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Design, optimization and characterization of glutathione loaded-alginate microspheres for topical antiaging

[Diseño, optimización y caracterización de microesferas de glutatión cargado de alginato para el antienvejecimiento tópico]

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Abstract

Context: Glutathione in the reduced form (GSH) is the predominant intracellular form, which acts as a strong antioxidant. However, it has low skin permeability due to the high hydrophilicity. Hence, the objective of this study was to prepare GSH by using microspheres delivery system and adding surfactant to overcome the barrier function of the skin.

Aims: To investigate the effect of polymer and surfactant on the characteristics and release profile of GSH–alginate microspheres.

Methods: GSH-alginate microspheres were prepared using ionotropic gelation method by aerosolisation. A randomized full factorial design was applied to prepare four different formulations of glutathione loaded alginate microspheres. Design was applied for all formulations to study about effect of independent variables of polymer and crosslinker on the entrapment efficiency (EE), drug loading (DL), particle size, yield, and *in vitro* drug release profile. For release study, microspheres formulas were also compared to microspheres, which applied into gel base.

Results: The GSH-alginate microspheres had a high EE ranging from 34.74 ± 0.07% to 56.63 ± 0.36%, with small particle sizes ranging from 1.89 ± 0.03 µm to 2.42 ± 0.08 µm, and drug loading ranging from 5.72 ± 0.05% to 6.23 ± 0.02%. The kinetic analysis of all release profiles was found to follow Higuchi's diffusion model. EE, DL, particle size, and yield variables had a significant effect on the dependent variables ($p<0.05$), and flux had no significant effect on the dependent variables ($p>0.05$).

Conclusions: All formulas produced high yield and encapsulation efficiency and small size particles. From the 2^2 randomized full factorial design, there was showed that the combination of the use of surfactant and polymer concentration significantly affected DL and EE.

Keywords: characteristics; design; glutathione-alginate microspheres; release profile; surfactant.

Resumen

Contexto: El glutatión en la forma reducida (GSH) es la forma intracelular predominante, que actúa como un fuerte antioxidante. Sin embargo, tiene una baja permeabilidad de la piel debido a la alta hidrofilicidad. Por lo tanto, el objetivo de este estudio fue preparar GSH mediante el uso de un sistema de suministro de microesferas y agregar surfactante para superar la función de barrera de la piel.

Objetivos: Investigar el efecto del polímero y el surfactante sobre las características y el perfil de liberación de las microesferas de alginato de GSH.

Métodos: Se prepararon microesferas de alginato de GSH usando un método de gelificación ionotrópica por aerosolización. Se aplicó un diseño factorial completo al azar para preparar cuatro formulaciones diferentes de microesferas de alginato cargadas con glutatión. El diseño se aplicó a todas las formulaciones para estudiar el efecto de las variables independientes de polímero y reticulante en la eficiencia de atrapamiento (EE), la carga del fármaco (DL), el tamaño de partícula, el rendimiento y el perfil de liberación del fármaco *in vitro*. Para el estudio de liberación, las fórmulas de microesferas también se compararon con las microesferas que se aplicaron en la base de gel.

Resultados: Las microesferas de alginato de GSH tenían una EE alta en el rango de 34,74 ± 0,07% a 56,63 ± 0,36%, con pequeños tamaños de partículas que variaron de 1,89 ± 0,03 µm a 2,42 ± 0,08 µm, y la carga de fármaco varió de 5,72 ± 0,05% a 6,23 ± 0,02%. Se encontró que el análisis cinético de todos los perfiles de liberación sigue el modelo de difusión de Higuchi. Las variables de EE, DL, tamaño de partícula y rendimiento tuvieron un efecto significativo en las variables dependientes ($p<0,05$), y el flujo no tuvo un efecto significativo en las variables dependientes ($p>0,05$).

Conclusiones: Todas las fórmulas produjeron alto rendimiento y eficiencia de encapsulación y partículas de pequeño tamaño. A partir del diseño factorial completo aleatorizado 2^2, se demostró que la combinación del uso de surfactante y la concentración de polímero afectó significativamente a DL y EE.

Palabras Clave: características; diseño; microesferas de glutatión-alginato; perfil de liberación; surfactante.

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INTRODUCTION

Glutathione (γ-glutamyl cysteinyl glycine) is a small, low-molecular weight, water-soluble thiol-tripeptide formed by three amino acids (glutamate, cysteine and glycine) (Kern et al., 2011). It is a ubiquitous compound with a biologically active sulfhydryl group contributed by the cysteine moiety that acts as the active part of the molecule (Watanabe et al., 2014). This sulfhydryl group allows for interaction with a variety of biochemical systems, hence the abbreviation "GSH" for its active form. Glutathione is one of the most active antioxidant systems in human physiology (Murray, 2009). However, the biomedical applications of glutathione remain limited due to relatively short half-life, labile properties, and rapid metabolism and elimination (Johnson et al., 2012). Nugrahaeni et al. (2018) studied the coefficient of glutathione partition with the addition of various surfactants of various HLBs approaching 2-3 according to the Log P skin with addition of HLB 7 surfactant which is a mixture of Span 80 and Tween 80. From the results of previous study, glutathione with the addition of HLB 7 surfactant was then formulated into alginate microspheres. Glutathione with surfactant penetration test results can decreased MMP-1 expression therefore it seems potential to use as a topical agent (Nugrahaeni et al., 2018). Glutathione has low oral bioavailability due to the action of the glutamyl transpeptidase (GGT) enzyme resulting in low absorption of the gastrointestinal tract when administered intravenous glutathione in the blood circulation. One attempt to avoid the first bait effect is to use a topical route.

Microspheres need to be optimized to control drug release into the skin and ensure that the drug remains localized at the on site of action and did not enter into the systemic circulation (Badilli et al, 2011). They acted as reservoir releasing an active ingredient during a prolonged period of time maintaining effective drug concentration in the skin and at the same time, reducing undesirable side effects (Basarkar et al., 2013). Microspheres can penetrate the skin and resolve the barrier of the stratum corneum by invasion through the intercellular lipid, they move according to the osmotic gradient from stratum corneum layer to a deep hydrated layer. The existence of the surfactant in their system helps in solubilising the lipid in stratum corneum and permits high penetration of microspheres (Aljaeid and Hosny, 2016).

Alginate is natural polysaccharides consisting of guluronic and mannuronic acid units. Sodium alginate have shown many uses in biomedical and pharmaceutical applications due to their low cost, low toxicity, biocompatibility and biodegradability. Alginate microspheres have been widely used as carriers for the controlled release of active agents due to their low immunogenicity and their mucoadhesive properties (Lee and Mooney, 2012). Divalent cations, e.g. Ca^{2+}, are frequency used for the purpose of ionic cross-linking to reduce the dissolution of the alginate matrix for many applications. Ca^{2+} ions are located between electronegative alginate molecules, like eggs in an egg-box. This is known as the 'egg-box' model (Islam et al., 2013). The aim of this study was to formulate and evaluate glutathione-alginate microspheres to enhance skin penetration and increase antiaging activity.

MATERIAL AND METHODS

Materials

Reduced L-glutation-≥ 98.0% (Sigma-Aldrich Inc); sodium alginate pharmaceutical grade (Sigma-Aldrich Inc); $CaCl_2.2H_2O$ pharmaceutical grade (Solvay Chemicals International); 5,5-dithiobis- (2-nitrobenzoicacid) (DTNB) Ellman's reagent (Sigma-Aldrich); sodium citrate pharmaceutical grade (Weifang Ensign Industry Co. Ltd.); Tween 80 (Merck); Span 80 (Merck); $NaH_2PO_4.2H_2O$ (Merck); $Na_2HPO_4.12H_2O$ (Merck).

Experimental design for optimization

A 2^2 randomized full factorial design was used in this study. In this design 2 factors were evaluated. Optimization of formulation ingredient was done in order to determine the values of

addition of surfactants (X1) and polymer concentration (X2) as shown in Table 1, which required getting an optimized formula with optimum values of drug loading (Y1), entrapment efficiency (Y2), particle size (Y3), flux of drug released profile (Y4) and yield (Y5) using Minitab version 16. A randomized full factorial design was applied to prepare four different formulations of glutathione loaded alginate microspheres.

Preparation of glutathion loaded alginate microparticles microspheres

Glutathione (GSH) of 2 g was dissolved in PBS (phosphate buffer solution) pH 6 ± 0.05 then surfactant (mixture of Tween 80 and Span 80) HLB 7 was added. The alginate-glutathione solution was sprayed into cross linking agent solution ($CaCl_2$) and was stirred at 1000 rpm for 2 hours. Microspheres were washed by centrifugation at 2500 rpm for 6 minutes and washed twice using distilled water. Glutathione-loaded alginate microspheres were then collected and freeze dried at -80°C for 29 hours. Formulas of glutathione-alginate microspheres were showed in Table 2.

Determination of entrapment efficiency

Glutathione-alginatemicrospheres of 120 mg was added into 50 mL sodium citrate 0.1 M, then it was stirred at 1000 rpm for 7 h to allow the separation the entrapped drug from the un-trapped drug, and was then analyzed spectrophotometrically at 407 nm using UV spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The percentage of EE of glutathione in the microspheres was calculated applying the following equation (1)(Trivedi et al., 2008).

$$EE = \frac{\text{Amount of Entrapped Glutathione}}{\text{Total Amount of Glutathione}} \times 100 \quad [1]$$

Determination of drug loading

To calculate drug loading, microspheres equivalent to 50 mg of drug were accurately weighed. The dried microspheres were dissolved in 10 mL methanol and volume was made up to 100 mL with water and sonicated for 15 min and kept overnight for 24 hours to extract the drug from microspheres. This resulting solution was then filtered through whatmann filter paper. Then 1 mL of this solution was withdrawn and diluted to 10 mL with water. The absorbance of resulting solution was measured using UV spectro-photometer against water as a blank (Venkatesan et al., 2011).

$$\text{Drug Loading} = \frac{\text{Amount of Entrapped Glutathione}}{\text{Total Amount of Dried Microspheres}} \times 100 \quad [2]$$

Determination of particle size

The particles were analyzed using an optical microscope (OPTILAB Viewer 2.2 by Micronos Nusantara, Indonesia). Prepared microspheres were placed in a glass slide and the mean microspheres size was calculated by measuring 300 microspheres using a calibrated ocular micrometer (Sinko, 2008).

Table 1. Formulation factors for the multilevel factorial design.

Independent factors	**Low**	**High**
X1 = addition of surfactans	Without surfactant tween 80 and span 80	With surfactant tween 80 and span 80
X2 = concentration of polymer	1.5% sodium alginate	2.5% sodium alginate
Dependent variables		**Goal**
Y1 = Entrapment Efficiency (EE%)		Maximize
Y2 = Drug Loading (DL%)		Maximize
Y3 = Particle Size		Maximize
Y4 = Yield (Y%)		Maximize
Y5 = Flux (µg/cm²/h)		Maximize

Table 2. Formula of glutathione-alginate microspheres.

Compound	Function	Concentration of compound			
		I	II	III	IV
Glutathione (g)	Active compound	0.5	0.5	-	-
Glutathione + surfactant HLB 7 (g)	Active compound	-	-	0.5	0.5
Alginate (%)	Polymer	1.5	2.5	1.5	2.5
$CaCl_2$ solution (M)	Crosslinker	1	1	1	1

Yield

Yield was calculated by procentage of total microspheres (grams) divided by total amounts of polymer and, surfactant, glutathione (grams) (Surini et al., 2009).

$$\text{Yield} = \frac{\text{Total Weight of Microspheres}}{\text{Total Weight of Drug and Polymer}} \times 100 \quad [3]$$

Surface morphological and topographic study

Surface morphology and shape of microspheres was studied using scanning electron microscopy (SEM), Model Carl Zeiss MA10, USA at suitable magnification at room temperature. The microphotographs were observed for morphological characteristics and to confirm spherical nature of the microspheres.

Preparation of GSH-alginate microspheres-loaded gel formulation

GSH microspheres (120 mg) was formulated using 1% carbopol 93 as a gelling agent. The microspheres dispersion was added with continous stirring to allow homogeneous distribution of GSH microspheres within the gel base. The dispersion was neutralized by addition of triethanolamine. Then the amount of added base was controlled was controlled to adjust the pH of preprared gel to pH 6.5 using pH meter, and the total weight was adjusted to 100 g using distilled water.

In vitro release of glutathione-alginate microspheres

The release testing of the glutathione loaded alginate microspheres in the gel-based and without gel-based was done using Franz's diffusion cell apparatus, and the results were analyzed by ANOVA using SPSS software (version 20). The experiment was done in triplicate and with measurement of means and standard deviations. The amount of GSH permeated was determined by spectrophotometric analysis at a wavelength of 407 nm against PBS pH 6.0 ± 0.05 (1:1 v/v) as a blank.

RESULTS AND DISCUSSION

Preparation of GSH-alginate microspheres

Glutathione-alginate microspheres were prepared and designed for a 2^2 randomized full factorial design to get optimized formula and study the effect of independent variables. Two different independent variables were used, which include: Addition of surfactans (X1), and concentration of polymer (X2) (Table 2). The independent variables were analyzed using Minitab 16 and four different formulations were obtained, as represented in table. All formulations were prepared using the ionotropic gelation technique and was then evaluated for entrapment efficiency, drug loading, particle size, yield, *in vitro* drug release profile.

Entrapment efficiency of GSH -alginate microspheres (Y1)

As shown in Table 3, it was found that the prepared GSH-alginate microspheres exhibited a good EE with values ranging from (34.74 ± 0.07%) for F1 to (56.63 ± 0.36%) for F4. Fig. 1 illustrated the effect of X1 dan X2 on the EE of GSH using Minitab plus software. As shown on the Pareto chart (Fig. 1A), X1, and X2 have significant effects on the entrapment efficiency, with p values of

0.000 and 0.000, respectively. The linier regression models for the EE% of GSH microspheres are represented in equation (4) as obtained from a randomized full factorial design study.

$$Y1 = 47.2 + 6.27\ X1 + 4.68\ X2 \quad [4]$$

The main effect plot for the EE (Fig. 1B) showed that the EE of GSH in prepared microspheres increased with increasing X1, and X2 increased. The same results were obtained through pareto chart (Fig. 1A), which illustrates the effect of two variables on EE.

The EE was increased in the case of concentration 2.5% of polymer alginate, as compared to 1.5% of polymer alginate, respectively. The entrapment efficiency of F4 formulation was higher as compared to other formulation. Overall, drug entrapment was found increasing with increased in polymer concentration due to its higher viscocity. These results are in good agreement with those of Bagade et al. (2013) who reported that the EE of praziquantel loaded microspheres was higher in case of drug: alginate polymer ratio 1:9 than in case of drug: alginate polymer ratio 1:3 (Bagade et al., 2014).

Drug loading (Y2)

The drug loading of GSH prepared microspheres was found in the range from (5.72 ± 0.05%) for F1 to (6.23 ± 0.02%) for F4 as shown in Table 3. As shown on the pareto chart (Fig. 2A), X1, and X2 have significant effects on the drug loading (DL), with p values of 0.000 and 0.000 respectively. The linier regression models for the DL of GSH microspheres are represented in equation (5) as obtained from a randomized full factorial design study.

$$Y2 = 6.03 + 0.165\ X1 + 0.0917\ X2 \quad [5]$$

The main effect plot for the DL (Fig. 2B) showed that the DL of GSH in prepared microspheres increased with increasing X1, and X2 increased. The same results were obtained through pareto chart (Fig. 2A), which illustrates the effect of two variables on DL. The drug loading of F4 formulation was higher as compared to other formulations. It was found that with increasing the drug-to polymer, the drug loading was increased. Theoretical concentration of drug in microsphere was evaluated to be 5.72 ± 0.05%, 6.00 ± 0.03%, 6.12 ± 0.05%, and 6.23 ± 0.02% w/v, respectively for F1 to F4. Actual drug loading increased as the theoretical drug loading increased, which is shown in Table 1. The amount of drug remaining and available for encapsulation increased as the theoretical drug loading increased. Consequently, the actual drug loading increased. As the molecular weight of the polymer increased, its hydrophobicity increased, leading to better precipitation of polymer at the boundary phase of the droplets (Trivedi et al., 2008).

Table 3. The designed formulations of GSH-alginate microspheres.

Formula	GSH (mg)	Surfactant (X1)	Polymer concentration (X2)
F1	500	-1	-1
F2	500	-1	1
F3	500	1	-1
F4	500	1	1

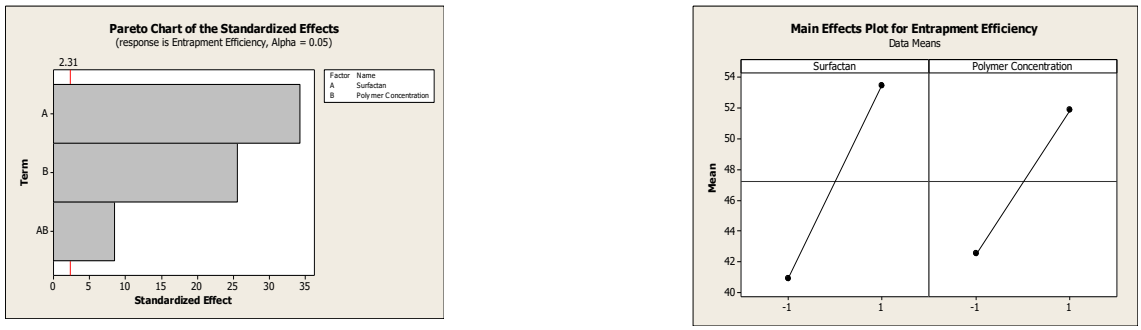

Figure 1. The effect of independent variables on the EE (Y1) including the Pareto chart **(A)**, and main plot effect **(B)**.

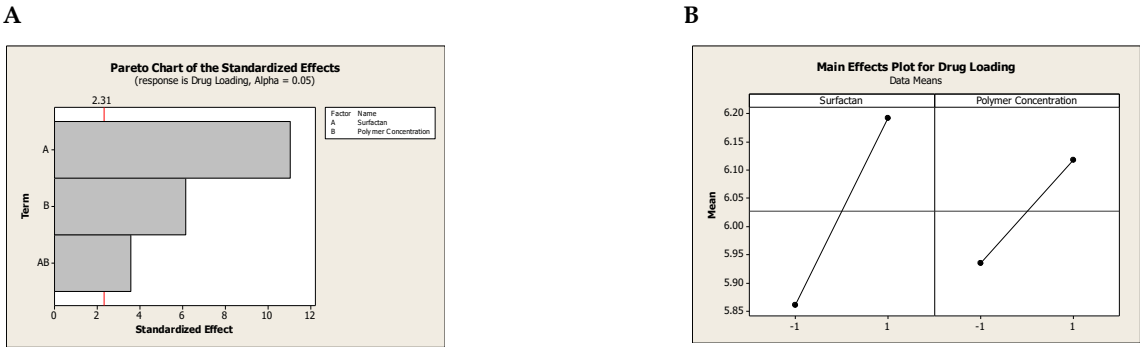

Figure 2. The effect of independent variables on the DL (Y2) including the Pareto chart **(A)**, and main plot effect **(B)**.

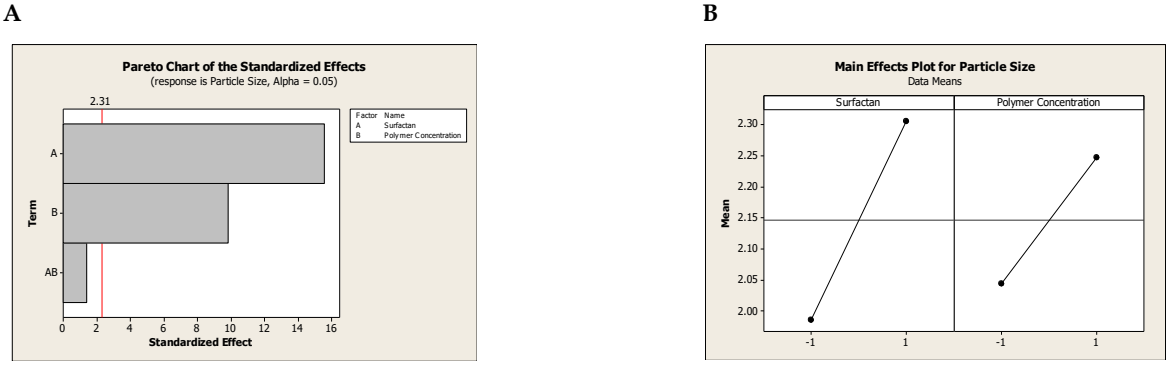

Figure 3. The effect of independent variables on the particle size (Y3) including the Pareto chart **(A)**, and main plot effect **(B)**.

Particle size analysis (Y3)

The particle size range for microspheres was found to be 1.89 ± 0.03 µm to 2.42 ± 0.08 shown in Table 3. As shown on the Pareto chart (Fig. 3A), X1, and X2 have significant effects on the particle size, with p values of 0.000; and 0.000, respectively. The linier regression models for the particle size of GSH microspheres are represented in equation (6), as obtained from a randomized full factorial design study.

$$Y3= 2.15 + 0.159\ X1 + 0.101\ X2 \quad [6]$$

The main effect plot for the particle size (Fig. 3B) showed that the particle size of GSH in alginate microspheres increased with increasing X1, and X2 increased. The same results were obtained through Pareto chart (Fig. 3A), which illustratedthe effect of two variables on particle size. Here, keeping drug ratio constant and varied polymer ratio as the polymer concentration increases viscosity, which influenced the interaction between disperse phase and dispersion medium that affects the size distribution of particle. If there was an increase in the amount of polymer concentration, there was an increase in relative viscosity so as a result increasedin mean particle size (Trivedi et al., 2008). From the results of particle size, it was found that all prepared GSH microspheres have a particle size less than 3 µm, and as such are effective for transdermal applications. It was noticed that the particle size of the microspheres increased with addition of surfactant (tween 80 and span 80) and this may be due to high viscosity of surfactants which increased the droplet size and results in increase in particle size.

Yield (Y4)

The percentage yield of the different formulations was found in the range of 88.80 ± 1.41% to 89.13 ± 0.09%, which is depicted in Table 3. As shown on the pareto chart (Fig. 4A), X1, and X2 have significant effects on the particle size, with p values of 0.018; and 0.046, respectively. The linear regression models for the particle size of GSH microspheres are represented in equation (7) as obtained from a randomized full factorial design study.

$$Y4 = 89.5 + 0.786\ X1 - 0.623\ X2 \quad [7]$$

From analysis of percentage of yield, loading, and encapsulation efficiency of glutathione-alginate microspheres, it was observed that as the polymer concentrationin the formulation increased, the yield also increased. The low percentage of yield in some formulation may be due to microspheres lost during the washing process. Percentage yield of all formulations was varies from 88.80 ± 1.41% to 89.13 ± 0.09%, the best formulation was F4 as given in Table 3.

Morphology study

The sustained release microspheres of glutathione prepared by ionotropic gelation (aerosolization method) were found to be almost spherical. SEM was performed on the prepared glutathione microspheres to access their surface and morphological characteristics as shown in Fig. 5. Formula F4 showed the smoothest and spherical morphology.

In vitro drug release study (Y5)

The flux of the different formulations was found in the range of 0.021 ± 0.002 µg/cm²/h to 0.008 ± 0.002, which is depicted in Table 4 and Fig. 6. The linier regression models for the flux of GSH microspheres are represented in equation (8) and flux of GSH microspheres-based gel in equation (9) as obtained from a randomized full factorial design study (Fig. 7). Flux resulted no significant differences between formulas ($p>0.05$).

$$Y5 = 0.0201 - 0.00167\ X1 - 0.00100\ X2 \quad [8]$$

$$Y5 = 0.0167 + 0.00239\ X1 - 0.00925\ X2 \quad [9]$$

The maximum *in vitro* release was evaluated to be 70.59 ± 0.03% over a period of 10 hours for formulation F1. The drug release was decreased with increasing alginate concentration. This may be due to increase in viscosity, which will increase the particle size and decrease the surface area. An increase in viscosity may also increased the diffusional path length, which might also be the reason for reduction in drug release. All the tested formulations of F1, F2, F3, F4, and all formulas-based gel provided good fit to the Higuchi model. According to this model, the drug releasedfrom these batches may be controlled by diffusion through the micropores (Nighute and Bhise, 2009).

Glutathione release from polymeric spheres can be explained by two mechanisms. The drug is released by diffusion from the encapsulating alginate microspheres. Secondly, the drug leaches out from the microspheres through the erosion

Table 4. Characterization of glutathione-alginate microspheres.

Formula	EE (%) (Y1)	Drug loading (%) (Y2)	Particle size (µm) (Y3)	Yield(%) (Y4)	Flux (µg/cm²/h) (Y5)
F1	34.74 ± 0.07	5.72 ± 0.05	1.89 ± 0.03	88.80 ± 1.41	0.021 ± 0.002
F2	47.19 ± 0.85	6.00 ± 0.03	2.06 ± 0.09	88.58 ± 0.98	0.026 ± 0.002
F3	42.80 ± 0.08	6.12 ± 0.05	2.08 ± 0.08	89.44 ± 2.03	0.017 ± 0.001
F4	56.63 ± 0.36	6.23 ± 0.02	2.42 ± 0.08	89.13 ± 0.09	0.008 ± 0.002

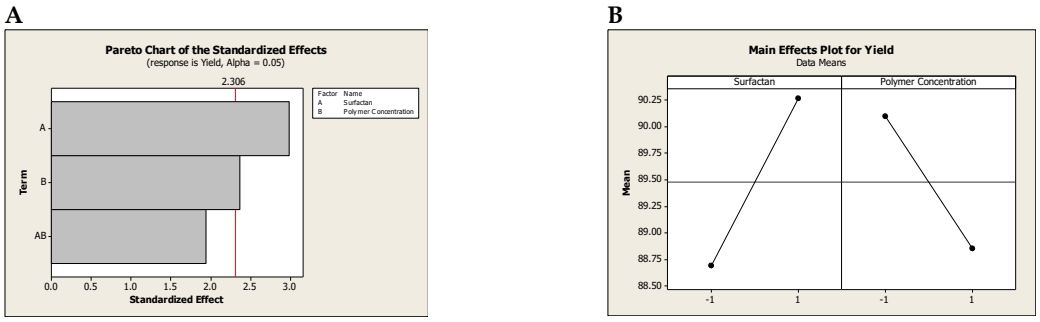

Figure 4. The effect of independent variables on the yield (Y4) including the Pareto chart **(A)**, and main plot effect **(B)**.

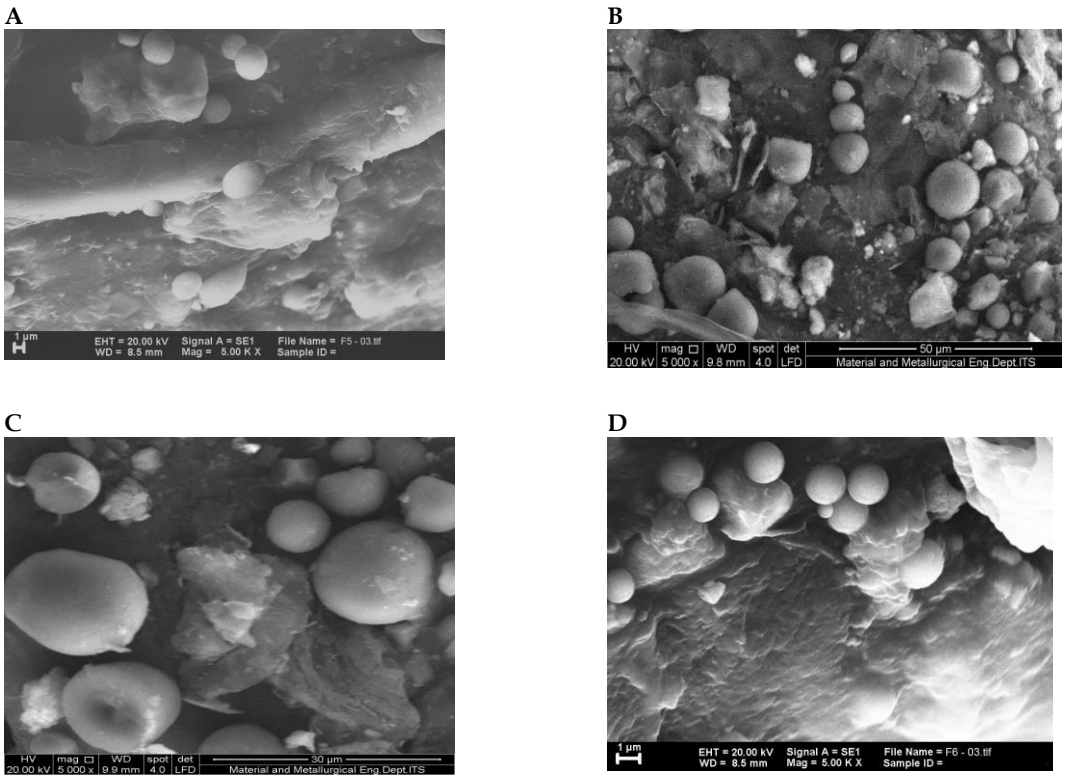

Figure 5. SEM images of F1**(A)**, F2**(B)**, F3**(C)**, F4**(D)**.

Table 5. Drug release kinetics of glutathione microspheres.

Formulation	Drug release kinetics coefficient of determination (R^2)			Flux (μg/cm²/h)
	Zero Order	First Order	Higuchi	
F1	0.978	0.856	0.989	0.021 ± 0.002
F2	0.986	0.839	0.994	0.026 ± 0.002
F3	0.988	0.893	0.989	0.017± 0.001
F4	0.924	0.645	0.994	0.008 ± 0.002
F1-gel based	0.978	0.845	0.989	0.019 ± 0.002
F2-gel based	0.984	0.826	0.994	0.005 ± 0.001
F3-gel based	0.988	0.889	0.989	0.034 ± 0.002
F4-gel based	0.931	0.643	0.995	0.011 ± 0.002

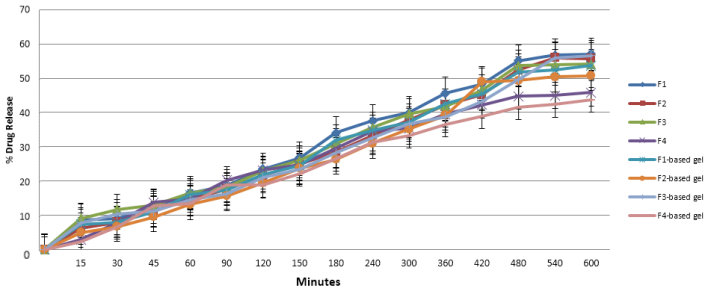

Figure 6. Drug release profile of glutathione-alginate microspheres.

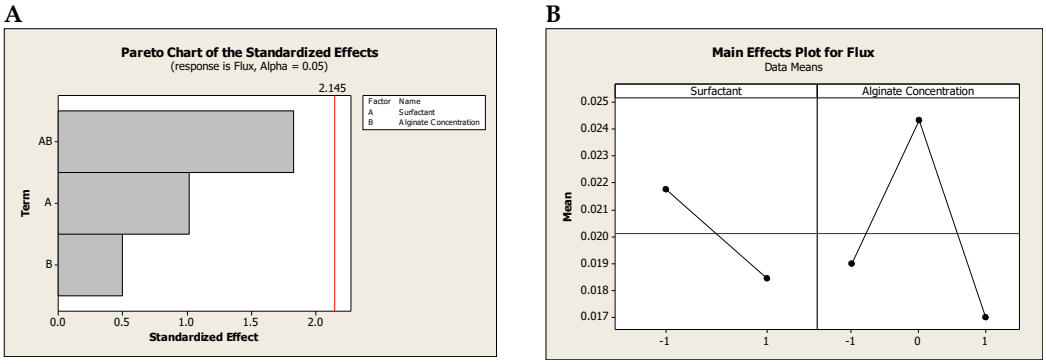

Figure 7. The effect of independent variables on the flux of *in vitro* drug release from alginate microspheres (Y5) including the Pareto chart **(A)**, and main plot effect **(B)**.

and/or degradation of the matrix. The latter phenomenon could be attributed to the removal of the cross-linker, calcium, from the microspheres. The swelling of alginate molecules increases matrix porosity and thus increases both diffusion and erosion. These findings comply well with the higher drug to polymer ratio used in formulation F1 (Sudhamani et al., 2010). Phosphate buffer has a chelating action due to the phosphate ions which helps further in the disruption of the matrix. Both

of our formulations exhibited a sustained release of glutathione over a period of 10 hours. A slower release pattern was observed for formulation containing higher amounts of the polymer, F4 and F4-based gel. Similar results were obtained for ropinirole hydrochloride loaded microspheres reported in a previous study (Avachat et al., 2011). Existence of gel and without gel of glutathione microspheres have significant effects on the entrapment efficiency, drug loading and, particle size and microspheres-based gel with paired sample T-test p values 0.004 <0.005. It was shown that drug release could be extended by increasing polymer proportion. Similarly, insulin and diaminopyridine microparticles were successfully prepared by solvent evaporation method and drug to polymer ratio was shown to affect microspheres characteristics and drug release profile (Rout and Nayak, 2009).

CONCLUSIONS

Glutathione-alginate microspheres were prepared successfully by using ionotropic gelation method by aerosolisation. Polymer-drug ratio influenced the particle size as well as drug release pattern of microspheres. All formulas produced high yield and encapsulation efficiency and small size particles. From the 2^2 randomized full factorial design, there was showed that combination of use of surfactant and polymer concentration significantly affected DL and EE but not for yield and particle size. Formula F4 using of 2.5% alginate and 1 M $CaCl_2$ with addition of surfactant at HLB 7 was selected as the optimized formula. The assessment of release kinetics showed that drug release from glutathione-alginate microspheres followed the Matrix-Higuchi model (diffusion-controlled drug release mechanism). This formulation can be potentially recommended for activity and stability test to further optimized as topical drug delivery system.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Aljaeid B, Hosny K (2016) Miconazole-loaded solid lipid nanoparticles: formulation and evaluation of a novel formula with high bioavailability and antifungal activity. Int J Nanomed 11: 441–447.

Avachat MA, Bornare NP, Dash RR (2011) Effect of process parameters sustained release microspheres of ropinirole hydrochloride. Acta Pharm 61: 363–376.

Badilli U, Sen T, Tarimci N (2011) Microparticulate based topical delivery system of clobetasol propionate. AAPS PharmSci Tech 12: 949–957.

Bagade OM, Dhole SN, Raskar VS, Pujari RR, Shete AM, Kharat PP, Vanave MD (2014) Formulation, statistical optimization and evaluation of praziquantel loaded microspheres by ionic gelation method. J Pharm Nanotechnol 2(4): 21–36.

Basarkar G, Shirsath G, Patil S (2013) Development of microspheres containing diclofenac diethylamine as sustained release topical formulation. Bull Pharm Res 3: 14–22.

Islam A, Taufiq Yap YH, Ravindra P, Moniruzzaman M, Chan ES (2013) Development of a procedure for spherical alginate-boehmite particle preparation. Adv Powder Technol 24: 1119–1125.

Johnson LR, Fayez KG, Johnathan DK, Juanita M, Hamid MS, Jackie W (2012) Physiology of the Gastrointestinal Tract, Fifth Edition. USA: Academic Press.

Kern JK, Geier DA, Adams JB, Garver CR, Audhya T, Geier MR (2011) A clinical trial of glutathione supplementation in autism spectrum disorders. Med Sci Monit 17 (CR6): 77–82.

Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. Prog Polym Sci 37: 106–126.

Murray RK (2009) Metabolism of xenobiotics. In: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA, editors. Harper's Illustrated Biochemistry. 28th ed. Michigan: McGraw-Hill, p. 612–613.

Nighute AB, Bhise SB (2009) Preparation and evaluation of rifabutin loaded polymeric microspheres. Res J Pharm Technol 2(2): 371–374.

Nugrahaeni F, Rosita N, Hariyadi DM (2018) Partition coefficient and glutathione penetration of topical antiaging: Preformulation study. Int J Drug Deliver Technol 8(2): 39–43.

Rout PK, Nayak BS. (2009). Formulation design, preparation of losartan potassium microspheres by solvent evaporation method and it's *in vitro* characterization. Arch Pharm Sci Res 1: 166–170.

Sinko PJ (2008) Martin's physical pharmacy and pharmaceutical sciences. 5th ed., New Delhi: Wolters.

Sudhamani T, Noveenkumarreddy K, Ravi Kumar VR, Revathi R, GanesanV (2010) Preparation and evaluation of ethyl cellulose microspheres of ibuprofen for sustained drug delivery. Int J Pharma Res Dev 1: 119–125.

Surini S., Angriani, Anwar E (2009) Study of mucoadhesive microspheres based on pregelatinized cassava starch as a new carrier for drug delivery. J Med Sci 9:249–256.

Trivedi P, Verma AML, Garud N (2008) Preparation and characterization of aceclofenac microspheres. Asian J Pharm 1: 110–115.

Venkatesan P, Manavalan R, Valliappan K (2011) Preparation and evaluation of sustained release loxoprofen loaded microspheres. J Basic Clin Pharma 2: 159–162.

Watanabe F, Hashizume E, Chan GP, Kamimura A (2014) Skin-lightening and skin-condition-improving effects of topical oxidized glutathione: A double-blind and placebo-controlled clinical trial in healthy women. Clin Cosmet Invest Dermatol 7: 267–274.

AUTHOR CONTRIBUTION:

Contribution	Hariyadi DM	Rosita N	Rahayu A
Concepts or ideas	x	x	
Design	x	x	
Definition of intellectual content	x	x	
Literature search	x	x	x
Experimental studies	x	x	x
Data acquisition	x	x	x
Data analysis	x	x	x
Statistical analysis	x	x	x
Manuscript preparation	x	x	x
Manuscript editing	x	x	x
Manuscript review	x	x	x

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