International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Issue 3, 2024

Original Article

# EFFECT OF POLYMER CONCENTRATION AND SURFACTANTS ON PHYSICAL CHARACTERISTICS, DRUG RELEASE AND ANTIOXIDANT ACTIVITY OF GLUTATHIONE-KAPPA CARRAGEENAN NANOSPHERES

**YUYUN NAILUFA*, BAMBANG WIDJAJA**

**Pharmacy Study Program, Hang Tuah University Surabaya, Surabaya-60271, Indonesia**
***Corresponding author: Yuyun Nailufa; *Email: yuyun.nailufa@hangtuah.ac.id**

***Received: 26 Aug 2023, Revised and Accepted: 01 Mar 2024***

**ABSTRACT**

**Objective:** Glutathione is one of the antioxidants widely used as an antiaging and skin lightener. Glutathione at a dose of 250 mg/d orally proved useful as an antiaging. At the same time, glutathione topical night cream is effective at a dose of 0.1% for the skin of Indonesian women. Glutathione is one of the antioxidants that has easily oxidized properties in storage. Research purpose to optimize the concentration of kappa carrageenan polymer and surfactan to obtain the optimal physical characteristics of nanosphere system analyzed based on size, PDI, yield, drug loading, entrapment efficiency, dissolution and antioxidant activity.

**Methods:** The most commonly used method of making nanospheres is ionotropic gelation because it has proven effective, easy, and easy to apply. Ionotropic gelation is depend on the tendency of polyelectrolytes to cross connect to develop hydrogel beads often called gelispheres in the existence of counter ions. Nanospheres were prepared by aerosolization ionotropic gelation technique followed by freeze-drying. This method uses carrageenan polymers of 0.5% and 1.0% with the addition of surfactant as a stabilizer. Evaluation parameters are particle size, entrapment efficiency, drug loading, drug release and antioxidant activity.

**Results**: The results of the nanospheres obtained were tested physically and drug activity. Nanospheres successfully formed, with size 382.67±52.24 nm, F2 325.20±4.62 nm, F3 495.39±30.61 nm, and F4 409.80±4.11 nm. The greater the polymer concentration, the gre ater the value of entrapment efficiency and drug content in the nanosphere. The morphology of the nanosphere is quite good, spherical, with a smooth surface. The release profile shows that glutathione release is quite good but takes a long time, namely F1 73.91±2.17%, F2 75.91±2.76%, F3 78.56±2.82%, and F4 79.56±1.34% in 480 min or 8 h. Antioxidant activity of glutathione-Kappa carrageenan nanospheres with the DPPH method showed that nanospheres have medium or medium category antioxidant activity.

**Conclusion:** The most optimal formula is F4 with 1% kappa-carrageenan concentration and 0.6% KCl.

**Keywords:** Nanosphere, Glutathione, Kappa carrageenan, Poloxamer 188, Ionotropic gelation


DOI: https://dx.doi.org/10.22159/ijap.2024v16i3.49242 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

## INTRODUCTION

Glutathione is still the best skin-lightening and anti-aging product for women in Asia. Glutathione at a dose of 250 mg/d orally proved useful as an anti-aging [1]. While glutathione topical night cream is effective at a dose of 0.1% for the skin of Indonesian women [2]. Glutathione is one of the antioxidants that has easily oxidized properties in storage. Glutathione is also easily oxidized in the digestive system, so it needs to be modified with a system that can maintain glutathione stability. One such system is the nanosphere. Nanosphere is a nanoencapsulation method made by adding a polymer to envelop the active ingredients of drugs so that they are not exposed to the environment, which can damage it by trapping the drug in its interior structure, binding to it, or absorbing it on its surface and can provide control release [3].

In the formation of nanosphere systems, polymer is needed. Carrageenan was chosen as a polymer to utilize the results of seaweed cultivation. Ionotropic gelation is the easiest and cheapest method of making nanospheres at room temperature. This method requires crosslinkers [4]. The crosslinkers used for kappa carrageenan are $K^+$, $Na^+$, $Rb^+$, $Cs^+$, and $Li^+$ [5]. The nanoparticle system has a nanosize, making it less stable in this study. Surfactants were added to determine the effect of surfactants on the nanosphere system. The wet nanospheres that have formed need to be dried. The drying method with high temperatures can damage glutathione, so in this study, the frozen drying method's nanosphere system that was still wet was dried.

Research purpose to optimize the concentration of kappa carrageenan polymer and surfactan to obtain the optimal physical characteristics of nanosphere system analyzed based on size, PDI, yield, drug loading, entrapment efficiency, dissolution and antioxidant activity. The best formula continued for the manufacture topical dosage form. Glutathione can be used as an antiaging through antioxidant mechanisms.

## MATERIALS AND METHODS

### Materials

The materials used to make nanosphere in this research were L-Glutathione Reduced ≥ 98% (Sigma-Aldrich Inc), Kappa Carrageenan (Japan), KCl p.a (Merck), poloxamer 188, $KH_2PO_4$ p. a (Merck), $Na_2HPO_4$ p.a (Merck), maltodextrin, and demineralized water (Bratachem Company).

### Method

The optimization design used was a randomized complete factorial design $2^2$ with variations in the polymer concentration, namely kappa carrageenan and potassium chloride, to obtain optimal nanosphere. It was carried out by observing physical characteristics and activity such as yield, drug loading, entrapment efficiency, particle size, PDI, dissolution, morphology, and antioxidant activity.

How to make nanospheres by ionic gelation method with aerosolization technique: Carrageenan kappa weighed with concentrations of 0.5% and 1.0%. The 1% glutathione in each formula is put in the solution and stirred using a 1000 rpm magnetic stirrer. KCl 0.3% and 0.6% are made 100 ml apart as crosslinks. The crosslinking solution is sprayed into glutathione-kappa carrageenan solution while stirring at 1000 rpm using a magnetic stirrer until it runs out, then stirring for up to 3 h at a speed of 1000 rpm. The formed nanospheres were centrifuged for 6 min at a rate of 3000 rpm and washed two times with distilled water. Before drying it is

suspended to within 5% maltodextrin. Centrifuged nanospheres are filtered and dried using a freeze-dryer for 30 h [6].

**Particle size and polydispersity index**

The particle size of the nanosphere was measured using the Malvern Particle Size Analyzer (PSA) and the polydispersity index was also determined to evaluate the uniformity of particle size [7].

**Drug loading, entrapment efficiency, and yield**

The glutathione drug loading in the nanosphere system and entrapment efficiency were evaluated following several procedures. These included the preparation of a stock solution of 300 ppm glutathione by dissolving 15 mg of glutathione in 50 ml of phosphate buffer pH 7.4. Subsequently, a standard curve solution was prepared from the stock solution and serially diluted with phosphate buffer pH 7.4±0.05, 10.0 ml in a 10.0 ml volumetric flask to obtain concentrations of 30 ppm, 60 ppm, 90 ppm, 120 ppm, and 150 ppm. The absorbance of 0.2 ml of glutathione solution at each concentration was observed using a spectrophotometer to determine the maximum wavelength in the 200-500 nm range. The drug loading of glutathione in the nanosphere system was determined by adding 50 mg of glutathione-kappa carrageenan nanosphere to 50 ml of phosphate buffer pH 7.4, allowing it to stand for 24 h. The nanosphere-buffer mixture was filtered and stirred at 1000 rpm for 2 h. The absorbance of the sample solution was observed with a spectrophotometer at a wavelength of 200-500 nm. The drug loading and entrapment efficiency were calculated, as well as the yield according to the formula by [8].

$$\text{Drug loading (\%)} = \frac{\text{Drug weight in nanosfer}}{\text{Nanosphere Weight}} \times 100\ \%$$

$$\text{Entrapment efficiency (\%)} = \frac{\text{Drug weight in the nanosphere}}{\text{Weight of drug used in the Nanosphere formulationr}} \times 100\ \%$$

$$\textit{Yield}\ (\%) = \frac{\text{The total weight of the nanosphere}}{\text{Drug and polymer weight}} \times 100\ \%$$

**Drug release test**

The associated thermodynamic activity of the formulation determines the drug release test. It can be demonstrated using a diffusion cell system generally used in *in vitro* drug release research. Release of glutathione from the nanosphere using saline phosphate components up to pH 6.0. The dry nanosphere of±50 mg each was put into a vessel in the Franz Diffusion Cell donor compartment while the receptor compartment below was filled with 21 ml of phosphate buffer solution pH 6.0±0.05. The experimental temperature was set to 32 °C±0.5 °C. The magnetic stirrer is rotated at 500 rpm and immediately recorded as a time to zero. At minutes 0, 15, 30, 45, 60, 90, 120, 150, 180, 240,300, 360, 420, and 480, samples were retaken as much as 1.5 ml, then replaced with the same amount, then shaken and allowed to stand for 15 min. Sample snippets were filtered using 0.45 µm Millipore filter paper and observed sample absorption with UV-Vis spectrophotometer at 200-500 nm wavelengths. Glutathione levels are determined by entering the absorbance price of the sample into the glutathione standard curve equation that has been made previously [8].

**Morphology**

Nanosphere morphology was evaluated using a scanning electron microscope (SEM) with a magnification of 5000x [9].

**Antioxidant activity**

Antioxidant activity can be known by measuring the ability to inhibit oxidation reactions. The DPPH method is in demand because it is considered effective, simple, easy, and cheap [10].

**RESULTS AND DISCUSSION**

**Formula**

The glutathione-kappa carrageenan nanosphere formula was optimized using the randomized complete factorial design $2^2$ method with variations in kappa-carrageenan polymer and surfactant concentration. The concentrations of kappa carrageenan were set at 0.5% and 1%, while that of surfactant, with or without surfactants. Details of the formula are presented in table 1.

**Table 1: Glutathione-kappa carrageenan nanosphere formula**

| Material name | Material function | Formula (%) | | | |
|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 |
| Glutathione | Active ingredients | 1 | 1 | 1 | 1 |
| Kappa carrageenan | Polymer | 0.5 | 0.5 | 1 | 1 |
| Poloxamer 188 | Surfactant | - | 5 | - | 5 |
| Potassium chloride | Cross linker | 0.6 | 0.6 | 0.6 | 0.6 |
| Demineralized water | Solvent | ad 100 | | | |

The four glutathione-kappa carrageenan nanosphere formula optimization results produced physical characteristics as shown in tables 2 and 3.

**Table 2: Physical characteristics of the glutathione-kappa carrageenan nanosphere**

| Formula | Particle size (nm) | PDI | Drug loading (%) | Entrapment efficiency (%) | *Yield (%)* |
|---|---|---|---|---|---|
| F1 | 382.67±52.24 | 0.542±0.20 | 19.51±0.71 | 44.65±0.66 | 79.08±0.97 |
| F2 | 325.20±4.62 | 0.129±0.01 | 21.12±0.75 | 45.74±0.43 | 78.14±0.93 |
| F3 | 495.30±30.61 | 0.487±0.16 | 32.64±1.67 | 55.35±2.52 | 81.19±2.29 |
| F4 | 409.80±4.11 | 0.138±0.03 | 34.49±1.65 | 56.20±2.03 | 82.63±1.48 |

Data were expressed as mean±SD, n=3

**Particle size and polydispersity index**

Based on the results of *multilevel factorial* design statistical tests with a completely random design with independent variables, A is a polymer, namely carrageenan, and B is a surfactant, namely poloxamer 188, each obtained the results of p-value (sig) 0.001 and p-value (sig) 0.004 (<0.05) to show a significant influence. While the interaction between the two has a p-value (sig) of 0.448 (>0.005), it indicates no significant effect on particle size.

In the Pareto chart fig. 1. the carrageenan polymer crosses the reference line of 2.306, meaning that the polymer significantly affects the particle size of the nanosphere. Chart B, i.e., poloxamer surfactant 188, also crosses the reference line 2.306. Thus, the concentration of the polymer significantly affects the particle size of the nanosphere. Meanwhile, the AB chart showing the interaction between polymers and surfactants does not cross the reference line 2.306, so the interaction between polymers and surfactants does not significantly affect the particle size of the nanosphere. The value of the polymer standardized effect is greater than that of the standardized effect of surfactant concentration. That is, the influence of the polymer on particle size is greater than the influence of surfactant.


**Fig. 1**: **Particle size pareto chart**

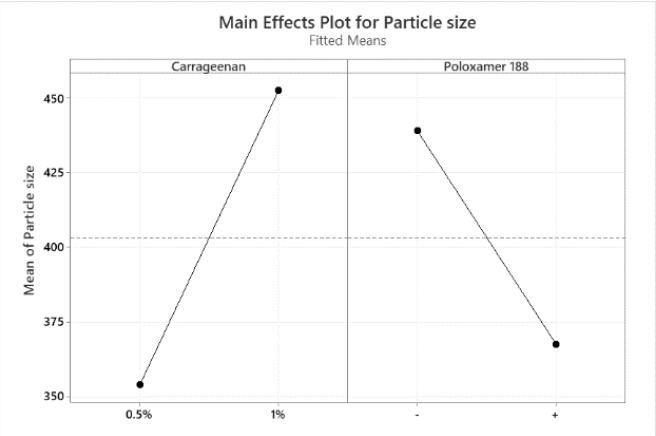


**Fig. 2: Main effects particle size plot**

Based on the results of the main effects plot fig. 2 it can be concluded that there are differences in nanosphere particle sizes caused by differences in polymer concentrations. A polymer concentration of 0.5% resulted in an average particle size of nanospheres of less than 400 nm. At a polymer concentration of 1%, it produces an average particle size above 450 nm. Thus, the higher the polymer concentration, the greater the particle size of glutathione-alginate microspheres. From the main effects plot of surfactants, it can be concluded that there are differences in particle size caused by the presence of surfactants. The absence of additional poloxamer 188 (-) resulted in an average particle size of less than 450 nm. Meanwhile, adding poloxamer 188 (+) resulted in an average particle size below 375 nm. Thus, the particle size is getting smaller with the addition of surfactants. High surfactant concentration decreases surface tension and stabilizes newly developed surfaces during homogenation and production of smaller particles [11]. Previous research without the use of surfactants resulted in smaller entrapment efficiencies and less uniform particle sizes with a sufficiently large polydispersity index value [12].

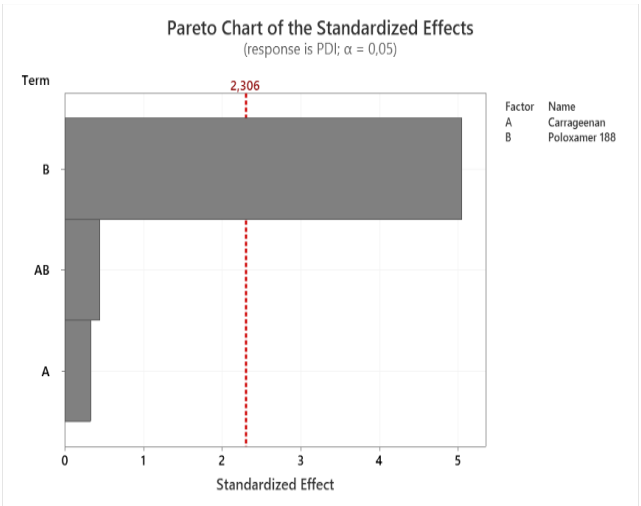


**Fig. 3**: **PDI pareto chart**

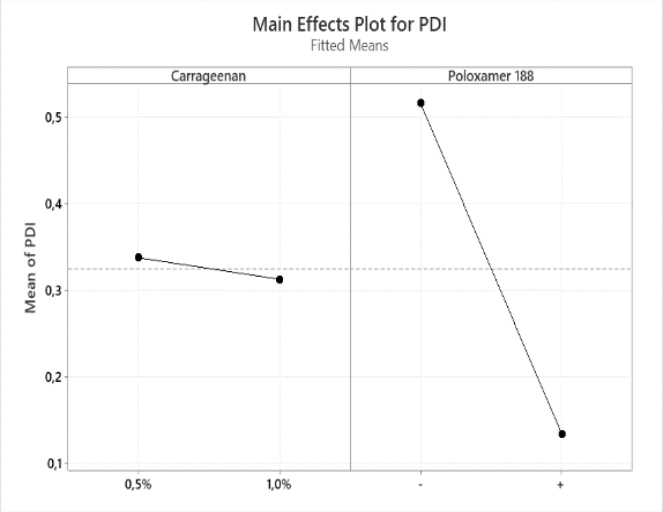


**Fig. 4: Main effect plot for PDI**

The Pareto chart fig. 3 shows that surfactant alone affects the PDI value because only chart B crosses the reference line 2.306. Based on the main effect plot of PDI, it is also proven that poloxamer 188 provides a higher PDI value shift. Poloxamer 188 is a surfactant that can help stabilize particle size because this surfactant will affect surface tension. Surfactants can lower the interface voltage of a system.

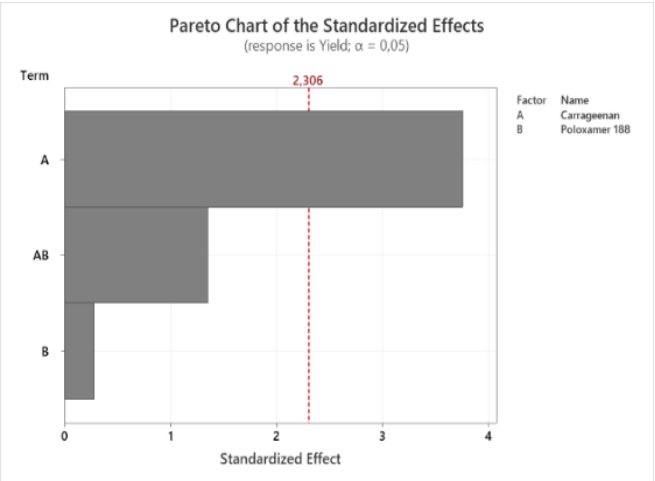


**Fig. 5: Yield pareto chart**

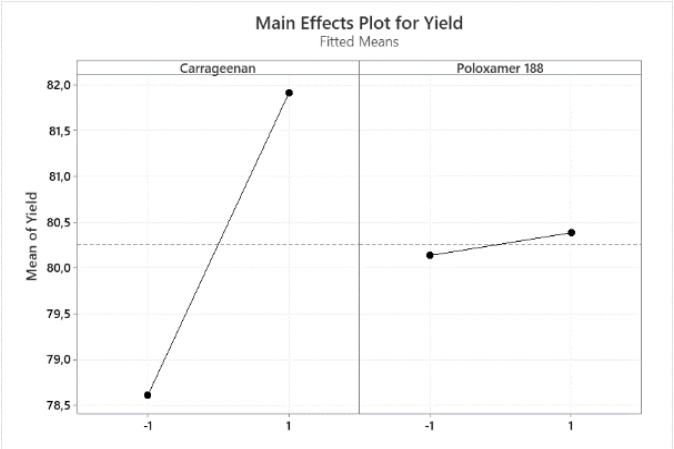


**Fig. 6: Main effects plot for yield, based on the pareto chart fig. 5 and main effect plot of yield fig. 6, it is proven that what has a significant influence on the yield value is the concentration of carrageenan polymer**

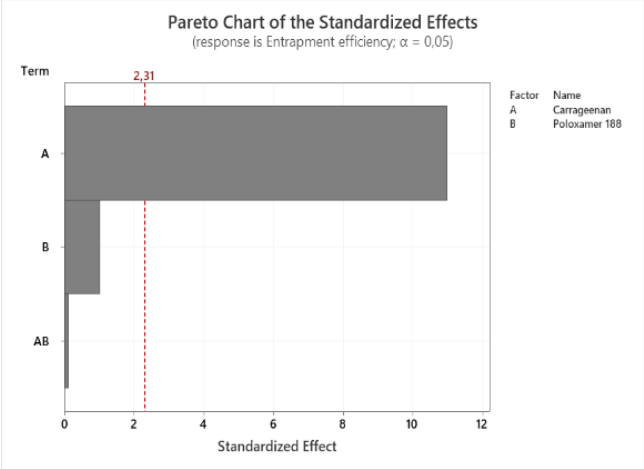


**Fig. 7: Entrapment efficiency (EP) pareto chart**

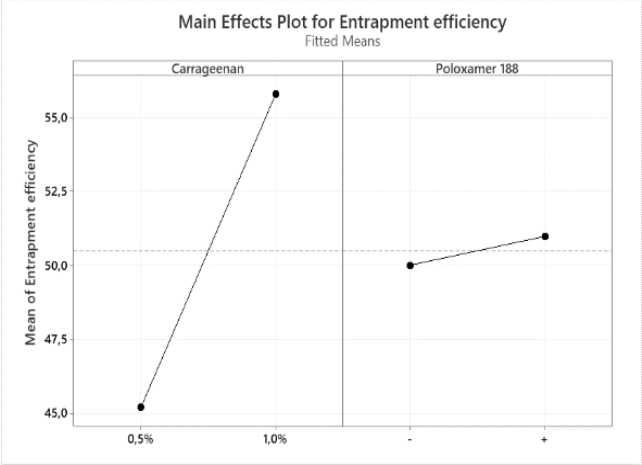


**Fig. 8: Main effects plot for EP**

Based on the results of statistical tests, the value of entrapment efficiency (EP) with multilevel factorial design shows that with the independent variable A is a polymer, carrageenan and B is a surfactant, namely poloxamer 188, each of which obtained the result of p-value (sig) 0.000 (<0.05) so that it shows a significant influence while p-value (sig) 0.341 (>0.05) so that it shows no significant impact. The interaction between the two has a p-value (sig) of 0.905 (>0.05), indicating no significant effect on the efficiency of the trapper. And evidently, only chart A crosses the reference line 2.31 on the Pareto chart. Added poloxamer, as a surfactant to polysaccharide (alginate or kappa-carrageenan) was found to increase the degree of encapsulation of poorly soluble drugs [13]. In this study, it was seen that the kappa-carrageenan polymer plays a role in increasing the efficiency of entrapment.

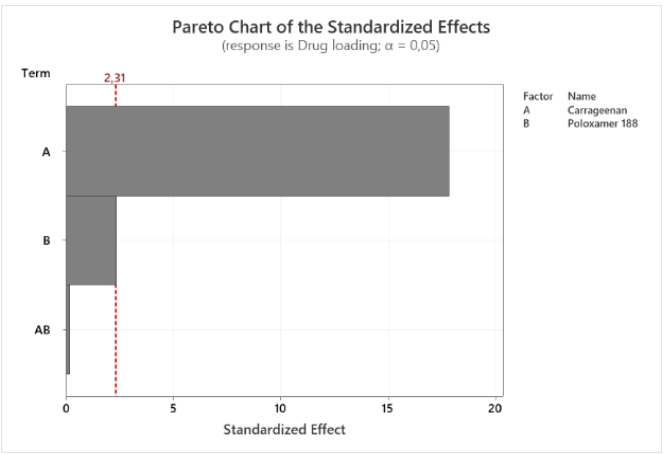


**Fig. 9: Drug loading pareto chart**

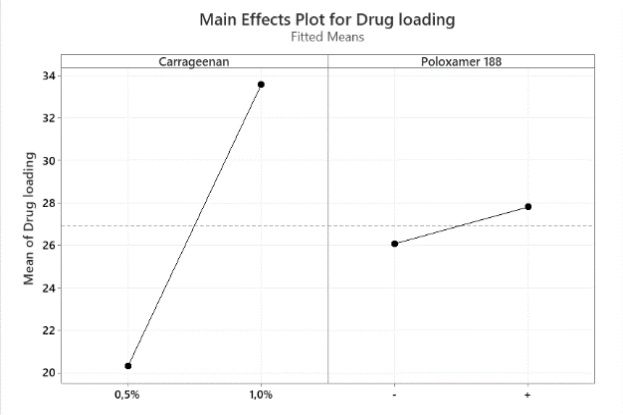


**Fig. 10: Main effect plot for drug loading**

Based on the results of statistical tests of drug levels or drug loading (DL) above with multilevel factorial design shows that with independent variables A is a polymer, carrageenan, and B is a surfactant, namely poloxamer 188, each obtained the results of p-value (sig) 0.000 and p-value (sig) 0.048 (<0.05) so that it shows a significant influence. While the interaction between the two has a p-value (sig) of 0.879 (>0.05), it indicates no significant effect on drug levels.

In addition to physical characteristics tests, the tests that need to be carried out are release tests with diffusion media of 0.01 M phosphate buffer solution, pH 6.0±0.05. The choice of diffusion media is because, later, this preparation will be aimed at the topical route so that it is adjusted to the skin's pH. This test uses a cellophane membrane cut with a size of 3x4 $cm^2$ soaked with demineralized water for one night (±12 h). The membrane is drained before use.

The soaked cellophane membrane is placed at the top of the receptor compartment and set with parafilm. Demineralized water and magnetic stirrer are fed through the sampling pipe of the receptor compartment. The donor-receptor compartment is tightly connected to the parafilm and then immersed in a container with water as high as the receptor compartment, placed on a stirrer with an adjustable heater. Franz diffusion cells consist of donor compartments and these receptors are erected with small clamps connected to the stative.

A dry nanosphere of±50 mg each was fed into vessels in the Franz Diffusion Cell donor compartment, while the receptor compartment below was filled with 21 ml of phosphate buffer solution pH 6.0±0.05. The experimental temperature was set to 32 °C±0.5 °C. The magnetic stirrer is rotated at 500 rpm and immediately recorded as a time to zero. At minutes 0, 15, 30, 45, 60, 90, 120, 150, 180, 240,300, 360, 420, and 480, samples were retaken as much as 1.5 ml, then replaced with the same amount, then shaken and allowed to stand for 15 min. The sample was observed for absorbance with a UV-Vis spectrophotometer at its maximum wavelength. The glutathione concentration in the sample is calculated using the glutathione standard curve equation.

The preparation of 0.01M phosphate buffer solution pH 6.0±0.05 is made in the following way: 1.530 grams of $Na_2HPO_4.2H_2O$ is put into a 1-liter measuring flask. Then, add $CO^2$-free water until the mark. In another measuring flask, a solution of $Na_2HPO_4.2H_2O$ was made through 0.9020 grams of $Na_2HPO_4.2H_2O$ dissolved in $CO^2$-free water and put into a 250 ml measuring flask. After that, add $CO^2$-free water until the mark line. $Na_2HPO_4.2H_2O$ solution was taken as much as 831.9470 ml, while $Na_2HPO_4.2H_2O$ solution was taken as much as 168.0530 ml so that a mixture solution of 1 liter was obtained, which was then measured pH using a pH meter. If the pH is smaller than 6.0, add a solution of 0.01 M $Na_2HPO_4.2H_2O$ until it reaches a pH of 6.0±0.05. 300 ppm glutathione stock solution was made by dissolving 15 mg of glutathione into a pH 6.0±0.05 phosphate solution of 50 ml.

Standard raw solutions are prepared by means of 300 ppm glutathione solution and a dilution series is carried out. Successively dilution of 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml with phosphate buffer solution pH 6.0±0.05, 10.0 ml in a 10.0 ml measuring flask until concentrations of 30 ppm, 60 ppm, 90 ppm, 120 ppm, and 150 ppm were obtained. 0.2 ml of glutathione solution of each concentration was mixed with 2.3 ml of dapar phosphate solution pH 6.0±0.05 and observed spectra to determine the maximum wavelength. Furthermore, the absorption of the raw solution at various concentrations at the maximum wavelength obtained was observed and the regression equation y = bx+a was determined. The glutathione release profile can be seen by making a curve of the relationship between the cumulative amount of glutathione released (µg/$cm^2$) over time.

The determination of the maximum wavelength of glutathione in a solution of 0.01 M phosphate pH 6.0±0.05 was carried out using a working standard solution of concentrations of 60 ppm, 90 ppm, and 120 ppm observed absorption with a UV-Vis spectrophotometer at wavelengths of 250 ppm-500 ppm. Based on the determining results, the maximum wavelength obtained is 405 nm. The results of determining the standard curve can be seen in table 3 below:

**Table 3: Relationship of glutathione concentration to absorption in λ 405**

| Concentration (ppm) | Absorbance |
|---|---|
| 30 | 0.96320 |
| 60 | 0.39966 |
| 90 | 0.54154 |
| 120 | 0.69531 |
| 150 | 0.79209 |
| 180 | 0.97524 |

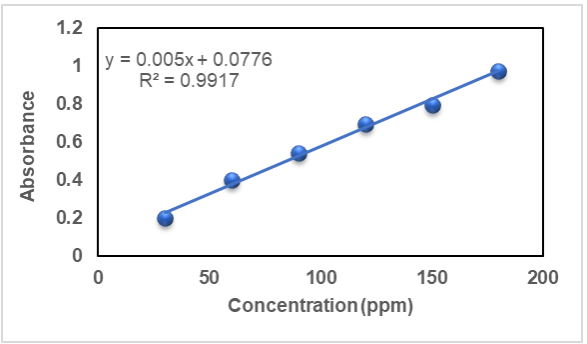


**Fig. 11: Curve of glutathione raw solution in phosphate buffer pH 6**

In this release test, the levels obtained are calculated and determined the cumulative amount released at each time (minutes).

The results of the discharge test examination can be seen in table 4 below

**Table 4: Glutathione-kappa carrageenan nanosphere drug release**

| Time (min) | % Glutathione unleashed | | | |
|---|---|---|---|---|
| | F1 | F2 | F3 | F4 |
| 0 | 0±0 | 0±0 | 0±0 | 0±0 |
| 15 | 9.12±1.23 | 8.98±0.98 | 5.22±2.45 | 6.22±1.28 |
| 30 | 13.11±1.09 | 14.28±1.07 | 10.54±2.11 | 11.54±1.67 |
| 45 | 14.85±1.87 | 15.22±2.19 | 14.98±2.67 | 15.45±1.79 |
| 60 | 21.28±2.11 | 23.58±1.26 | 20.62±1.26 | 22.93±1.25 |
| 90 | 26.15±2.02 | 25.75±1.89 | 27.65±1.22 | 29.45±2.83 |
| 120 | 37.18±1.98 | 36.68±1.56 | 35.28±1.90 | 39.68±1.98 |
| 150 | 42.23±1.65 | 44.23±2.87 | 43.25±2.06 | 45.25±1.20 |
| 180 | 45.82±1.04 | 46.56±0.88 | 45.02±2.24 | 46.22±1.28 |
| 240 | 55.25±1.28 | 55.95±1.65 | 54.94±1.67 | 55.94±1.92 |
| 300 | 56.54±1.33 | 58.77±1.49 | 56.17±1.69 | 58.87±0.91 |
| 360 | 65.77±1.87 | 64.23±2.08 | 62.94±1.44 | 63.54±0.89 |
| 420 | 68.72±2.87 | 67.12±3.11 | 66.91±2.35 | 68.21±2.10 |
| 480 | 73.91±2.17 | 75.91±2.76 | 78.56±2.82 | 79.56±1.34 |

Data were expressed as mean±SD, n=3

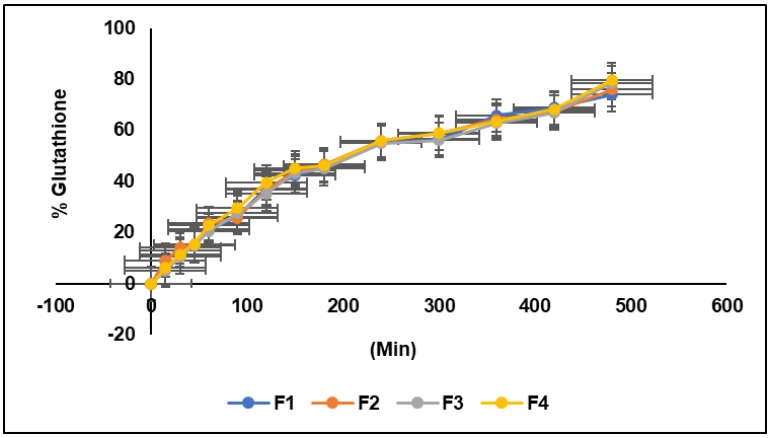


**Fig. 12: Drug release profile of glutathione-kappa carrageenan nanosphere, data were expressed as mean±SD, n=3**

Based on the nanosphere release test profile fig. 12. it can be seen that nanospheres with a lower carrageenan concentration of 0.5% release glutathione faster than nanospheres with a carrageenan concentration of 1%. Higher polymer concentrations will cause the surface of the nanosphere to be thicker, as evidenced by a larger particle size and more dense nanosphere suspension. Kappa carrageean is a polymer for controlled release. In addition, kappa carrageenan combined with poloxamer can form hydrogels of thermosensistive [14].

Nanosphere aforementioned also views shape its morphology from F2 and F4. Test results Morphology get views at picture at below:

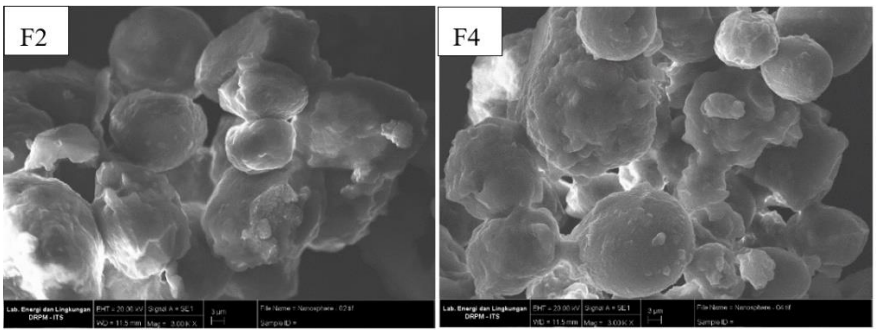


**Fig. 13: Morphology of glutathione-kappa carrageenan nanosphere F2 and F4 at 3000x magnification**

Antioxidant activity can be known by measuring the ability to inhibit oxidation reactions. Metodae is effective because it is simple, easy, and cheap, including DPPH (2,2 diphenyl-1-picrylhydrazyl). 50 mg of nanospheres were dissolved into a 50 ml mixture of methanol: aquades (6:4). The solution was diluted to a concentration series of 10–150 ppm. Then, % inhibition is calculated using the following formula [15]:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100\%$$

The percentage of inhibition obtained is then made a linear relationship with the concentration series to cause a damping of 50%. In this study, IC50 values were obtained at F2 of 196, 253 ppm and F4 of 210.180 ppm. Based on these data, it can be concluded that the Glutathione-Kappa Carrageenan nanosphere has antioxidant activity in the medium category.

## CONCLUSION

Glutathione-Kappa Carrageenan nanosphere was successfully formulated and obtained the appropriate particle size, namely nanoparticles with F1 size 382.67±52.24 nm, F2 325.20±4.62 nm, F3 495.39±30.61 nm, and F4 409.80±4.11 nm. Entrapment efficiency is quite good, ranging from 44.65±0.66-56.20±2.03% with a drug content value of 19.51±0.71-34.49±1.65. The greater the polymer concentration, the greater the value of entrapment efficiency and drug content in the nanosphere. The morphology of the nanosphere is quite good, spherical, with a smooth surface. The release profile shows that glutathione release is quite good but takes a long time, namely F1 73.91±2.17%, F2 75.91±2.76%, F3 78.56±2.82%, and F4 79.56±1.34% in 480 min or 8 h. It shows that the nanosphere system can aim for controlled release or lepping slowly, and the drug will last a long time in the skin tissue so that effectiveness increases. Test of glutathione-Kappa carrageenan nanospheres' antioxidant activity with the DPPH method showed that nanospheres have medium or medium category antioxidant activity.

## ACKNOWLEDGMENT

I would like to express my sincere thanks to Head of Pharmacy Study Program, Hang Tuah University Surabaya for providing invigorative and conductive environment in the college.

## FUNDING

Hang Tuah University Surabaya

## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interest in this research.

## REFERENCES

1. Weschawalit S, Thongthip S, Phutrakool P, Asawanonda P. Glutathione and its antiaging and antimelanogenic effects. Clin Cosmet Investig Dermatol. 2017;10:147-53. doi: 10.2147/CCID.S128339, PMID 28490897.
2. Etnawati K, Adiwinarni DR, Susetiati DA, Sauchi Y, Ito H. The efficacy of skin care products containing glutathione in delivering skin lightening in Indonesian women. Dermatol Reports. 2019;11:8013. doi: 10.4081/dr.2019.8013.
3. Geyik G, Isıklan N. Design and fabrication of hybrid triple-responsive κ-carrageenan-based nanospheres for controlled drug delivery. Int J Biol Macromol. 2021;192:701-15. doi: 10.1016/j.ijbiomac.2021.10.007, PMID 34637816.
4. Lushchak VI. Glutathione homeostasis and functions: potential targets for medical interventions. J Amino Acids. 2012;2012:1-26. doi: 10.1155/2012/736837.
5. Tanusorn N, Thummarungsan N, Sangwan W, Lerdwijitjarud W, Sirivat A. Influence of carrageenan molecular structures on electromechanical behaviours of poly(3-hexylthiophene)/carrageenan conductive hydrogels. Int J Biol Macromol. 2018;118(B):2098-107. doi: 10.1016/j.ijbiomac.2018.07.066, PMID 30009911.
6. M HD, EH, NS. Development of carrageenan polymer for encapsulation of ciprofloxacin HCl: *in vitro* characterization. Int J Drug Deliv Technol. 2019;9(1):89-93. doi: 10.25258/ijddt.9.1.14.
7. Boltnarova B, Kubackova J, Skoda J, Stefela A, Smekalova M, Svacinova P. PLGA based nanospheres as a potent macrophage-specific drug delivery system. Nanomaterials (Basel). 2021;11(3):749. doi: 10.3390/nano11030749, PMID 33809764.
8. Hariyadi DM, Rosita N, Rahayu A. Design, optimization and characterization of glutathione loaded-alginate microspheres for topical antiaging. J Pharm Pharmacogn Res. 2019;7(1):223-33. doi: 10.56499/jppres19.554_7.4.223.
9. Vladar AE, Hodoroaba VD. Characterization of nanoparticles by scanning electron microscopy. Micro Nano Technol. 2020;2:7-27.
10. Shahidi F, Zhong Y. Measurement of antioxidant activity. J Funct Foods. 2015;18:757-81. doi: 10.1016/j.jff.2015.01.047.
11. Zirak MB, Pezeshki A. Effect of surfactant concentration on the particle size, stability and potential zeta of beta carotene nano lipid carrier. Int J Curr Microbiol Appl Sci. 2015;4(9):924-32.
12. Nailufa Y, Widjaja B, Najih YA, Rakhma D. Optimization of kappa carrageenan polymer concentration and potassium chloride crosslinker on physical characteristics of glutathione-kappa carrageenan nanosphere. Int J App Pharm. 2023;15:128-33. doi: 10.22159/ijap.2023.v15s2.24.
13. Postolovic K, Ljujic B, Kovacevic MM, Dordevic S, Nikolic S, Zivanovic S. Optimization, characterization, and evaluation of carrageenan/alginate/poloxamer/curcumin hydrogel film as a functional wound dressing material. Mater Today Commun. 2022;31:103528. doi: 10.1016/j.mtcomm.2022.103528.
14. Dinh L, Hong J, Min Kim D, Lee G, Jung Park E, Hyuk Baik S. A novel thermosensitive poloxamer-hyaluronic acid- kappa-carrageenan-based hydrogel anti-adhesive agent loaded with 5-fluorouracil: a preclinical study in sprague-dawley rats. Int J Pharm. 2022;621:121771. doi: 10.1016/j.ijpharm.2022.121771, PMID 35487401.
15. Sirivibulkovit K, Nouanthavong S, Sameenoi Y. Paper-based DPPH assay for antioxidant activity analysis. Anal Sci. 2018;34(7):795-800. doi: 10.2116/analsci.18P014, PMID 29998961.