JPPIPA 9(11) (2023)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Formulation of Glutathione-Alginate Microspheres for Diabetes Mellitus Dietary Supplementation

Asti Rahayu[1*], Nadya Ambarwati[1], Desta Ayu Cahya Rosyida[2]

[1] Pharmacy of Bachelor Degree, University of PGRI Adi Buana Surabaya, Surabaya, Indonesia.
[2] Midwifery of Bachelor Degree, University of PGRI Adi Buana Surabaya, Surabaya, Indonesia.

Received: August 2, 2023
Revised: October 27, 2023
Accepted: November 25, 2023
Published: November 30, 2023

Corresponding Author:
Asti Rahayu
astirahayu@unipasby.ac.id

DOI: 10.29303/jppipa.v9i11.4877

Abstract: The microsphere is a multi-molecule drug conveyance framework made to accomplish controlled drug conveyance to further develop bioavailability and dependability and target medications to explicit destinations at a given rate. Glutathione as a model for microspheres as dietary supplementation can restore GSH synthesis and lower oxidative stress and oxidant damage in the face of persistent hyperglycemia. The biomedical utilization of glutathione stays restricted because of its moderately short half-life, shaky properties, fast digestion, discharge, and high hydrophilicity. Hence, the motivation behind this study was to get ready GSH utilizing a microsphere with polysorbate 80 and sorbitan monooleate 80, beat skin obstruction capability, and research the properties and delivery profile of GSH-stacked alginate microspheres. It was to do. GSH microspheres were ready by ionotropic gelation technique. The produced microspheres were broken down in vitro for the proficiency of ensnarement (EE%), drug stacking (DL%), molecule size, yield, and medication discharge profile to acquire a streamlined plan. The pre-arranged GSH Microspheres had a high EE% going from (41.50 ± 1.12%) for F1 to (50.36 ± 0.86%) for F2, with little molecule sizes running (2.02 ± 0.09 µm) for F1 to (2.19 ± 0.16 µm) for F2, and drug stacking going from (5.81 ± 0.13%) for F1 to (6.15 ± 0.09%) for F2. The transition profile was tracked down in the scope of 0.018 ± 0.001 µg/cm²/h to 0.030 ± 0.002µg/cm²/h. GSH microspheres were ready by aerosolization utilizing the ionotropic gelation strategy. The GSH microspheres were assessed in vitro for the effectiveness of entanglement (EE%), drug stacking (DL%), molecule size, yield, and medication discharge profile to acquire a streamlined plan.

Keywords: Aerosolization; Alginate; Characteristics; Glutathione; Microspheres; Release Profile; Surfactant.

Introduction

Uncontrolled hyperglycemia significantly increases oxidative stress and ROS production, whereas lowering blood glucose levels reduces oxidative stress. Glycemic control may reduce the likelihood of diabetic microvascular complications by reducing oxidative stress (Tesauro et al., 2015). However, despite numerous attempts to implement evidence-based guidelines, the majority of patients are unable to achieve the glycemic goals (e.g., reducing oxidative stress through glycemic control) due to practical limitations., A1C 7%) that the American Diabetes Association encourages (Lagman et al., 2015). According to the Diabetes Study, diabetes is still the most common cause of blindness, renal failure, and amputations (Bermejo et al., 2021). Due to a lack of precursors, patients with uncontrolled type 2 diabetes have severely reduced glutathione synthesis. In the face of persistent hyperglycemia, dietary GSH supplementation can restore GSH synthesis and reduce oxidative stress and oxidant damage (Pimson et al., 2014).

The tripeptide, γ-L-glutamyl-L-cysteinyl-glycine known as glutathione (GSH), is the main low sub-atomic weight cancer prevention agent orchestrated in cells. It is combined with the consecutive expansion of cysteine

How to Cite:
Example: Susilawati, S., Doyan, A., Muliyadi, L., & Hakim, S. (2019). Growth of tin oxide thin film by aluminum and fluorine doping using spin coating Sol-Gel techniques. *Jurnal Penelitian Pendidikan IPA, 1*(1), 1-4. https://doi.org/10.29303/jppipa.v1i1.264

to glutamate followed by the expansion of glycine (Labarrere & Kassab, 2022). The sulfhydryl bunch (−SH) of the cysteine is engaged with decrease and formation responses that are typically thought to be the main elements of GSH. These responses give the means the o evacuation of peroxides and numerous xenobiotic compounds; notwithstanding, GSH is likewise engaged with guidelines of the cell cycle (Noctor et al., 2011) & (Pizzorno, 2014) Glutathione is one of the significant cancer prevention agent frameworks in human science (Watanabe et al., 2014) In any case, the biomedical utilization of glutathione stays restricted because of its moderately short half-life, unsteady properties, and fast digestion and discharge (Said, 2012). The expansion of different HLBs of surfactants moving toward 2-3 as indicated by the Log P skin with an expansion of HLB 7 surfactant which is a combination of Sorbitan monooleate 80 and Polysorbate 80 was considered. Glutathione, which has been tried for surfactant entrance, may lessen MMP1 articulation and might be utilized as a skin drug (Nugrahaeni et al., 2018). Glutathione has low oral bioavailability because of the activity of the catalyst glutamyl transpeptidase (GGT), and when glutathione is directed intravenously, it is less ingested from the gastrointestinal parcel, so one endeavor to stay away from the underlying bait impact is to utilize the neighborhood course (Byeon et al., 2019).

Microspheres are multiparticulate drug delivery systems that are prepared to obtain prolonged or controlled drug delivery to improve bioavailability, and stability and to target the drug to specific site at a predetermined rate (Saralidze et al., 2010). Sodium alginate, a hydrophilic biopolymer got from earthy-colored ocean growth has been viewed as profoundly encouraging as for drug conveyance due to its high organic well-being (Thakur et al., 2018). Synthetically, it is a polysaccharide made out of fluctuating extents of D-mannuronic corrosive (M) and L-guluronic corrosive (G) buildups which are organized in MM or GG blocks scattered with MG blocks. Alginate microspheres are broadly utilized as supported discharge transporters for drugs because of their low immunogenicity and mucosal bond (Hecht & Srebnik, 2016). Its uncommon property of outlining water-insoluble calcium alginate gel through ionotropic gelation with Ca^{2+} particles under fundamental and delicate conditions has made it possible to exemplify both macromolecular trained professionals (Cerciello et al., 2017). Divalent cations (Ca^{2+}) are much of the time used for ionic cross-associating with decrease the crumbling of alginate structures in various applications. Ca^{2+} particles are sandwiched between electronegative alginate molecules, like eggs in an egg box. This is known as the "egg box" model (Bruchet & Melman, 2015). The point of this study was to decide the impact of surfactant expansion for portrayal, and delivery profile of GSH stacked alginate microspheres for effective antiaging.

Reduced L-Glutation ≥ 98.0% (Sigma Aldrich Inc); Sodium alginate (Sigma Aldrich Inc); $CaCl_2.2H_2O$ (Solvay Chemicals International); 5,5-dithio-bis-(2-nitrobenzoicacid) (DTNB) Ellman's Reagent (Sigma Aldrich); Sodium citrate (Weifang Ensign Industry Co. Ltd.); Polysorbate 80 (Merck); Sorbitan Monooleat 80 (Merck); $NaH_2PO_4.2H_2O$ (Merck); $Na_2HPO_4.12H_2O$ (Merck); Distilled water

Method

Preparation of GSH Loaded Alginate Microspheres using Surfactant

Two grams of glutathione (GSH) was broken down in phosphate cradle (pH 6,11 ± 0.05) and afterward HLB7, a combination of surfactants (polysorbate 80 and sorbitan monooleate 80), was added. Glutathione alginate arrangement was showered onto the cross-connecting specialist arrangement ($CaCl_2$) and the combination was mixed at 1000 rpm for 2 hours. The microspheres were washed by centrifugation at 2500 rpm for 6 minutes and two times utilizing a water distiller. Then, glutathione-stacked alginate microspheres were gathered and lyophilized at 80 ° C for 29 hours (Razmjooee et al., 2022). The formula as shown as in the equation (1).

Preparation of Glutathione Loaded Alginate Microspheres

Zero point five (0.5) grams of glutathione was broken up in a 2% alginate arrangement. The alginate-glutathione blend was made into a splash arrangement with a crosslinker ($CaCl_2$) and mixed at 1000 rpm for 2 hours. Microspheres were washed by centrifugation at 2500 rpm for 6 minutes and two times with refined water. Glutathione-stacked alginate microspheres were gathered and lyophilized at 80 ° C for 29 hours (Zhuang et al., 2023).

Determination of Efficiency of entrapment

Microspheres containing 120 mg of medication were precisely weighed to ascertain drug load. The dried microsphere was broken down in 50 ml of sodium citrate 0.1 M and blended at 1000 rpm for 7 hours to remove the medication from the microsphere. The subsequent arrangement was then separated through Whatman channel paper. The absorbance of the subsequent arrangement was estimated at 407 nm regarding water as a clear utilizing an UV spectrophotometer (Dhakar et al., 2010).

$$EE = \frac{\text{Amount of Entrapped GS}H}{\text{Amount of Glutathione}} x100\% \quad (1)$$

Determination of Drug Loading

Microspheres containing 120 mg of medication were precisely weighed to compute drug load. The dried microsphere was broken up in 50 ml of sodium citrate 0.1 M and blended at 1000 rpm for 7 hours to separate the medication from the microsphere. The subsequent arrangement was then sifted through Whatman channel paper. The absorbance of the subsequent arrangement was estimated at 407 nm as for water as a clear utilizing an UV spectrophotometer (Wei et al., 2016).

$$\text{Drug Loading} = \frac{\text{Amount of Entrapped } GSH}{\text{Total Amount of Dried Microspheres}} x100\% \quad (2)$$

Determination of Particle Size

Particles were broke down utilizing magnifying instrument (Micronos Nusantara, OPTILAB Viewer 2.2, Indonesia). The arranged microspheres were put on a glass slide and the normal microsphere size was determined by estimating 300 particles utilizing an adjusted eye micrometer (Yang et al., 2015).

Yield

Yields were calculated as a percentage of total microspheres (grams) divided by the total amount (grams) of polymer and detergent GSH (Meshram et al., 2016).

$$\text{Yield} = \frac{\text{Total Weight of Microspheres}}{\text{Total Weight of Drug and Polymer}} x100\% \quad (3)$$

Surface Morphological Testing

The surface morphology and state of the microsphere was inspected utilizing a checking electron magnifying instrument (SEM), model Carl Zeiss MA10, USA at room temperature at a suitable amplification. Micrographs were analyzed for morphological elements to affirm the sphericity of the microsphere (Corti & others, 2020).

In-Vitro Release of Glutathione-Alginate Microspheres

Discharge trial of gel-based and non-gel-based glutathione-stacked alginate microspheres were performed utilizing a Franz dissemination cell gadget and the outcomes were investigated by ANOVA utilizing SPSS programming (variant 20). The analysis was performed multiple times and the mean and standard deviation were estimated. How much GSH not set in stone by spectrophotometric examination at a frequency of 407 nm for PBS pH 6.0 ± 0.05 (1: 1 v/v) as a clear (Hariyadi & Islam, 2020).

Result and Discussion

Glutathione-alginate microspheres were explored to get streamlined recipe and study the impact of autonomous factors, expansion of surfactant. The free factor is IBM SPSS Statistic 20ver. Was investigated utilizing. As displayed in the table, two unique plans were acquired. All definitions were arranged utilizing ionotropic gelation innovation and restored for 2 hours. All details were assessed for catch proficiency, drug stacking, molecule size, yield, and in vitro drug discharge profile (Hariyadi et al., 2019).

Table 1. Formulation of GSH-Alginate Microspheres

Compounds	Function	Compound Concentration	
		I	II
GSH	Active Compound	0.5 g	-
GSH +surfactant HLB 7	Active Compound	-	0.5 g
Sodium Alginate	Polymer	2%	2%
$CaCl_2$ Solution	Cross-linker	1 M	1 M

One of the benefits of microspheres is that they protect the drug from chemical degradation, chemical degradation, and cleavage by photodegradation. Therefore, it turns out to be optimal for protein drug delivery (Salem et al., 2020). As shown in Table 2, the capture efficiency of GSH microspheres was found to be in the range of F1 (41.50 ± 1.12) to F2 (50.36 ± 0.86). As the independent T-test shows, the addition of detergent has a p-value of 0.000 and has a significant effect on capture efficiency (EE). It was found that the capture efficiency of the F2 preparation was higher than that of F1, and that the addition of the surfactants of polysorbate 80 and sorbitan monooleate 80 increased the viscosity and thus increased the capture of the drug. These results are in good agreement with the results of EE of Microsphere loaded with cefixime was reported to be higher for the drug: alginate with detergent polysorbate 80 and sorbitan monooleate 80 (Escudero et al., 2014).

The medication heap of the F2 readiness was higher than that of different arrangements. The hypothetical centralization of medication in microspheres was assessed to be 5.72 ± 0.05% for F1 and 6.00 ± 0.03% w/v for F2. As shown by the free T-test, the expansion of cleanser fundamentally affects drug stacking (DL) with a p-worth of 0.018. The medication heap of F2 has expanded. This is because of the better adjustment of the inner drops with the expansion of surfactant 21.

The expansion of surfactant builds the consistency, which influences the communication between the scattered stage and the scattering medium, which influences the size dissemination of the particles. The molecule size results show that all GSH microspheres created have a molecule size of under 3 µm and are successful for percutaneous ingestion. The molecule size of microspheres was found to increment with the

expansion of surfactants (polysorbate 80 and sorbitan monooleate 80), which expanded the bead size and brought about an expansion in molecule size. It could be because of the great consistency of the specialist.

Table. 2. Characterization of Glutathione-Alginate Microspheres

Formula	EE (%)	Drug Loading (%)	Particle Size (µm)	Yield(%)	Flux (µg/cm²/h)
F1	41.50 ± 1.12	5.72 ± 0.05	1.89 ± 0.03	88.80 ± 1.41	0.018 ± 0.001
F2	50.36 ± 0.86	6.00 ± 0.03	2.06 ± 0.09	88.58 ± 0.98	0.030 ± 0.002

From the percent yield analysis of glutathione dinate microspheres, it was observed that the addition of detergent in the formulation increased and the yield also increased. The low yields of some formulations may be due to microspheres lost during the washing process (Meimaridou, 2007). Percent yields for all formulations varied from 88.80 ± 1.41% for F1 to 89.13 ± 0.09% for F2.

FTIR otherworldly investigation was performed on cleanser free microspheres (F1) and cleanser added microspheres (F2) and sans drug microspheres. This study affirms the cross-connecting of alginate GSH blended microspheres with CaCl2 and the shortfall of compound responses between the medication and the polymer. The wavenumber of the carboxyl gatherings of F1 and F2 was 1423.52 cm-1. 1421.25 cm1. At 1614 cm1, certain glulonic fingerprints vanished, affirming the development of bonds by the cross-connecting response among alginate and $CaCl_2$ (cross-connecting specialist). This incorporates particle trade between the carboxyl gathering of gluronic corrosive and the cross-connecting specialist Ca^{2+} (Dhakar et al., 2010).

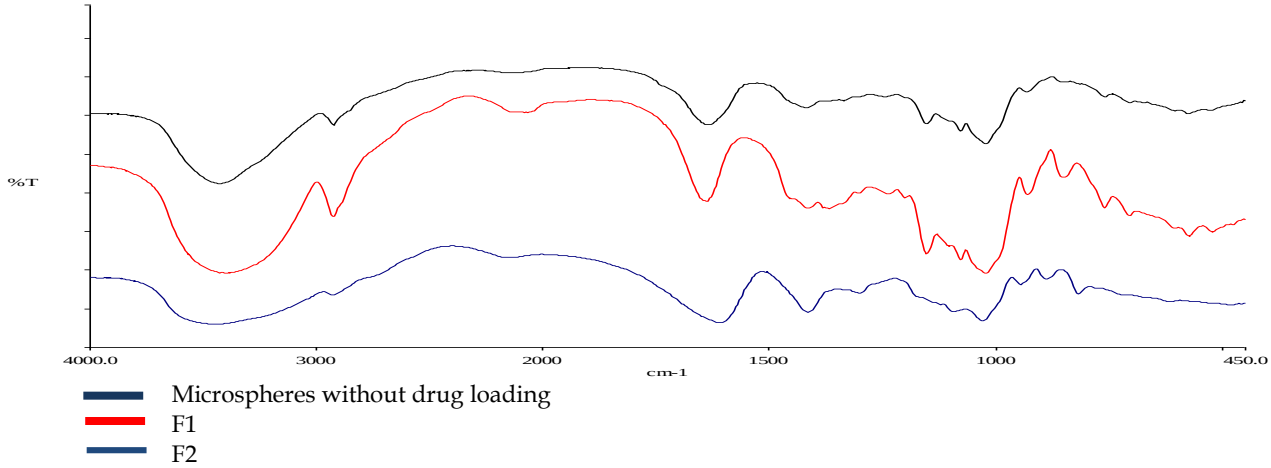

Figure 1. FTIR spectral of Microspheres Glutathione-Alginate

In vitro discharge was evaluated at 64.16 ± 0.04% for F1 and 55.20 ± 0.07% for drug item F1 north of 10 hours. The expansion of the cleansers polysorbate 80 and sorbitan monooleate 80 decreased drug discharge. This might be because of an expansion in thickness, which increments molecule size and diminishes surface region. Expanded thickness can likewise build the length of the dissemination pathway, which may likewise be the justification for the decreased medication discharge. The arrival of glutathione from polymer dots can be made sense of by two components (Ku & Gan, 2019). The medication is set by dissemination free from the exemplified alginate microspheres. Second, the medication is filtered out of the microsphere by disintegration and/or corruption of the lattice. The last peculiarity might be because of the expulsion of calcium crosslinkers from microspheres. The expanding of alginate particles builds the porosity of the lattice and consequently increments both dispersion and disintegration (Feng et al., 2023). These discoveries are in great concurrence with the higher medication to-polymer proportion utilized in definition F1. Phosphate cradle has a chelating impact by phosphate particles, which further speeds up the deterioration of the grid. Both of our definitions showed supported arrival of glutathione north of 10 hours. For definitions containing higher measures of polymers, F2 and F2-based gels, a more slow delivery design was noticed. Comparative outcomes were acquired for microspheres stacked with ropinirole hydrochloride detailed in past examinations.

Conclusion

Glutathione microspheres were effectively ready by aerosolization utilizing the ionotropic gelation strategy. Polymer/drug proportion impacted molecule size and medication discharge design in microspheres. All equations delivered high return and exemplification

productivity and little size particles. From the free Test, It was shown that the mix of surfactant and polymer fixation use fundamentally affected DL and EE, however not on yield and molecule size. For the improved recipe, we picked recipe F2, which utilized 2.0% alginate and 1 M CaCl 2 and added a cleanser to HLB7. Assessment of delivery energy showed that medication discharge from glutathione dinate microspheres followed the Matrix Higuchi model (dissemination controlled drug discharge component). This detailing might be suggested for movement and steadiness testing for additional streamlining as a skin drug conveyance framework.

Acknowledgments
Place acknowledgments, including information on grants received, before the references, in a separate section, and not as a footnote on the title page

Author Contributions
The authors of this paper consist of three people, namely A.R, N.A, and D.A.C.R. This paper was able to be completed because of the collaboration of the writing team at each stage.

Funding
This research received no external funding.

Conflicts of Interest
The authors declare no conflict of interest.

References

Bermejo, S., García-Carro, C., & Soler, M. J. (2021). Diabetes and renal disease—should we biopsy? *Nephrology Dialysis Transplantation*, *36*(8), 1384–1386. https://doi.org/10.1093/ndt/gfz248

Bruchet, M., & Melman, A. (2015). Fabrication of patterned calcium cross-linked alginate hydrogel films and coatings through reductive cation exchange. *Carbohydrate Polymers*, *131*, 57–64. https://doi.org/10.1016/j.carbpol.2015.05.021

Byeon, J. C., Lee, S.-E., Kim, T.-H., Ahn, J. Bin, Kim, D.-H., Choi, J.-S., & Park, J.-S. (2019). Design of novel proliposome formulation for antioxidant peptide, glutathione with enhanced oral bioavailability and stability. *Drug Delivery*, *26*(1), 216–225. https://doi.org/10.1080/10717544.2018.1551441

Cerciello, A., Del Gaudio, P., Granata, V., Sala, M., Aquino, R. P., & Russo, P. (2017). Synergistic effect of divalent cations in improving technological properties of cross-linked alginate beads. *International Journal of Biological Macromolecules*, *101*, 100–106. https://doi.org/10.1016/j.ijbiomac.2017.03.077

Corti, M., & others. (2020). *Development of biocompatible materials for the immobilization of macromolecules of pharmaceutical interest*. Retrieved from https://iris.unipv.it/bitstream/11571/1321846/2/TESI COMPLETA 21 gennaio 2020.pdf

Dhakar, R. C., Maurya, S. D., Sagar, B. P. S., Bhagat, S., Prajapati, S. K., & Jain, C. P. (2010). Variables influencing the drug entrapment efficiency of microspheres: A pharmaceutical review. *Der Pharmacia Lettre*, *2*(5), 102–116. Retrieved from https://ssrn.com/abstract=3774651

Escudero, I., Geanta, R. M., Ruiz, M. O., & Benito, J. M. (2014). Formulation and characterization of Tween 80/cholestherol niosomes modified with tri-n-octylmethylammonium chloride (TOMAC) for carboxylic acids entrapment. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *461*, 167–177. https://doi.org/10.1016/j.colsurfa.2014.07.042

Feng, J., Yap, X. Y., Gao, J., Gan, C. L., Wang, R., & Qian, S. (2023). Rapid self-sealing of macro cracks of cementitious composites by in-situ alginate crosslinking. *Cement and Concrete Research*, *165*, 107074. https://doi.org/10.1016/j.cemconres.2022.107074

Hariyadi, D. M., & Islam, N. (2020). Current status of alginate in drug delivery. *Advances in Pharmacological and Pharmaceutical Sciences*, *2020*. https://doi.org/10.1155/2020/8886095

Hariyadi, D. M., Rosita, N., & Rahayu, A. (2019). Design, optimization and characterization of glutathione loaded-alginate microspheres for topical antiaging. *Journal of Pharmacy \& Pharmacognosy Research*, *7*(4), 223–233. Retrieved from https://www.researchgate.net/publication/340771084_Design_optimization_and_characterization_of_glutathione_loaded-_alginate_microspheres_for_topical_antiaging

Hecht, H., & Srebnik, S. (2016). Structural characterization of sodium alginate and calcium alginate. *Biomacromolecules*, *17*(6), 2160–2167. https://doi.org/10.1021/acs.biomac.6b00378

Ku, J. W. K., & Gan, Y.-H. (2019). Modulation of bacterial virulence and fitness by host glutathione. *Current Opinion in Microbiology*, *47*, 8–13. https://doi.org/10.1016/j.mib.2018.10.004

Labarrere, C. A., & Kassab, G. S. (2022). Glutathione: A Samsonian life-sustaining small molecule that protects against oxidative stress, ageing and damaging inflammation. *Frontiers in Nutrition*, *9*, 2653. https://doi.org/10.3389/fnut.2022.1007816

Lagman, M., Ly, J., Saing, T., Kaur Singh, M., Vera Tudela, E., Morris, D., Chi, P.-T., Ochoa, C., Sathananthan, A., & Venketaraman, V. (2015). Investigating the causes for decreased levels of glutathione in individuals with type II diabetes. *PloS One*, *10*(3), e0118436. https://doi.org/10.1371/journal.pone.0118436

Meimaridou, E. (2007). *Calcium oxalate modulation of tubular epithelial cell mitochondria: oxidative vulnerability due to restricted glutathione homeostasis*. University of London, University College London (United Kingdom).

Meshram, B. M. S., Mishra, A., & Pathak, A. (2016). Formulation and evaluation of microspheres of Rabiprazole sodium. *International Journal of Advances in Pharmaceutics*, *53*, 76–83. https://doi.org/10.7439/ijap

Noctor, G., Queval, G., Mhamdi, A., Chaouch, S., & Foyer, C. H. (2011). *Glutathione. Arabidopsis Book*.

Nugrahaeni, F., Hariyadi, D. M., & Rosita, N. (2018). Partition coefficient and glutathione penetration of topical antiaging: preformulation study. *International Journal of Drug Delivery Technology*, *8*(2), 39–43. Retrieved from https://repository.unair.ac.id/86276/

Pimson, C., Chatuphonprasert, W., Jarukamjorn, K., & others. (2014). Improvement of antioxidant balance in diabetes mellitus type 1 mice by glutathione supplement. *Pak. J. Pharm. Sci*, *27*(6), 1731–1737. Retrieved from https://scholar.google.com/scholar?hl=id&as_sdt=0%2C5&q=Pimson+C%2C+Chatuphonprasert+W%2C+Jarukamjorn+K.+Improvement+of+antioxidant+balance+in+diabetes+mellitus+type+1+mice+by+glutathione+supplement.+Pakistan+journal+of+pharmaceutical+sciences.+2014+Nov+1%3B27%3A1731–7.+&btnG=

Pizzorno, J. (2014). Glutathione. Integrative Medicine. *A Clinician's Journal*, *13*(1), 8. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4684116/

Razmjooee, K., Oustadi, F., Golaghaei, A., & Nassireslami, E. (2022). Carboxymethyl chitosan--alginate hydrogel containing GSNO with the ability to nitric oxide release for diabetic wound healing. *Biomedical Materials*, *17*(5), 55013. https://doi.org/10.1088/1748-605X/ac877c

Said, H. M. (2012). *Physiology of the Gastrointestinal Tract, Two Volume Set*. Academic Press.

Salem, S., Sakir, M., Sahin, K., Korkmaz, I., Yavuz, E., Sarp, G., Onses, M. S., & Yilmaz, E. (2020). Low bandgap microsphere-like magnetic nanocomposite: an enhanced photocatalyst for degradation of organic contaminants and fabrication of SERS-active surfaces. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *589*, 124436. https://doi.org/10.1016/j.colsurfa.2020.124436 Get rights and content

Saralidze, K., Koole, L. H., & Knetsch, M. L. W. (2010). Polymeric microspheres for medical applications. *Materials*, *3*(6), 3537–3564. https://doi.org/10.3390/ma3063537

Tesauro, M., Nisticò, S., Noce, A., Tarantino, A., Marrone, G., Costa, A., Rovella, V., Di Cola, G., Campia, U., Lauro, D., & others. (2015). The possible role of glutathione-S-transferase activity in diabetic nephropathy. *International Journal of Immunopathology and Pharmacology*, *28*(1), 129–133. https://doi.org/10.1177/0394632015572564

Thakur, S., Sharma, B., Verma, A., Chaudhary, J., Tamulevicius, S., & Thakur, V. K. (2018). Recent progress in sodium alginate based sustainable hydrogels for environmental applications. *Journal of Cleaner Production*, *198*, 143–159. https://doi.org/10.1016/j.jclepro.2018.06.259

Watanabe, F., Hashizume, E., Chan, G. P., & Kamimura, A. (2014). Skin-whitening and skin-condition-improving effects of topical oxidized glutathione: a double-blind and placebo-controlled clinical trial in healthy women. *Clinical, Cosmetic and Investigational Dermatology*, 267–274. https://doi.org/10.2147/CCID.S68424

Wei, Y., Wang, Y., Zhang, H., Zhou, W., & Ma, G. (2016). A novel strategy for the preparation of porous microspheres and its application in peptide drug loading. *Journal of Colloid and Interface Science*, *478*, 46–53. https://doi.org/10.1016/j.jcis.2016.05.045

Yang, H., Kang, W., Liu, S., Bai, B., Zhao, J., & Zhang, B. (2015). Mechanism and influencing factors on the initial particle size and swelling capability of viscoelastic microspheres. *Journal of Dispersion Science and Technology*, *36*(11), 1673–1684. https://doi.org/10.1080/01932691.2014.1000463

Zhuang, H., Shao, J., Wu, P., Yu, G., Fu, K., Sun, Z., Cao, M., Liu, Y., & Zhou, Y. (2023). Nitric oxide releasing alginate microspheres for antimicrobial application. *International Journal of Biological Macromolecules*, *224*, 1244–1251. https://doi.org/10.1016/j.ijbiomac.2022.10.210