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Original Article

OPTIMIZATION OF KAPPA CARRAGEENAN POLYMER CONCENTRATION AND POTASSIUM CHLORIDE CROSSLINKER ON PHYSICAL CHARACTERISTICS OF GLUTATHIONE-KAPPA CARRAGEENAN NANOSPHERE

YUYUN NAILUFA, BAMBANG WIDJAJA*, YULI AINUN NAJIH, DITANURLITA RAKHMA

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

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ABSTRACT

Objective: The objective of the study to obtain the most optimal physical characteristics of glutathione-kappa carrageenan nanosphere in terms of particle size, moisture content, yield, drug loading and entrapment efficiency.

Methods: One of the drug delivery systems that can improve drug stability for antioxidants is nanosphere. Nanospheres were prepared using ionotropic gelation method with aerosol technique. This method is easy, fast, and relatively cost-effective to manufacture. This research was applied with optimization using a randomized full factorial design of 2^2 with differences in kappa carrageenan as polymer and potassium chloride concentrations as crosslinker. This study aims to obtain the most optimal physical characteristics of the nanosphere in terms of particle size, moisture content, yield, drug loading and entrapment efficiency.

Results: The glutathione-kappa carrageenan nanosphere had particle sizes ranging from 247.03 to 675.07 nm with spherical and smooth nanosphere. Furthermore, the entrapment efficiency value ranged from 25.50–35.61%, and drug loading of 6.84–10.16%. The concentration of kappa polymer affected particle size, moisture content, entrapment efficiency, and drug loading. This indicated that higher polymer concentration resulted in greater particle size, moisture content, entrapment efficiency, and drug loading.

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

Keywords: Nanosphere, Glutathione, Kappa carrageenan, Potassium chloride, Ionotropic gelation

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INTRODUCTION

The chemical compound glutathione is a highly potent antioxidant that possesses low bioavailability due to its easy degradation and oxidization. Glutathione is a potent antioxidant and a thiol tripeptide molecule found in human cells, which can be oxidized and damaged during the digestive process [1]. Oral glutathione also has low bioavailability due to its susceptibility to degradation as well as oxidation and can easily be damaged during storage [2]. Therefore, a system is needed to protect glutathione as a pharmaceutical raw material from oxidation during storage and increase its bioavailability when consumed orally. Previous studies have found that encapsulation in form of the nanosphere can improve the stability of glutathione due to its susceptibility to damage during the digestive process.

Nanosphere can act as a drug delivery system by entrapment efficiency the molecules within the interior structure, adsorbing them on the surface, or through covalent binding. Furthermore, it increases bioavailability, improves pharmacokinetics and protects the active drug substances from physical, chemical, or biological degradation, and allows for controlled release [3]. The type of polymer affects the formation of the nanosphere system that can protect active drug substances. The polymer can be derived from natural or synthetic materials. Polymer derived from natural offers several advantages such as renewability, biodegradability, good mechanical properties, and cost-effectiveness [4]. An example of a natural polymer is kappa carrageenan, which has good elasticity properties and forms hydrogels [5]. Therefore, it can optimally trap drugs during nanosphere entrapment. The concentration of kappa carrageenan that can be used for encapsulation processes is 2% [6]. The preparation methods of nanosphere effect their physicochemical characteristics. This makes it necessary to consider several important factors, including the desired particle size, stability of the active ingredient, reproducible release profiles, and the absence of toxic substances in the final product [7]. Meanwhile, ionotropic gelation is a cost-effective method that can produce small particle sizes and easily encapsulate drugs to protect them from the environment [8]. This method involves mixing two aqueous phases to produce an ionic interaction between different charges in both phases [9]. The ionotropic gelation method involves a crosslinker that transitions from liquid to gel due to ionic interaction at room temperature. The crosslinkers used for kappa carrageenan are K^+, Na^+, Rb^+, Cs^+, and Li^+ [10]. When using kappa carrageenan polymer and potassium chloride crosslinkers, the ionotropic gelation method can form stable, elastic, cohesive, transparent gels and produce smooth spherical particles [11].

Nanosphere needs to be dried during production, but the stability of the active ingredient must also be considered. Meanwhile, the freeze-drying technique can be used for drying to prevent the instability of the active ingredient at high temperatures. This research aimed to optimize the concentration of kappa carrageenan polymer and crosslinker to obtain the optimal physical characteristics of the nanosphere system analyzed based on size, polydispersity index (PDI), zeta potential, moisture content, drug loading, entrapment efficiency, and yield.

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), KH_2PO_4 p . a (Merck), Na_2HPO_4 p . a (Merck), and demineralized water (Bratachem, Indonesia).

Methods

In this research, ionotropic gelation with the aerosol technique was used to make the nanosphere. Kappa carrageenan was weighed with a concentration of 0.5% and 1.0%, followed by the addition of 1% glutathione to each formula and stirred using a magnetic stirrer at 1000 rpm. KCl of 0.3% and 0.6% were separately made as crosslinkers in 100 ml solution. The crosslinker solution was sprayed into the glutathione-kappa carrageenan solution, stirred

continuously at 1000 rpm using a magnetic stirrer until it was finished, and stirred for another 3 h at 1000 rpm. The resulting nanosphere was centrifuged for 6 min at 3000 rpm and washed twice with distilled water. The centrifuged nanosphere was filtered and dried using a freeze-dryer for 30 h [11].

Optimization design

The optimization design used was a randomized full factorial design 2^2 with variations in the concentration of the polymer, namely kappa carrageenan and potassium chloride, to obtain optimal nanosphere. This was carried out by observing physical characteristics such as drug loading, entrapment efficiency, particle size, PDI, zeta potential, moisture content, and particle morphology. Meanwhile, storage stability testing was conducted at temperatures of 8, 25, and 40 °C for 90 d.

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.

Zeta potential

The zeta potential was evaluated using the Malvern zeta sizer to determine the surface charge of nanosphere system in the colloid form. The magnitude of the zeta potential indicated the level of colloid stability.

Moisture content

The evaluation of moisture content was necessary to describe the wetness of a sample, as measured by the percentage of water content. The moisture content was determined using a moisture content meter from BEL engineering i-Thermo L Touch Ser. No IT1600760.

Drug loading, entrapment efficiency, and yield

The evaluation of the glutathione drug loading in nanosphere system and entrapment efficiency was carried out 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 nanosphere system was determined by adding 50 mg of glutathione-kappa carrageenan nanosphere to 50 ml of phosphate buffer pH 7.4 and allowed 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 [11].

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

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

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

Morphology

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

RESULTS AND DISCUSSION

Formula optimization

The optimization of the glutathione-kappa carrageenan nanosphere formula was conducted using the randomized full factorial design 2^2 method with variations in the concentration of kappa carrageenan polymer and potassium chloride crosslinker. The concentrations of kappa carrageenan were set at 0.5% and 1%, while that of potassium chloride was set at 0.3% and 0.6%. The details of the formula are presented in table 1**.** The result of physical characteristics as shown in Tables 2 and 3.

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

Material name	Material function	Formula (%)			
		F1	F2	F3	F4
Glutathione	Active ingredient	1	1	1	1
Kappa carrageenan	Polymer	0.5	0.5	1	1
Potassium chloride	Cross linker	0.3	0.6	0.3	0.6
Demineralized water	Solvent	ad 100			

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

Formula	Particle size (nm)	Polydispersity index	Potential zetas (mV)	Moisture content (%)
F1	247.03±25.00	0.630±0.08	-9.81±0.92	2.56±0.27
F2	358.30±43.92	0.500±0.06	-9.35±1.52	2.63±0.23
F3	501.63±2.50	0.560±0.050	-9.19±0.39	4.33±0.41
F4	675.07±114.49	0.640±0.060	-10.82±1.4	4.25±0.55

Data were expressed as mean±SD, n=3

Table 3: Physical characteristics of the glutathione-kappa carrageenan nanosphere

Formula	Drug loading (%)	Entrapment efficiency (%)	Yield (%)
F1	6.84±0.40	25.50±1.46	71.24±2.40
F2	7.02±0.83	25.55±1.15	76.04±3.09
F3	9.31±0.27	35.02±1.55	74.29±1.51
F4	10.16±0.63	35.61±1.46	75.56±3.98

Data were expressed as mean±SD, n=3

Particle size and polydispersity index

The difference in kappa-carrageenan polymer concentration affected the particle size of the nanosphere. The Main Effects Plot in fig. 1 showed that there was a difference in particle size of glutathione-kappa carrageenan nanosphere caused by the variation in kappa-carrageenan polymer concentration. A polymer concentration of 0.5% resulted in smaller average particle sizes, with F1 at 247.03 nm

and F2 at 358.30 nm. Meanwhile, a polymer concentration of 1.0% led to larger average particle sizes, with F3 at 501.65 nm and F4 at 675.07 nm. Formula with 1% Kappa carrageenan resulting larger particle size compare to 0.5%. and Formula with 0.6% KCl, resulting larger particle size compare to 0.3%, it is proven that the higher concentration of polymers and crosslinkers, the larger particle size. However, there was no effect on the polydispersity index due to changes in polymer or crosslinker concentration, as presented in fig. 2. This was because kappa carrageenan polymer swells when interacting with water, leading to an increase in moisture content. Hariyadi *et al.*, (2019) stated that when the concentration of polymer increases, the viscosity and particle size will also increase [11].

Potential zetas

Based on the multilevel factorial design statistical analysis results on a complete randomized design with the independent variables of KCl and kappa carrageenan polymer concentrations, it was found that each p-value (sig) was 0.419 and 0.550(>0.005), as presented in fig. 3.

Moisture content

The stability of particles can be affected by moisture content and cause microbial growth. Therefore, moisture content in nanoparticle should be less than 10%. In this research, the percentage of moisture content in nanosphere ranged from 2.56% to 4.33%. The high percentage occurred due to the ineffective or insufficient drying process. The variable that affected the percentage of moisture content according to the results of multilevel factorial design analysis was the polymer concentration, such as kappa carrageenan, as indicated by the p-value of 0.000 (<0.005). The Main Effects Plot graph showed that at a polymer concentration of 0.5%, the moisture content of glutathione-kappa carrageenan nanosphere was smaller compared to a concentration of 1.0%. Fig. 4 showed that a higher concentration of kappa carrageenan will lead to a greater moisture content of nanosphere. This was because kappa carrageenan can attract and trap water; hence, the higher the concentration of kappa carrageenan, the more water will enter the polymer. High moisture content in the granule, namely nanosphere, will cause difficulty in powder flowability due to interparticle cohesion [12].

Drug loading

The drug content in nanosphere is crucial because it provides therapeutic effects against disease. Glutathione-kappa carrageenan nanosphere contains drugs ranging from 6.84-10.16%. The statistical analysis of a multilevel factorial design of a complete randomized design with independent variables of KCl and kappa carrageenan polymer concentrations resulted in p-values (sig) of 0.160 (>0.005) and 0.000 (<0.005), respectively. This indicated a significant effect on drug loading when the concentration of kappa carrageenan was increased from 0.5% to 1.0%.

However, the increase in crosslinker concentration from 0.3% to 0.6% did not affect drug loading. The interaction between crosslinker and kappa carrageenan did not affect the entrapment efficiency with a p-value (sig) of 0.347, as presented in fig. 5. Drug loading is the drug content, specifically glutathione in the glutathione-kappa carrageenan nanosphere. The drug loading percentage can be measured using a UV spectrophotometer following the Lambert-Beer law at a maximum wavelength (range 200-500 nm).

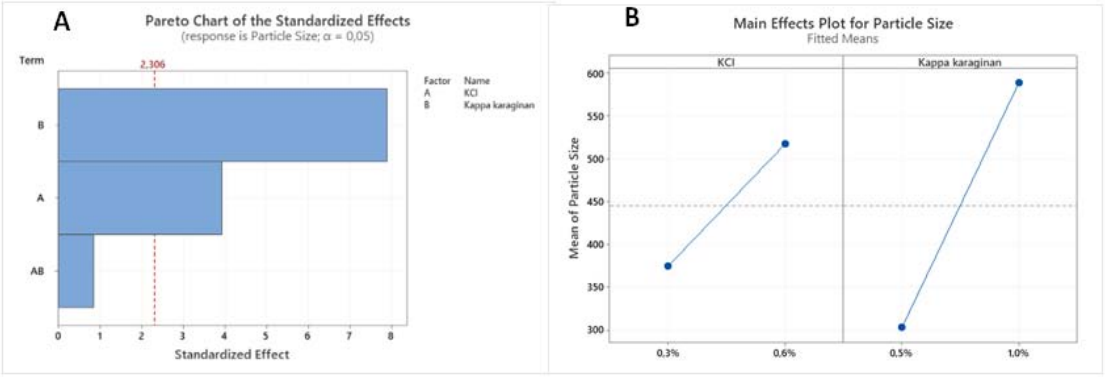

Fig. 1: The effect of independent variables on particle size in pareto chart (A) and main effect plot (B). The main effect plot for particle size (fig. 1B) showed that particle size of glutathione in prepared nanospheres increased with increasing kappa carrageenan and KCl increased. The same results were obtained through pareto chart (fig. 1A), which illustrates the effect of two variables on particle size.

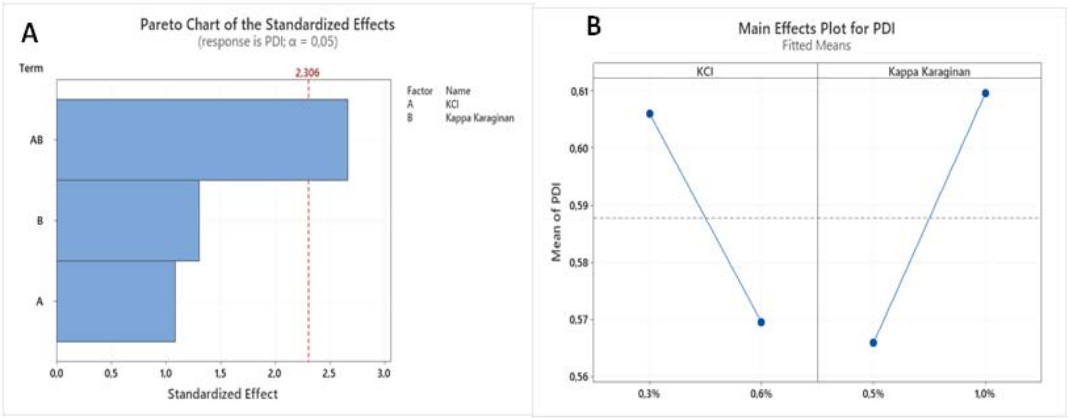

Fig. 2: The effect of independent variables on PDI on pareto chart (A) and main effect (B). The main effect plot for PDI (fig. 2B) showed that PDI of glutathione in prepared nanospheres increased with increasing kappa carrageenan and KCl increased. The same results were obtained through pareto chart [fig. 2A], which illustrates the effect of two variables on PDI

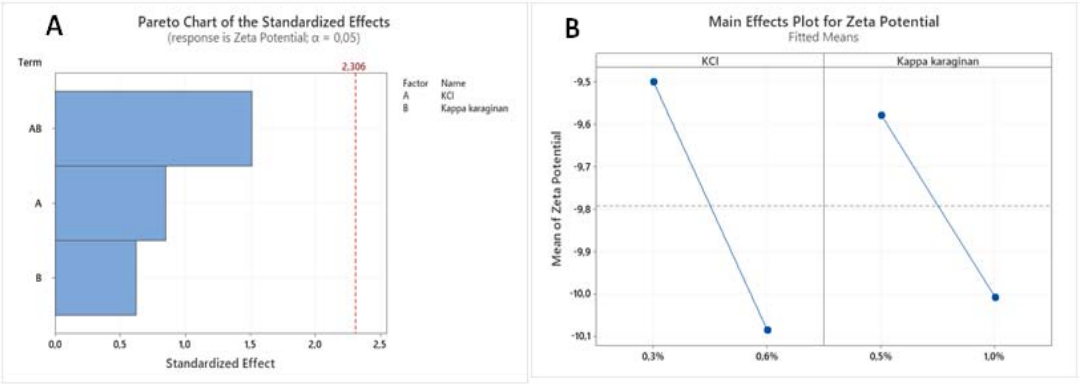

Fig. 3: The effect of independent variables on zeta potential in pareto chart (A) and main effect (B). The pareto chart for the zeta potential [fig. 3A] showed that the zeta potential of glutathione in prepared nanospheres increased with increasing kappa carrageenan and KCl but resulted no significant differences between formulas (p>0.05) because does not cross the red line

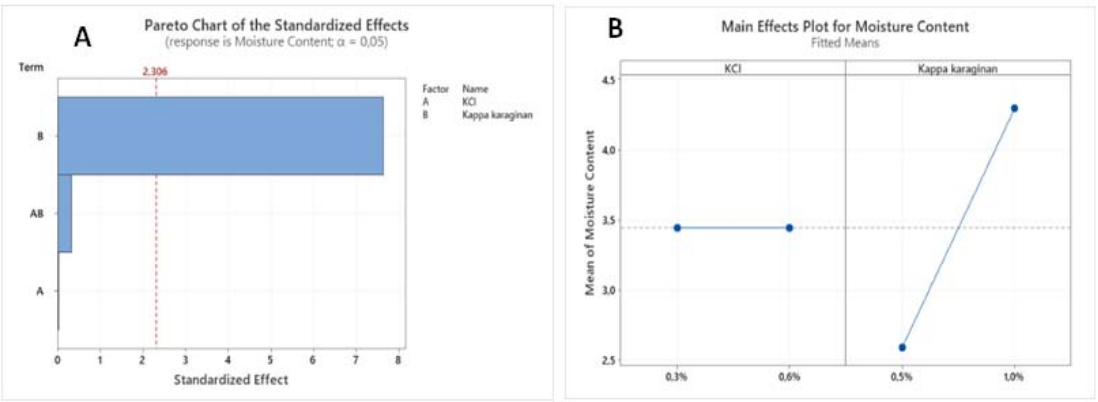

Fig. 4: The effect of independent variables on moisture content in pareto chart (A) and main effect (B). The main effect plot for the moisture content [fig. 4B] showed that the moisture content of glutathione in prepared nanospheres increased with increasing kappa carrageenan. The same results were obtained through pareto chart [fig. 4A], which illustrates the effect of kappa carrageenan variables on moisture content

Entrapment efficiency

Fig. 6 showed that the multilevel factorial design of a completely randomized design with the independent variables of KCl and kappa carrageenan polymer concentrations obtained p-values (sig) of 0.711 (>0.005) and 0.000 (<0.005), respectively. This indicated a significant effect on the entrapment efficiency when increasing the concentration of kappa carrageenan from 0.5% to 1.0%. However, increasing the concentration of the crosslinker from 0.3% to 0.6% did not affect the entrapment efficiency. The interaction between crosslinker and kappa carrageenan had no significant effect on the entrapment efficiency, with a p-value of 0.749. The percentage of entrapment efficiency ranges from 25.50% to 35.61%. Formulas F1 and F2 had almost the same results, with an average entrapment efficiency of 25.50% and 25.55%, respectively, due to their identical kappa carrageenan concentration, which was 0.5%, as indicated by the multilevel factorial design. The Main Effects Plot also revealed that there were differences in the entrapment efficiency of glutathione-kappa carrageenan nanosphere due to variations in the concentration of kappa carrageenan polymer. At a polymer concentration of 1.0%, formulas F3 and F4 resulted o entrapment efficiency of 35.02% and 35.61%, respectively. The higher the polymer concentration, the greater the percentage of entrapment efficiency of glutathione-kappa carrageenan nanosphere, as more drug was trapped within the polymer [11].

The results showed high entrapment efficiency in formulas F3 and F4 with 1% kappa carrageenan concentration. This indicated that higher polymer concentration can cause more active drug ingredients to be trapped in the polymer. Furthermore, a higher crosslinker concentration in F4 led to greater entrapment efficiency because the polymer and the crosslinker effectively bound and strengthened the bonds formed in nanosphere system, reducing the likelihood of drug leakage.

Yield

The yield obtained was 71.24-75.56%, which was in a good category and worth developing. This indicated that the ionotropic gelation method used, with 0.5% and 1.0% kappa carrageenan as the polymer and the addition of KCl as a crosslinker at 0.3% and 0.6% was relatively good. The aerosol method was used for manufacturing, where the carrageenan and glutathione solution was sprayed with a spray nozzle into the KCl solution. The droplets from the spray nozzle bonded with KCl and formed particles, which were stirred at 1000 rpm for 2 h to form nanoparticle. The multilevel factorial design analysis of the completely randomized design with independent variables of KCl concentration and kappa carrageenan concentration yielded p-values (sig) of 0.108 and 0.465 (>0.005), respectively, indicating no significant effect on yield. The interaction between both variables had a p-value of 0.323 (>0.005), which also indicated no significant effect on the yield, as illustrated in fig. 7.

Morphology

The Scanning Electron Microscope (SEM) is an electron microscope type used to obtain information on a nanomaterial's morphology, texture, and topography. The SEM uses a focused beam of high-energy electrons to produce various signals from the surface of nanomaterial. The advantage of this technique is that it directly measures the particle size of particles without being affected by

factors such as temperature and refractive index. Based on the morphology examination, all four formulas appeared to have spherical particles. However, their surfaces still need to be smoother, as there were visible scratches on the particle surfaces due to the vacuum process during the freeze-drying method. The spherical and round shape can improve flow properties, increase particle stability, and reduce the likelihood of particle agglomeration.

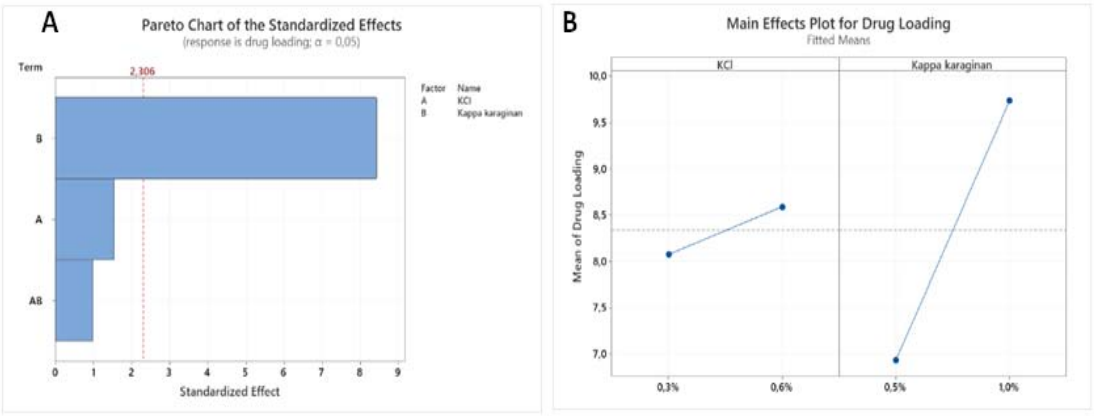

Fig. 5: The effect of independent variables on drug loading or drug content on pareto chart (A) and main effect (B). The main effect plot for the drug loading [fig. 5B] showed that the drug loading of glutathione in prepared nanospheres increased with increasing kappa carrageenan. The same results were obtained through pareto chart [fig. 5A], which illustrates the effect of kappa carrageenan variables on drug loading

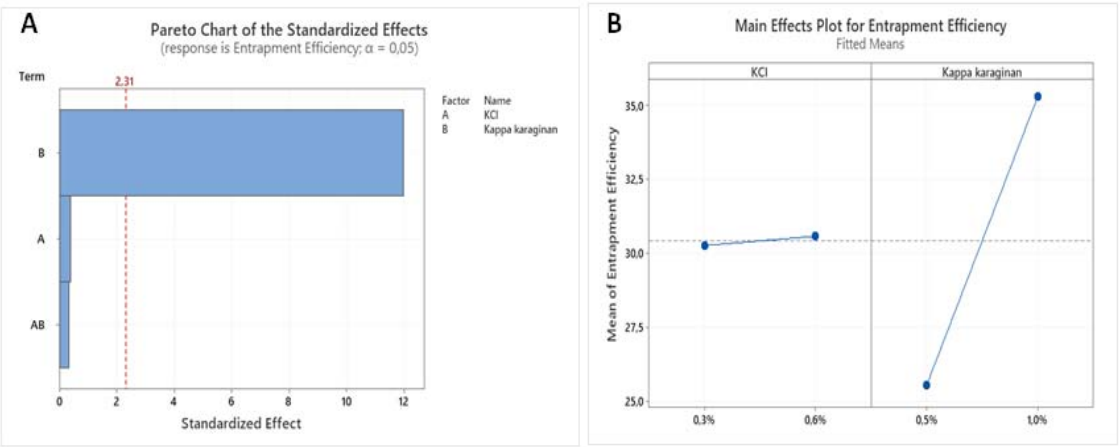

Fig. 6: The effect of independent variables on entrapment efficiency in pareto chart (A) and main effect (B). The main effect plot for the entrapment efficiency [fig. 6B] showed that the entrapment efficiency of glutathione in prepared nanospheres increased with increasing Kappa carrageenan. The same results were obtained through pareto chart [fig. 6A], which illustrates the effect of kappa carrageenan on entrapment efficiency

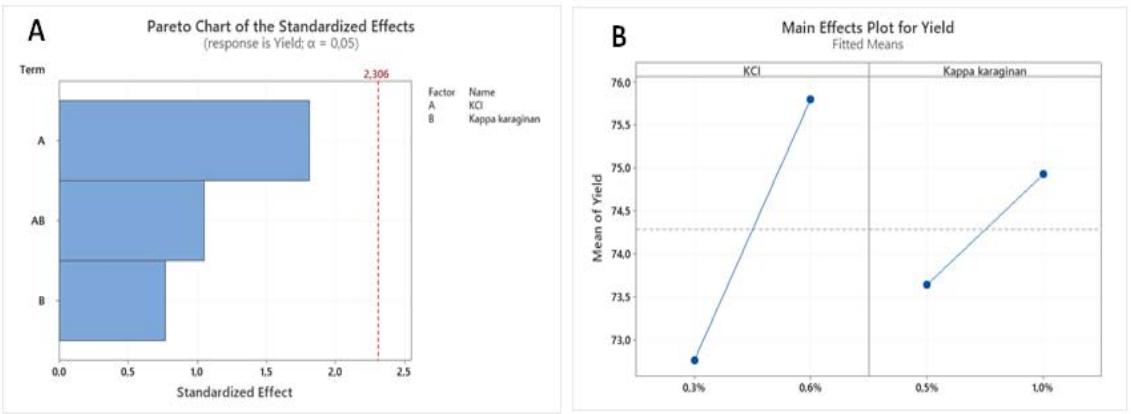

Fig. 7: The effect of independent variables on the yield on the pareto chart (A) and main effect (B). The main effect plot for yield [fig. 7B] showed that the EE of glutathione in prepared nanospheres increased with increasing kappa carrageenan and KCl increased. The same results were obtained through pareto chart [fig. 7A], which illustrates the effect of two variables on yield

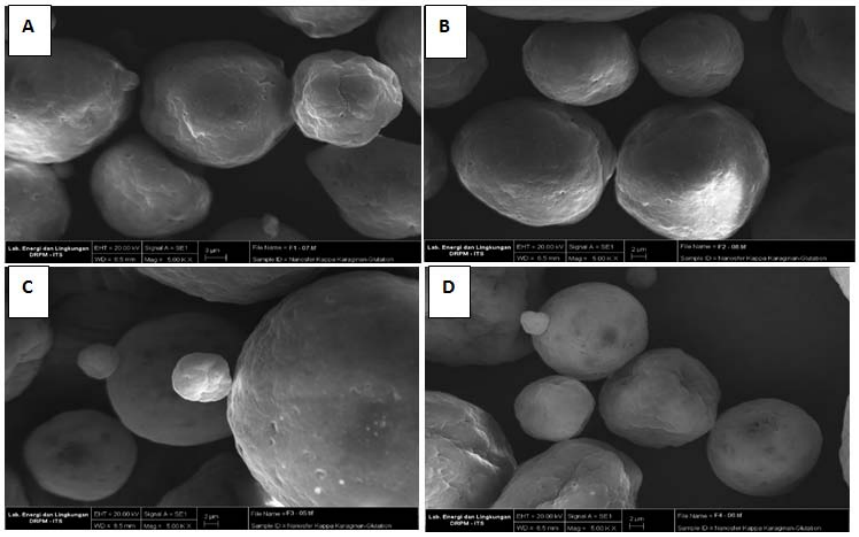

Fig. 8: Morphology of glutathione-kappa carrageenan nanosphere (A) F1, (B) F2, (C) F3, and (D) F4

CONCLUSION

The optimization of glutathione-kappa carrageenan nanosphere production using a variety of polymer and crosslinker concentrations through ionotropic gelation aerosol technique successfully produced a spherical nanosphere with nano-size ranging from 247.03-675.07 nm. The values of entrapment efficiency ranged from 25.50-35.61%, with a drug loading of 6.84-10.16%. The most optimal formula is F4 with 1% kappa-carrageenan concentration and 0.6% KCI. This study showed that to improve the entrapment efficiency and drug loading, it is necessary to optimize the formula by increasing the polymer concentration. The stirring speed and time are important factors to obtain smaller particle sizes. However, there is the possibility of changes in pressure due to the less tightness of the spray nozzle cover used during spraying.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

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

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