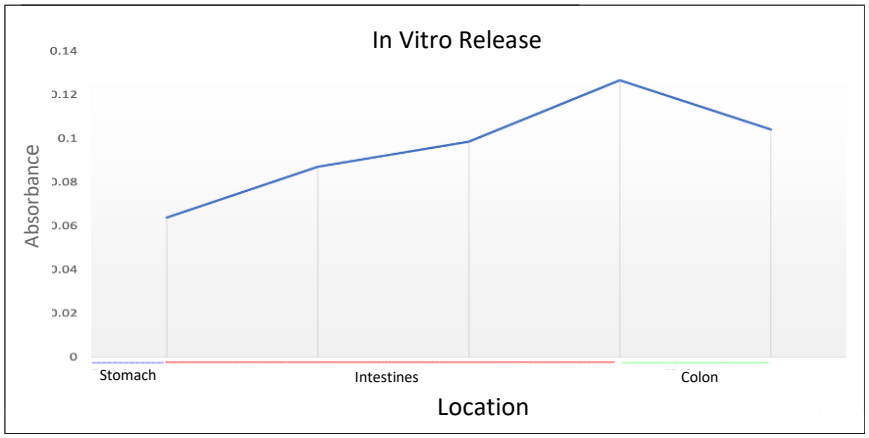


Figure 6. In Vitro Release Chart

4. CONCLUSION

Nanoencapsulation of SARS-CoV-2 oral vaccine with a combination of chitosan and alginate polymers has an optimal formula at a concentration of 1.75% chitosan; 0.1% NaTPP, and 0.05% alginate with a stirring speed of 1150 rpm and a duration of 60 minutes as measured by

%transmittance and %EE. PSA evaluation results showed 94.4% had a size of 83.81 nm with homogeneous particle distribution and zeta potential indicating a stable system that supports mucoadhesive power. Physicochemical stability evaluation from day 0 to 7 showed no significant changes in organoleptics and formula content. SEM showed spherical particle morphology but less uniform. FTIR showed the presence of chitosan, alginate, and vaccine protein functional groups. The release profile of the formula was in accordance with the target achievement, namely having the highest release in the intestine and being able to pass through acidic pH conditions well. The SARS-CoV-2 oral vaccine with chitosan and alginate polymers with the optimum formula has great potential to be developed so that it can be an alternative choice for the community.

Funding: We would like to thank the Ministry of Education, Culture, Research and Technology; the Directorate of Learning and Student Affairs; and Udayana University for funding our research.

Acknowledgments: We are also grateful to all those who have helped in the collection of data and information needed in writing this journal. Finally, we hope that this article can provide benefits and inspiration for readers who are interested in the topic of nanoencapsulation, vaccines, and pharmaceutical innovation.

Conflicts of interest: The authors declare no conflict of interest

References

[1] Kwong, K.W.Y., Xin, Y., Lai, N.C.Y., Sung, J.C.C., Wu, K.C., Hamied, Y. K., Sze, E.T., and Lam, D.M.K., "Oral vaccines: A better future of immunization", *Vaccines*, vol. 11, pp. 1-17, May.2024, doi: 10.3390/vaccines11071232.

[2] Jazayeri, S.D., Lim, H.X., Shameli, K., Yeap, S.K., and Poh, C.L., "Nano and microparticles as potential oral vaccine carriers and adjuvants against infectious diseases", *Frontiers in Pharmacology.* Vol.12, pp. 1-15, Jun.2021, doi: doi.org/10.3389/fphar.2021.682286.

[3] Slabakova, Y., Gerasoudis, S., Miteva, D., Peshevska-Sekulovska, M., Batselova, H., Snegarova, V., ... and Velikova, T., "SARS-CoV-2 variant-specific gastrointestinal symptoms of COVID-19: 2023 update," *Multidisciplinary Digital Publishing Institute (MDPI)*. Vol. 14, pp. 431-435, Dec.2023, doi: 10.3390/gastroent14040032.

[4] Baker, P.J.,"Advantages of an oral vaccine to control the COVID-19 pandemic," *Elsevier Inc*. Vol. 135, pp. 133-134, Feb.2022, doi: 10.1016/j.amjmed.2021.08.037.

[5] Chau, E. C. T., Kwong, T., Pang, C., Chan,L., Yao, X., Tam, J., Chan, S., Leung, G., Tai, W., and Kwan, Y., "A novel probiotic-based oral vaccine against SARS-CoV-2 omicron variant B.1.1.529," *International Journal of Molecular Sciences*. Vol. 24, pp. 1-14, Sep. 2023, doi: 10.3390/ijms241813931.

[6] Li, S., Zhang, H., Chen, K., Vu, S., Jung, S., He, N., Zheng, Z.,and Lee, M., "Application of chitosan/alginate nanoparticle in oral drug delivery systems: prospects and challenges," *Drug Delivery*. Vol. 29, pp. 1142–1149, Apr.2022, doi: 10.1080/10717544.2022.2058646.

[7] Cao,P. F. Y. Han, L. Grøndahl, Z. P. Xu, and L. Li, "Enhanced oral vaccine efficacy of polysaccharide-coated calcium phosphate nanoparticles," *American Chemical Society Omega.* Vol. 5, pp. 18185–18197, Jul. 2020, doi: 10.1021/acsomega.0c01792.

[8] Sun, Z., Li, W., Lenzo, J., Holden, J., McCullough, M., O'Connoer, A., O;Brien-Simpson, N., "The potential of calcium phosphate nanoparticles as adjuvants and vaccine delivery vehicles," *Frontiers in Materials*. Vol.8, pp. 1-17, Dec. 2021,. doi: 10.3389/fmats.2021.788373.

[9] Xing,L., Fan, Y., Zhou, T., Gong, J., Cui, L., Cho, K., Choi, Y., Jiang, H., and Cho, C., "Chemical modification of chitosan for efficient vaccine delivery," *Molecules.* Vol. 23, pp. 1-19, Jan.2018, doi: 10.3390/molecules23020229.

[10] Auliasari, N., Hanifa, H., and Permatasari, A.,"Formulasi dan karakterisasi sistem penghantaran nanopartikel α-mangostin dengan kitosan-alginat sebagai polimer,"*Prosiding Seminar Nasional Diseminasi Penelitian.* Vol. 3, pp. 222-228, Sep.2023. Available: https://ejurnal.universitas-bth.ac.id/index.php/PSNDPV3/article/download/1154/877.

[11] Onuigbo, E., Iseghohimhen, J. Chah, K., Gyang, M., and Attama, A., "Chitosan/alginate microparticles for the oral delivery of fowl typhoid vaccine: innate and acquired immunity," *Vaccine.* Vol. 36, pp. 4973–4978, Aug. 2018, doi: 10.1016/j.vaccine.2018.05.087.

[12] Akib, N. I. , Sahumena, M. H., Dawu, Y., Aspadiah, V., Hafizah, I. , and Ritonga, H.," Optimasi kadar fenilbutazon dalam pembawa vesikular etosom (optimization of concentration of phenylbutazone in ethosomes vesicular carrier)," *Medula.* Vol. 7,pp. 88-96, Apr.2020, EISSN: 2443-0218.

[13] Ways, T. M. M., Lau, W. M., and Khutoryanskiy, V. V.,"Chitosan and its derivatives for application in mucoadhesive drug delivery systems," *Polymers.* Vol. 10, pp. 1-37, Mar.2018, doi: 10.3390/polym10030267.

[14] Desai, N., Rana, D., Salave, S., Gupta, R., Patel, P., Karunakaran, B., Sharma, A., Giri, J., Benival, D., Kommineni, N., "Chitosan: A potential Biopolymer in drug delivery and biomedical applications," *Multidisciplinary Digital Publishing Institute (MDPI)*. Vol. 15, pp. 1-69, Apr.2023, doi: 10.3390/pharmaceutics15041313.

[15] Azevedo, M., Bourbon, A., Vicente, A. and M. A. Cerqueira, M., "Alginate/chitosan nanoparticles for encapsulation and controlled release of vitamin B2," *International Journal Biological Macromolecules*. Vol. 71, pp. 141–146, Nov. 2014, doi: 10.1016/j.ijbiomac.2014.05.036.

[16] Nnamonu, L.A., Sha'Ato, R., and Onyido,I., "Alginate reinforced chitosan and starch beads in slow release formulation of imazaquin herbicide—preparation and characterization," *Materials Sciences and Applications*. Vol. 3, pp. 566–574, Jul.2012, doi: 10.4236/msa.2012.38081.

[17] Ilangovan, R., Subha V., Ravindran, R.S.E., Kirubanandan, S., and Renganathan, S., "Nanomaterials: Synthesis, physicochemical characterization, and biopharmaceutical applications," *In Micro and Nano Technologies*, pp. 33–70, 2021, doi: 10.1016/B978-0-12-820569-3.00002-5.

[18] Zhang, W., Taheri-Ledari, R., Ganjali, F., Mirmohammadi, S., Qazi, F., Saeidirad, M., KashtiAray, A., Zarei-Shokat, S., Tian, Y., Maleki, A., "Effects of morphology and size of nanoscale drug carriers on cellular uptake and internalization process: a review," *Royal Society of Chemistry*. Vol. 13, pp. 80-114, Dec.2022, doi: 10.1039/d2ra06888e.

[19] Amin, M.K. and Boateng, J.S., "Enhancing stability and mucoadhesive properties of chitosan nanoparticles by surface modification with sodium alginate and polyethylene glycol for potential oral mucosa vaccine delivery," *Marine Drugs*, Vol. 20, pp. 1-22, Mar.2022, doi: 10.3390/md20030156.

[20] Liu, J., Tu, L., Cheng,M., Feng, J., and Jin, Y., "Mechanisms for oral absorption enhancement of drugs by nanocrystals,"*Journal of Drug Delivery Science and Technology.*Vol. 56, pp. 1-11, Apr.2020, doi: 10.1016/j.jddst.2020.101607.

[21] Honary, S. and Zahir, F., "Effect of zeta potential on the properties of nano-drug delivery systems - A review (Part 2)," *Tropical Journal of Pharmaceutical Research*, Vol. 12, pp. 265–273, Apr.2013, doi: 10.4314/tjpr.v12i2.20.

[22] Septiano, A.F. and Setyaningsih, N. E. "Analisis citra hasil scanning electron microscopy energy dispersive x-ray (SEM EDX) komposit resin timbal dengan metode contrast to noise ratio (CNR)," *Indonesian Journal of Mathematics and Natural Sciences.* Vol. 44, pp. 81-85. Oct. 2021. Available: http://journal.unnes.ac.id/nju/index.php/JM

[23] Istianah, "Enkapsulasi asam folat,"*Thesis.* Syarif Hidayatullah Islamic University, Jakarta, Nov. 2019. Available: https://repository.uinjkt.ac.id/dspace/bitstream/123456789/48415/1/ISTIANAH-FST.pdf.

[24] Banerjee, A., Qi, J., Gogoi, R., Wong, J., and Mitragotri, S., "Role of nanoparticle size, shape and surface chemistry in oral drug delivery," *Journal of Controlled Release*, Vol. 238, pp. 176–185, Sep. 2016, doi: 10.1016/j.jconrel.2016.07.051.

[25] Nandiyanto, A.B.D., Ragadhita, R., and Fiandini, M., "Interpretation of fourier transform infrared spectra (FTIR): a practical approach in the polymer/plastic thermal decomposition," *Indonesian Journal of Science and Technology*, Vol. 8, pp. 113–126, Apr.2023, doi: 10.17509/ijost.v8i1.53297.

[26] LibreTexts, "Infrared spectroscopy sbsorption table", URL: https://chem.libretexts.org/Ancillary_Materials/Reference/Reference_Tables/Spectroscopic_Reference_Tables/Infrared_Spectroscopy_Absorption_Accestable tanggal 1 Juli 2024

[27] Weng, J., Tong, H., Chow, S.F., "In vitro release study of the polymeric drug nanoparticles: development and validation of a novel method," *Pharmaceutics*. Vol. 12, pp. 1–18, Aug. 2020, doi: 10.3390/pharmaceutics12080732.