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Research Article

Formulation, Characteristic Evaluation, Stress Test and Effectiveness Study of Matrix Metalloproteinase-1 (MMP-1) Expression of Glutathione Loaded Alginate Microspheres and Gel

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ABSTRACT

Background: The present study aimed to formulate and evaluate the stability, characteristics and effectiveness of glutathione-loaded alginate microspheres through increased lipophilicity using surfactant with a Hydrophylic-Lipophilic Balance (HLB) value equal to 7. The selection of glutathione as an antioxidant was based on its prominent role in maintaining intracellular redox balance. Alginate was used as the polymer, while calcium chloride constituted a cross-linking agent and Tween and Span were employed as surfactants.
Methods: The study applied an ionotropic gelation-aerosolization method. Microspheres were characterized by their morphology, size, drug loading, entrapment efficiency and yield. Stress testing utilized a forced degradation method, while an effectiveness study of glutathione incorporated a Matrix Metalloproteinase I (MMP-1) parameter on mouse skin. Glutathione-microspheres, to which had been added surfactants with a HLB value equal to 7, were compared to those without surfactants.
Results: Microspheres demonstrated both high yield and encapsulation efficiency. From the stability study conducted, it was evident that the glutathione-microspheres with additional surfactant were more stable than glutathione with surfactant, but without microspheres. Similarly, the glutathione-microspheres with additional surfactant were more stable than the glutathione without surfactant. The *in vivo* effectivity showed lipophilic glutathione microspheres were able to decrease MMP-1 expression in the dermis tissue of mice.
Conclusion: The results of freeze-dried glutathione-loaded alginate microspheres with surfactant with a HLB value equal to 7 can be utilized as potential glutathione delivery systems.

Introduction

Glutathione (L-γ-glutamyl-L-cysteinyl-glycine) (GSH) is a low molecular weight thiol-tripeptide that plays a prominent role in maintaining intracellular redox balance.[1] Glutathione is a ubiquitous compound of the biologically active sulfhydryl group provided by the cysteine moiety that acts as the active part of the molecule.[2] The sulfhydryl group promotes interaction with a variety of biochemical systems to form glutathione in its predominant intracellular form, which acts as a potent antioxidant and defends against toxic compounds and xenobiotics.[3] However, the biomedical applications of glutathione remain limited due to its relatively short half-life, labile properties and rapid metabolism and elimination.[3] A study was carried out into the lipophilicity increase of glutathione using mixed surfactants of Tween 80 and Span 80.[4] The addition of surfactants with an HLB value equal to 7 affected the lipophilicity of glutathione resulting in similarity to Log P lipophilicity of the skin (Log P 2-3).[4] Therefore, a surfactant system was needed in order to improve the stability of glutathione.

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 µm to 1000 µm (1 mm)). Encapsulation system in the microspheres can be used to protect sensitive materials to environmental conditions such as light, oxygen, water, and temperature, such as glutathione. By using microspheres delivery system it is expected that the active substance will be protected and will be also able to penetrate the dermis layer of the skin. Microspheres can stabilize and protect a drug from degradation, while preserving its biological activity and enhancing its bioavailability.[5] Moreover, they offer prolonged or controlled drug delivery, improved bioavailability and stability.[5-6] Sodium alginate, used in drug delivery systems, is a linear copolymer with a polysaccharide backbone comprising two repeating carboxylated monosaccharide units (mannuronic acid and guluronic acid).[7]

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Table 1. Formula of Glutathione-Ca Alginate Microspheres with or without surfactant.

Compounds	Function	Concentration of Compund	
		I	II
Glutathione	Active Compound	-	0.5 g
Dried Glutathione+surfactant HLB 7	Active Compound	0.5 g	-
Alginate	Polymer	2% (b/v)	2% (b/v)
$CaCl_2$ Solution	Crosslinker	1 M	1 M

Formula I: Dried glutathione+surfactant HLB 7 0.5g; Alginate 2% (b/v);1M $CaCl_2$ Solution
Formula II: Glutathione; Alginate 2% (b/v); 1M $CaCl_2$ Solution

Alginate can be cross-linked by external gelation methods allowing the alginate-drug solution to be extruded as microspheres into a $CaCl_2$ solution. Alginates have guluronate (G) and mannuronate (M) monomer units. Gelling of the alginate occurs when divalent cations participate in the interchain bonding between guluronate units (G-blocks), giving rise to a three-dimensional network in the form of a gel. The "eggbox" model has been formulated to explain the nature of this interaction.[7-8] Sodium alginate forms gel microspheres by crosslinking with Ca^{2+} ion.[8-9] This study applied an aerosolization technique, previously used to encapsulate drug and proteins, which crosslinked alginate polymer and $CaCl_2$ crosslinker to encapsulate a drug model by spraying followed by freeze-drying.[10] The advantages of aerosolization techniques include its ability to produce a simple, rapid, non-toxic and cost-effective method.[10,11] In cases of antioxidant use, denaturation or stability issues of the antioxidants could be avoided.[8,10,11]

The use of alginate microspheres in the field of biotechnology and the pharmaceutical industry is currently widespread due to their unique properties of high biocompatibility and biodegradability.[12] The present study was aimed to formulate and evaluate the characteristics and stability of the glutathione and freeze-dried glutathione-loaded alginate microspheres with surfactants with a HLB value equal to 7. The microspheres were evaluated for size, morphology, encapsulation efficiency, loading and yield. Stress Testing was studied using a forced degradation method[13]. Effectiveness study of glutathione described as MMP-1 parameter will be studied on animal's skin.

Materials and Methods

Chemical Reagents

The following pharmaceutical grade chemical reagents were used: Glutathione (Sigma-Aldrich Inc); Sodium alginate (Sigma-Aldrich Inc); $CaCl_2.2H_2O$ (Merck); Sodium citrate (Merck); Tween 80 (Merck); Span 80 (Merck); $NaH_2PO_4.2H_20$ (Merck); $Na_2HPO_4.12H_20$ (Merck) and Aquadest.

Formulation of Glutathione-Loaded Alginate Microspheres

2g of Glutathione was dissolved in 20ml of phosphate buffer solution pH 6±0.05. 0.5g of surfactant (mixed tween 80 and span 80) with a HLB value equal to 7 was added before freeze-drying was conducted for 30 hours at -26 °C. The preparation of alginate microspheres used ionotropic gelation method involving aerosolization. The alginate microspheres formulas were summarized in Table 1.

Gel Formulation

The carbomer (0.05 g) was dispersed in a preheated aquadest (5 g) before being cooled and propylene glycol and triethanolamine added through continuous stirring. Carbomer concentration was 1% w/w. The glutathione-alginate microspheres were then added and stirred continuosly, while the pH was checked. Gel formulation was shown in Table 2.

Stress Test of Glutathione-Alginate Microspheres

The glutathione microspheres' stress test involved storage in an oven at 50°C, 60°C or 80 °C and 75% RH for five days.[13] Organoleptic observations (color, odor and taste), drug loading and percentage of entrapment were subsequently performed on days 1, 3 and 5.

Effectiveness Study of MMP-1 of Glutathione-Alginate Microspheres and Gel

MMP-1, known as collagenase-1, is a zinc and calcium dependent endopeptidase, produced and released by both dermal fibroblasts and keratinocytes, which functions to break down collagens. The effectiveness study of MMP-1 consisted of the following stages: 30 balb/c mice were prepared by first shaving those areas of their backs to be irradiated.

Table 2. Gel formula of gluthahione-ca alginate microspheres and blank microspheres.

Compound	Formula I (g)	Formula II (g)	Formula III (g)
Glutathione	-	-	-
Glutathione Microspheres	Equal 0.2	Equal 0.2	-
Carbomer	0.05	0.05	0.05
Propylene glycol	0.5	0.5	0.5
Triethanolamine	0.025	0.025	0.025
Aquadest ad	5	5	5

Formula I: Gel Formula Glutathione Microspheres $_{\text{+ surfactant HLB 7}}$
Formula II: Gel Formula Glutathione Microspheres $-_{\text{surfactant}}$
Formula III: Formula gel base

A dose of 60 mJ/m^2 UV radiation was administered with interval every two days (at days 1,3,5,7,9,11, and 13), mice were then prepared its skin biopsy for histopatholgy examination (skin tissue of skin biopsy of mouse diameter 5 mm and depth until sub-cutaneous). Then histopathology examination on fibroblasts which expressing MMP-1 were determined under microscope with measurements taken by means of a calibrated lux meter. The determination of MMP-1 levels (%) was done by counting Fibroblasts expressing MMP-1 divided with total fibroblasts in the field of view.[14] The subjects were divided into three groups: Group I: glutathione microspheres gel with increased lipophilicity, Group II: glutathione gel, Group III: gel base.

Characterization of Glutathione-Alginate Microspheres

Size and morphology: Size was determined by means of optical microscopy, while morphology was investigated using a Scanning Electron Microscopy (SEM). The 300 particles of wet microspheres were measured using an optical microscope. The particles were firstly grouped to identify the smallest and largest within all the samples, by dividing them into several intervals and classes. The average diameter was then determined using the following equation:

$$\text{D average} = {}^{\sum \text{nd}}\!/_{\sum \text{n}} \qquad \text{Eq. (1)}$$

where:

n = number of particles observed

d = particle size

For freeze-dried microspheres, SEM was used to determine morphology and size by firstly placing them on an adhesive material containing metal grains, for example Platinum (Pt). The gold in the chamber was then evaporated in order to coat the entire surface of the microspheres with its vapor. The surface of the gold-coated microspheres was subsequently observed by means of SEM.

Determination of Glutathione-Alginate Microspheres

The drug content of alginate microspheres was quantified by breaking the microspheres formed into 120 mg with 50 ml Na Citrate over seven hours. From standard curves, microspheres were calculated in terms of entrapment efficiency, glutathione content and yield.[15] The results obtained were calculated based on the percentage of glutathione content of each formula using the equation below:

$$\%\text{Drug Loading} = \frac{\text{Weight of glutathione in microspheres}}{\text{Total weight of dry microspheres}} \times 100 \qquad \text{Eq. (2)}$$

Determination of Glutathione Entrapment Efficiency

Entrapment efficiency was calculated based on the glutathione content in microspheres using the following equation:

$$\text{Entrapment efficiency} = \frac{\text{Weight of glutathione in microspheres}}{\text{Theoretical weight of glutathione}} \times 100 \qquad \text{Eq. (3)}$$

Determination of Yield

The percentage recovery was calculated from the total number of dry microspheres produced compared to the amount of sodium alginate-glutathione added during the manufacturing process. From the calculation results, it was possible to quantify the yield of microspheres.

$$\%\ \text{Yield} = \frac{\text{Total weight of dry microspheres}}{\text{Total weight of glutathione and polymer}} \times 100 \qquad \text{Eq. (4)}$$

Results

The microencapsulation process can protect the active ingredient against chemical or enzymatic degradation.[16] The formulas were divided into two types of microspheres, namely glutathione-alginate microspheres with increased lipophilicity through the addition of surfactants and glutathione-alginate microspheres to which surfactants had not been added. The production of glutathione-alginate microspheres with increased lipophilicity was achieved by, firstly, adding surfactants to glutathione prior to the encapsulation process by means of alginate and a crosslinker. Another formula of glutathione-alginate microspheres without added surfactants was compared to glutathione-alginate microspheres which had not been subjected to lipophilicity enhancement. The production of microspheres employed an ionotropic gelation method including aerosolization. During microspheres formulation, the excess $CaCl_2$ that did not react with sodium alginate was removed since it can decrease entrapment efficiency.[9] Maltodextrin, as a lyoprotectant, was intended to stabilize the microspheres against the pressure exerted during the freeze-drying step of any water replacement process.[17] Maltodextrin replaces water molecules by forming hydrogen bonds between maltodextrins and polar groups on microspheres surfaces on conclusion of the drying process. Consequently, microspheres will be protected from mechanical stress and can prevent aggregation during the freeze-drying process. Maltodextrin also plays a role in the formation of microspheres surfaces.[9]

An evaluation of the characteristics of microspheres was performed in terms of their size, shape and surface, IR spectrophotometry, entrapment efficiency, glutathione loadings and degree of yield. The evaluation of the size distribution of wet microspheres involved use of a 300-particle optical microscope. The size of the blank microspheres was confirmed as 1.34 µm and both formulas of microspheres (F1 and F2) showed larger particle sizes of 1.40 µm and 1.58 µm respectively compared to blank microspheres (Table 3). Blank microspheres were smaller compared to the two formulas of microspheres. Three formulas had a polydispersity index of 0.003. The resulting polydispersity index of less than 0.3 indicated that the sample had a narrow distribution (monodisperse) or uniformly stated size.[18]

A study of the shape and surface of wet microspheres can be seen in Figure 1A, while one of dry microspheres can be seen in Figure 1B. An investigation into the shape and surface of the microspheres by Scanning Electron Microscope (SEM) confirmed that the F1 microspheres had an uneven surface. This was because a certain degree of moisture cannot be eradicated after the sublimation process involving water and surfactant glutathione. This was evidenced from the moisture content level of 8.25% in F1, while that in F2 was lower than 2.65%. The sublimation process of F1 was not optimal because microspheres contained a surfactant with the potential to attract water. F2 microspheres possessed a smooth surface. The F1 and F2 microspheres surfaces became spherical due to the addition of maltodextrin which closes the cavities or pores which had increased in number and size during the freeze-drying process by forming hydrogen bonds with polar groups on the microspheres surface.[19]

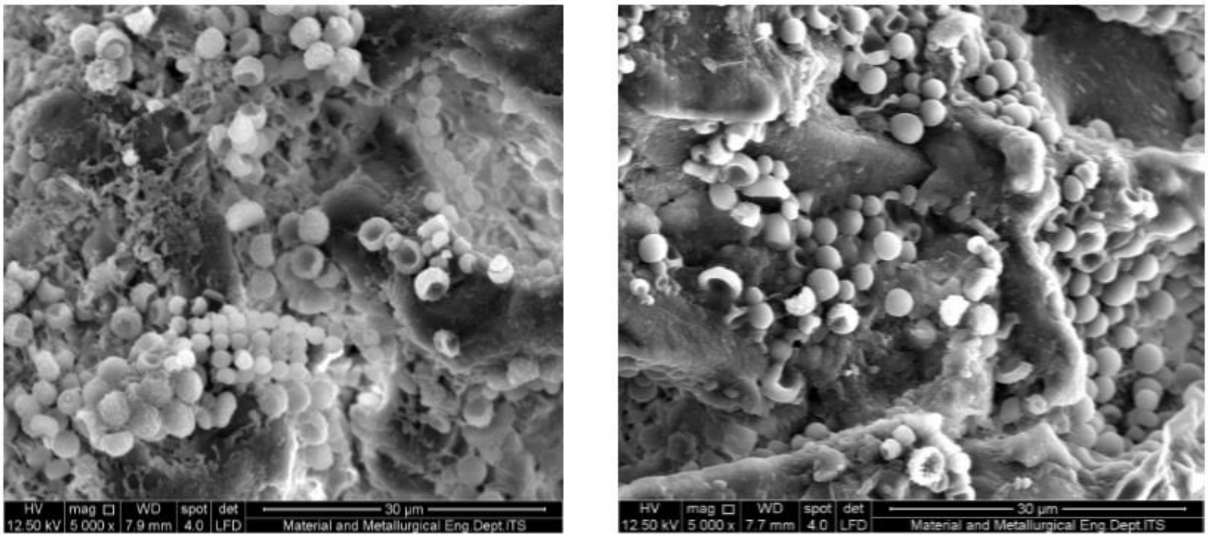
The surface of those microspheres which was not containing surfactants became smooth and spherical as the evaporated water was replaced by maltodextrin. Meanwhile, in F1, which contained surfactant during the lyophilization process, it remained possible for water to be retained in the surfactant.

The results of the overlay of an IR spectrum inspection of the glutathione-alginate microspheres can be seen in Figure 2. The interaction was characterized by shifts in wave numbers, loss of guluronate fingerprint absorption and an uptake of carboxylic salt groups (1614 cm^{-1}) of Na alginate due to cross-linked reactions with $CaCl_2$. From the results of the second IR-spectra examination of the formulas, the absorption of glutathione-specific groups still existed in all formulas. This means that glutathione was absorbed in the microspheres system without reacting with alginates.

Table 3. Particle size distribution of glutathione-Ca alginate microspheres and blank microspheres.

Distance size (µm)	**Average of distance size (µm)**	**Blank Microspheres**		**F1**		**F2**	
		n	**n×d**	**n**	**n×d**	**n**	**n×d**
0.64 – 1	0.76	69	52.44	31	23.56	3	2.28
1.01 – 1.37	0.94	115	136.8	24	22.56	47	44.18
1.38 – 1.74	1.29	76	124.8	124	159.9	97	125
1.75 – 2.11	1.68	34	57.12	98	164.6	85	142.8
2.12 – 2.48	2.22	2	4.6	18	39.96	36	79.9
2.49 - 2.85	2.41	1	2.41	5	12.05	28	67.5
2.86 – 3.22	3.10	2	6.2			1	3.1
3.23 – 3.59	3.39	1	3.39			3	10.2
Average Diameter (µm)			**1.34**	**1.40**		**1.58**	
Polydispersity Index			**0.003**	**0.003**		**0.003**	

The average diameter of Glutathione-Ca Alginate Microspheres was obtained from 300 particles of wet microspheresmeasured with an optical microscope.
Formula I: Glutathione Microspheres $_{\text{+ surfactant HLB 7}}$
Formula II: Glutathione Microspheres $-_{\text{surfactant}}$
n×d: number of particles × average of distance size



A

B

Figure 1. The morphology of the shape and surface of the microspheres (A) F1 (GSH HLB 7), (B) F2 (GSH) observed through 5000x magnification scanning electron microscopy (SEM).

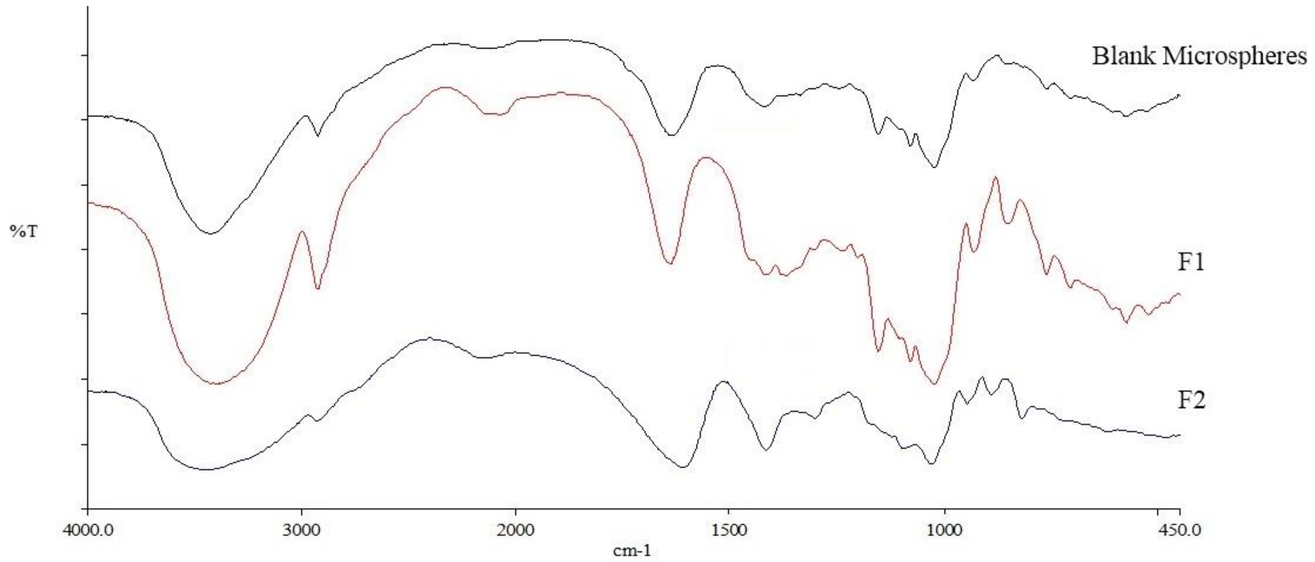


Figure 2. IR spectrum of glutathione-alginate microspheres.

On examination of specific Na-alginate group uptake, guluronate fingerprints were absent from all formulas. While the uptake of carboxylate salt groups was still present in all formulas, one of the two absorptions of 1614 cm^{-1} was absent. The wavelengths of the carboxylic salt group in F1 and F2 were 1423.52 cm^{-1} and 1421.25 cm^{-1} respectively. The loss of one uptake within the carboxylic salt group and the guluronate fingerprint was due to a crosslinking reaction between the alginate and $CaCl_2$ crosslinker involving ion exchange between the carboxylic group of guluronate acid and Ca^{2+} of crosslinkers.

The drug loading of glutathione in microspheres can be seen in Table 4 which also contains an analysis of entrapment efficiency and glutathione yield in microspheres. The determination of entrapment efficiency and drug loading used Na citrate 0.5 M pH 8.5. Na citrate solution was chosen as the medium within which the mechanism breaks down microspheres by replacing Ca^{2+} in crosslinked Ca carboxylic linkage with $Na^{+,}$ thus rendering alginate soluble and causing glutathione to be re-dissolved. The data resulting from the examination of the glutathione content of microspheres produced was expressed as percentages. F1 was 8.623% ± 0.12 and F2 was 7.81% ± 0.22.

Table 4. Drug loading, entrapment efficiency and yield of gluthatione-Ca alginate microspheres.

Formula	Drug Loading ± SD (%)	EE ± SD (%)	Yield ± SD (%)
1	8.623 ± 0.12	54.46 ± 2.4	94.03 ± 3.07
2	7.81 ± 0.22	48.49 ± 2.37	93.34 ± 3.65

Formula I: Glutathione Microspheres $_{\text{+ surfactant HLB 7}}$
Formula II: Glutathione Microspheres $-_{\text{surfactant}}$
The values are written as an average value mean ± SD of drug loading, entrapment efficiency and yield of triplicates.
EE: Encapsulation efficiency; SD: Standad Deviation

The entrapment efficiency result for F1 was 54.46% ± 2.40, while that for F2 was 48.49% ± 2.37. From these results, it could be seen that 500mg of the drug could not be entrapped entirely by alginate at a concentration of 2% w/v. The resulting drug content remained relatively low. This was possibly due to the production of alginate microspheres using the current concentration of alginate and $CaCl_2$ only being able to encapsulate this maximum capacity. Therefore, it is recommended to further optimize certain ratios of alginate polymer and crosslinker concentrations as a means of encapsulating a higher amount of glutathione. In this study, the 2% alginate concentration or 1M $CaCl_2$ may need to increase so as to entrap and load larger amounts of the drug. Moreover, in order to produce optimum entrapment and drug loading efficiency, microspheres must consist of the necessary amount of both polymer and crosslinker to establish an optimal hydrogel composition which needs further experiments using several molar ratio composition.[20,21]

The ANOVA test results obtained did not differ significantly ($p>0.05$) because the concentration of alginate and $CaCl_2$ used within both formulas was equal. Moreover, the addition of surfactant to glutathione did not affect microspheres entrapment efficiency. An analysis of F1 microspheres recovery confirmed yields as being 94.03% ± 3.07 (F1) and 93.34% ± 3.65 (F2). From these results, it could be seen that no difference in yield between F1 and F2 was existed because the amount of alginate and $CaCl_2$ concentrations in both formulas was equal. The determination of yield recovery was aimed to quantify the extent of dry microspheres recovery of initial compounds added during the manufacture of microspheres (polymers and drug).[22] In future research, it is advisable to investigate the maximum capacity of the microspheres in order to obtain high drug loading, high entrapment efficiency and high yield by varying concentrations of alginate polymer and $CaCl_{2.}$ Furthermore, the conducting of an in vitro release test is highly recommended.

Microspheres were subsequently subjected to stress tests which aimed at determining the stability of glutathione after microencapsulation (Figure 3, Table 5 and 6).

Table 5. Log Ct vs GSH remaining remaining after storage at temperatures of 50°C 0, 60°C and 80°C.

Days	Temperature 50°C				Temperature 60°C				Temperature 80°C			
	Log Ct GSH	**Log Ct Microsphere GSH$_{\text{-surf}}$ (F2)**	**Log GSH $_{\text{+ Surf}}$**	**Log Microsphere GSH$_{\text{+Surf}}$ (F1)**	**Log Ct GSH**	**Log Ct Microsphere GSH$_{\text{-surf}}$ (F2)**	**Log GSH $_{\text{+Surf}}$**	**Log Microsphere GSH$_{\text{+Surf}}$ (F1)**	**Log Ct GSH**	**Log Ct Microsphere GSH $_{\text{-surf}}$ (F2)**	**Log GSH $_{\text{+ Surf}}$**	**Log Microsphere GSH$_{\text{+Surf}}$ (F1)**
0	2.356	2.343	2.406	2.410	2.358	2.344	2.406	2.411	2.378	2.410	2.340	2.410
1	2.355	2.342	2.405	2.409	2.357	2.341	2.406	2.410	2.357	2.406	2.339	2.402
3	2.348	2.337	2.404	2.407	2.343	2.333	2.404	2.407	2.274	2.343	2.26	2.36
5	2.337	2.295	2.402	2.405	2.33	2.289	2.400	2.404	2.175	2.293	2.17	2.32
Regression curve (y=ax+b)												
a	0.003	0.0091	0.0008	-0.0009	0.0005	0.0100	-0.0012	0.0014	0.0414	-0.0248	0.0357	0.0186
b	2.357	2.35	2.406	2.41	2.36	2.3505	2.406	2.411	2.389	2.419	2.3575	2.415
r	-0,9804	-0,9878	-0,9796	0,9742	-0,9877	-0,9190	-0,9566	-0,9983	-0.993	-0.987	-0.979	-0.994
K	0.0085	0.0209	0.0018	0.0020	0.0133	0.023	0.0027	0.0032	-0.095	-0.057	-0.082	-0.043
Ln K	-4.767	-3.868	-6.319	-6.18	-4.319	-3.772	-5.914	-5.744	-2.35	-2.862	-2.501	-3.14

GSH: Glutathione; Surf: Surfactant

From the results of stress test, it was known that glutathione belonged to the first order because the plot of log Ct to t produced a straight line or linearity approaching 1. Based on the value of each compound, it was evident that the glutathione plus surfactant microspheres were more stable than glutathione with surfactant only. In addition, the glutathione with surfactant was more stable than the glutathione without surfactant (Figure 3). This was in accordance with the microencapsulation purpose of protecting glutathione from oxidation reactions.[23]

Table 6. Stability Linearity Curve of 0 order and 1st order at a temperature of 80°C.

Compounds	**Linearity (r)**	
	Zero Order	**1st Order**
GSH $_{\text{- surf}}$	-0.9972	-0.9930
Microspheres GSH $_{\text{-surf}}$ (F2)	-0.9871	-0.9897
GSH $_{\text{+ Surf}}$	-0.9847	-0.9799
Microspheres GSH$_{\text{+Surf}}$ (F1)	-0.9889	-0.9941

GSH: Glutathione; Surf: Surfactant

Based on the linearity of the stability value (r), it was apparent that the 1st order reaction was more linear (the value of r was close to 1), indicated that the reaction order of this microspheres system followed 1st order. Therefore, the determination of the constant value of glutathione degradation (k) used an equation formula of the 1st order. The averages produced by an MMP1-1 test are presented in Table 7. Based on the statistical analysis of variance (ANOVA) within an MMP-1 expression test, a p-value (sig) of 0.000, less than 0.050, was obtained. The resulting glutathione-alginate microspheres, both those with additional surfactants and those without surfactants, were then mixed into the gel base for penetration evaluation. It was possible to evaluate the penetration test result from the MMP-1 level. The formula was able to penetrate when showing decreased levels of MMP-1 in mouse skin, having been exposed to ultra violet (UV) irradiation every two days, through the application of a dosage of 60 mJ/m^2 during each irradiation. The gel was applied to the skin twice a day, 20 minutes before irradiation (to give the topical absorption time into the skin) and four hours after irradiation (reactive oxygen species (ROS) initiated four hours after exposure). Topical application of the material was occurred on a day without irradiation. The gel base was chosen because the microspheres were hydrophilic. Thus, it was appropriate to use gel as a carrier basis since it is elastic, easy to wash and has a cooling effect when applied to the skin on which it can readily be spread.

In this study, the average MMP-1 expression in the treatment group smeared with glutathione microspheres gel with increased lipophilicity was lower than in either the glutathione gel without microspheres or control groups (Figure 4). The average level of MMP-1 expression in the control group consisting exclusively of gelling base was 72.03%, whereas the MMP-1 level of gel consisting of glutathione-alginate microspheres with increased lipophilicity was 15.44%. One-way ANOVA and post-hoc tests on the glutathione lipophilicity gel of the control group confirmed a significant increase.

Table 7. Study of MMP-1 expression of glutathione-Ca alginate microspheres.

Group	**MMP-1 Expression (%)**	**SD**
1	15.44	3.83
2	55.12	5.85
3	72.03	0.59

The values are presented as average mean values ± SD of percentage of MMP-1 expression. Each group consisted of triplicates. MMP-1: Matrix Metalloproteinase I; SD: Standard Deviation

Therefore, the results of the control group with glutathione lipophilicity gel increased significantly. It demonstrated that lipophilic glutathione microspheres were able to decrease MMP-1 expression in the dermis tissue of mice. This was due to formulas having increased their lipophilicity near the 2-3 ^{P}log with the result that they penetrated the stratum corneum and entered the dermis network. Lipophilic glutathione increased on the gel microspheres. Increased MMP-1 expression was occurred after the skin of the mice was exposed to radiation for two weeks because the energy from UV radiation damages cell membranes and proteins. This, in turn, produces reactive oxygen species (ROS) which induce expression of proinflammatory cytokines binding to cell surface receptors including receptors of epidermal growth factor, interleukin (IL)-1, insulin keratinocyte growth factor and tumor necrosis factor (TNF).[24-25]

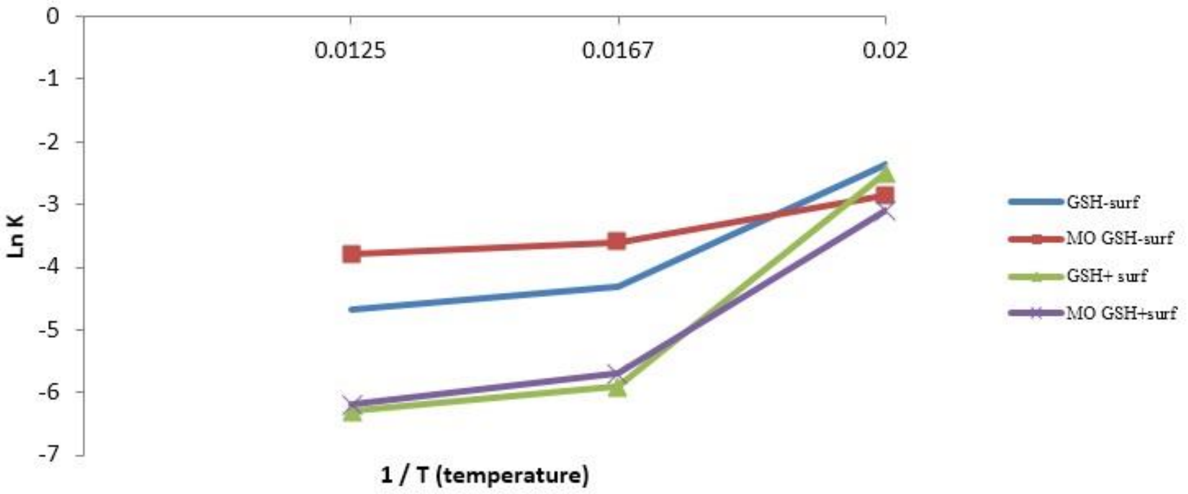


Figure 3. Correlation between Ln K and 1/ T (temperature) of glutathione and glutathione microspheres. The data represented is Mean ± SD. N average = three formulas in each group.

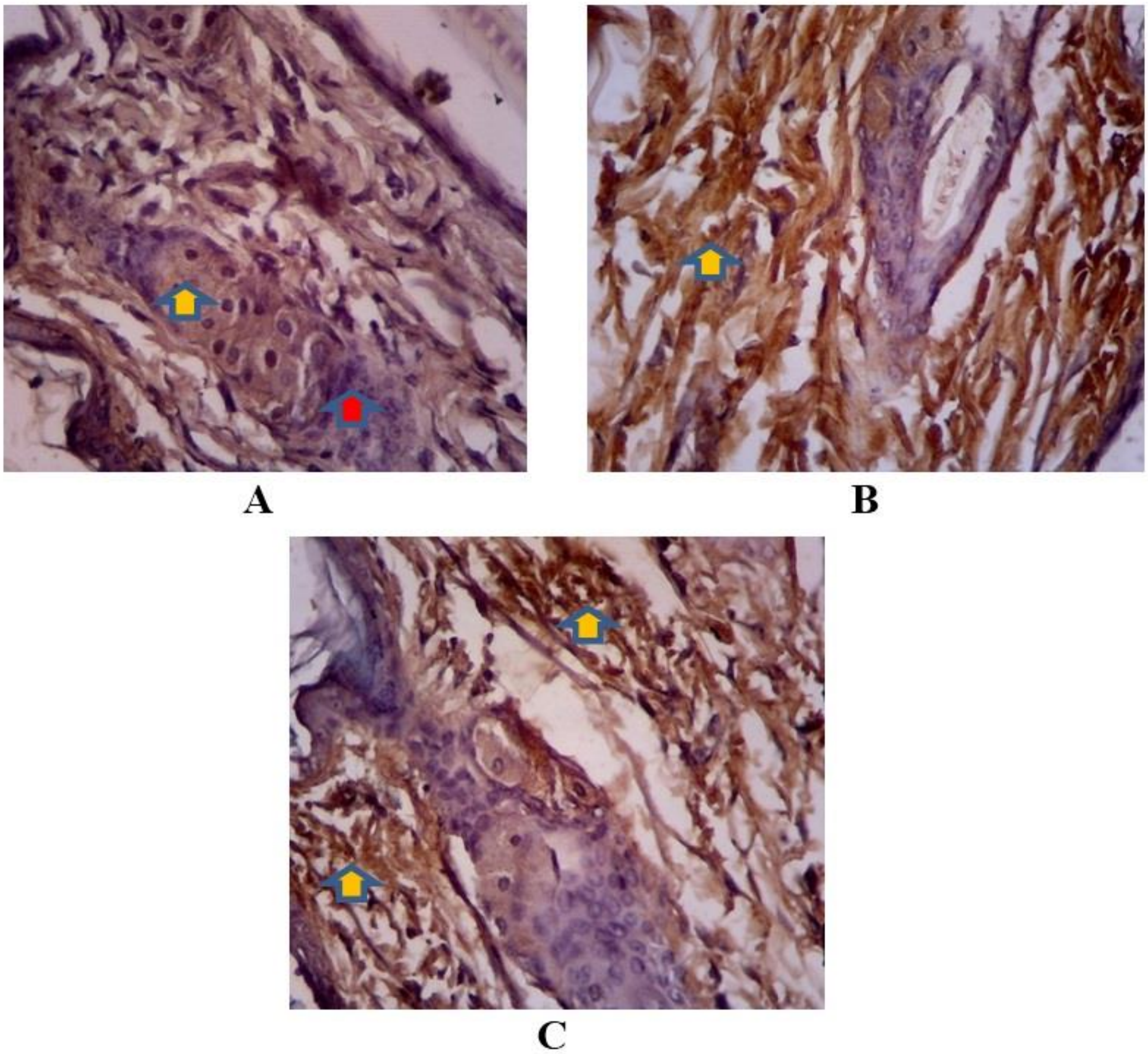


Figure 4. MMP-1 Expression of histology dermis networks of mice withımmunohistochemical staining (A) Glutathione microspheres gel +surf, (B) Gel glutathione microspheres -surf, (C) Gel base (Magnification 400x). The yellow arrow indicates fibroblasts cells expressing MMP-1 (the surrounding cytoplasm is purplish to brownish), while the red arrows indicate fibroblast cells (cytoplasm around the bluish-colored) not expressing MMP-1

Discussion

According to the results of this study, morphology changes were observed in the glutathione-alginate microspheres with the addition of surfactant compared to those without surfactant. Glutathione-alginate microspheres with the addition of surfactant with a HLB value equal to 7 possessed an almost spherical and smooth surface, whereas that of the glutathione microspheres without surfactant was not spherical and contained a number of holes. Almost spherical microspheres morphology was formed suggesting that non-spherical microspheres that may require a higher concentration of maltodextrin lyoprotectant which protects against mechanical stress and prevents aggregation during the freeze-drying process. This result was in accordance with those of a previous study using maltodextrin lyoprotectant to stabilize microsphere surfaces and improve dissolution properties.[9, 26]

In terms of resistance to oxidation, the glutathione plus surfactant microspheres were more resistance than either the glutathione with surfactant only or the glutathione without surfactant. This was in accordance with the microencapsulation's purpose of protecting glutathione from oxidation reactions.[23]

In addition, to increase entrapment efficiency and drug loading, higher concentrations of alginate polymer and $CaCl_2$ may be needed for future study of the optimum encapsulation process during the crosslinking process. Crosslinking of longer duration may need to be considered. Higher percentages of drug loading and entrapment efficiency of alginate microspheres have been shown to be necessary by other researchers employing a larger amount of alginate and $CaCl_2$ and crosslinking time in excess of one hour.[27,28]

Consequently, the optimized lipophilic glutathione-loaded alginate microspheres produced high in vivo effectiveness by decreasing MMP-1 expression in the dermis tissue of mice and penetrating the stratum corneum and dermis.

Conclusion

Glutathione with surfactant loaded alginate microspheres has been successfully produced through aerosolization. The resulting small, spherical microspheres which were almost completely smooth were produced using alginate 2%. The enhanced lipophilicity of glutathione microspheres using surfactant with a HLB value equal to 7 was significantly more penetrative in nature than that of others, as indicated by the decreased levels of MMP-1.

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Conflict of interests

The authors claim that there is no conflict of interest.

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