



Optimization of chitosan and sodium tripolyphosphate as a carrier system for nanoparticles of ethanol extract of aloe vera (*Aloe vera* L.) as an antioxidant

Erin Efrilia^{1*}, Titik Sunarni¹, Ilham Kuncahyo¹

¹Master of Pharmacy, Universitas Setia Budi, Indonesia

Article Info

Article history:

Received January 9, 2025

Revised February 3, 2025

Accepted February 7, 2025

Keywords:

Antioxidant

Chitosan

Aloe vera

Nanogels

Sodium tripolyphosphate

ABSTRACT

Aloe Vera has phenolic active compounds that are proven to have potential antioxidant activity but are sensitive to light, easily oxidized and unstable at high temperatures. To overcome this, aloe vera is formulated into a chitosan and sodium tripolyphosphate carrier system. The interaction between chitosan and sodium tripolyphosphate for nanoparticle formation is strongly influenced by the ratio of both. The purpose of this study was to obtain the optimal formula of aloe vera extract nanoparticle preparation with chitosan and sodium tripolyphosphate carrier system with critical parameters including particle size, polydispersity index, zeta potential, sorption efficiency and antioxidant activity. Aloe vera was extracted with 96% ethanol using maceration method. Determination of chitosan and sodium tripolyphosphate concentration variation using simplex lattice design method. The results of the tests that have been carried out, the characteristics of aloe vera nanoparticle preparations with variations in the concentration of chitosan: sodium tripolyphosphate in runs 2 & 6 have the most optimal formula with the ratio of chitosan: sodium tripolyphosphate = (0.3: 0.1).

This is an open access article under the [CC BY-SA](https://creativecommons.org/licenses/by-sa/4.0/) license.



1. Introduction

Free radicals are highly reactive atoms or molecules with unpaired electrons. Various methods are used to prevent or repair the effects of free radicals. The body needs a

* Corresponding Author:

Erin Efrilia,

Master of Pharmacy,

Universitas Setia Budi,

Indonesia.

Email: efriliaerin@gmail.com

DOI: <https://doi.org/10.52465/johmpe.v3i1.532>

substance that can provide protection from free radical attacks, namely antioxidants. The use of synthetic antioxidants is starting to be limited because the results of research that has been done show that synthetic antioxidants such as BHT (Butylated Hydroxy Toluene) can poison experimental animals and are carcinogenic. Traditional medicinal plants found in Indonesia are very diverse, one example of a plant that contains antioxidants is the aloe vera plant [1]–[4].

Based on research conducted by Rohiyati et al.[5], it was concluded that 96% ethanol extract of aloe vera has an IC50 value of 49.03 ppm. Phenolic compounds found in aloe vera flesh show instability in various processing and storage conditions. Phenolic compounds are very sensitive to high temperatures, light exposure and oxidation. Phenolic effectiveness is highly dependent on maintaining the stability and activity of the active ingredient, in this context, encapsulation techniques can provide the necessary protection for bioactive compounds that are sensitive to oxidation and decomposition of natural extracts. Madan & Singh [6], stated that drug delivery is influenced by the size of the particles, due to the presence of a skin membrane barrier. The smaller the particle size, the easier it is to penetrate the skin membrane barrier and the better the effect. One way that can be used to increase the effectiveness of aloe vera extract and avoid oxidation and decomposition is to formulate it into a nanoparticle preparation.

Nanoparticles from natural polymer chitosan are widely applied to drug delivery systems because of their special properties such as biocompatible, biodegradable, mucoadhesive and increasing permeation. Biopolymers as ingredients in this drug formulation are common for several reasons, namely they are inert to active ingredients but compatible for combination and have special characteristics, for example in the use of various sugar polymer derivatives [7]–[9].

This study aims to optimize the chitosan nanoparticle carrier system with sodium tripolyphosphate using the ionic gelation method. Among the various methods of making chitosan nanoparticles, ionic gelation is a method that has attracted the attention of many researchers because the process is simple, does not use organic solvents, and can be easily controlled. The principle of particle formation in this method is the occurrence of ionic interactions between positively charged amino groups in chitosan with negatively charged polyanions forming a three-dimensional inter and/or intramolecular network structure. The most widely used polyanion crosslinker is sodium tripolyphosphate, because it is non-toxic and multivalent. This physical crosslinking process not only avoids the use of organic solvents, but also prevents the possibility of damage to the active ingredients that will be encapsulated in chitosan nanoparticles [9]–[11].

2. Method

2.1. Materials

The materials used in this study were aloe vera, chitosan (Brataco), DPPH (Brataco), sodium tripolyphosphate (Brataco), 95% ethanol (Onemed), acetic acid (Brataco), isopropyl myristate (technical, Brataco), DPPH, propylene glycol (Brataco), HPMC (Brataco),

methylparaben, aquadest (Ikapharmindo), ethanol (Brataco), Follin ciocalteu reagent (Merck), gallic acid (sigma-Aldrich), NaCl (Otsu), demineralized water (Brataco).

2.2. Tools

The tools used include a UV-Vis spectrophotometer (Genesys 10S UVVIS), magnetic stirrer (Thermo Scientific Cimarec), refrigerator (Polytron), glassware (Pyrex), blender (Miyako), maceration vessel, flannel cloth, sieve mesh no. 40, separating funnel, clamp & statip rod, filter paper, Buchner funnel (NefMedSupply), cuvette, micropipette (Drago Lab), Zetasizer Malvern (Zetasizer Nano-Zs 90, MPT-Z, UK), vernier caliper.

2.3. Extraction

Making 96% aloe vera ethanol extract is done by inserting one part of the leaf flesh that has been mashed with a blender into a macerator, adding 6 parts of 96% ethanol left for 5 days while continuing to stir repeatedly. The extract is filtered with flannel cloth and evaporated until the alcohol is gone with a rotary evaporator at low pressure until a thick extract is obtained.

2.4. Formulation of Aloe Vera Nanoparticles

Chitosan nanoparticles were made using the ionic gelation method, namely the polyelectrolyte complexation between positively charged chitosan and negatively charged tripolyphosphate. The combination of concentrations between chitosan and tripolyphosphate was determined using Simplex lattice design.

Table 1. Formulation of *Aloe vera* nanoparticles

Ingredients	Amount	Functions
Aloe vera extract	100 mg	Active ingredients
Chitosan	0,2% - 0,4%	Polycation
Tripolyphosphate	0,1% - 0,2%	Polyanion

Table 2. Optimisation simplex lattice design

Formula	Chitosan	Tripolyphosphate
Run 1	0,2	0,2
Run 2	0,3	0,1
Run 3	0,25	0,15
Run 4	0,2	0,2
Run 5	0,225	0,175
Run 6	0,3	0,1
Run 7	0,275	0,125
Run 8	0,25	0,15

Nanoparticles were made by: Chitosan solution with various concentrations according to Table 3 was dissolved in 1% acetic acid solution using a magnetic stirrer as much as 100 ml. Tripolyphosphate solution was made by dissolving various concentrations of sodium tripolyphosphate in distilled water using a magnetic stirrer as much as 100 ml. 100 mg of aloe vera extract was dispersed in 100 ml of distilled water, then mixed with 100 ml of chitosan solution and 100 ml of tripolyphosphate solution so that the amount of aloe vera

extract nanoparticles was obtained as much as 300 ml, mixing and reducing the size using a magnetic stirrer. After mixing for 5 minutes, the TPP solution was added to the mixture drop by drop slowly. Mixing and reducing the size using a magnetic stirrer was carried out again for 5 minutes [12].

2.5. Characterization of Nanoparticle Preparations

Characterization of nanoparticle preparations includes particle size, polydispersity index and zeta potential using the Malvern Zetasizer. Entrapment efficiency was carried out by dissolving 5 mL of aloe vera nanoparticles in 5 mL of ethanol, then centrifuged at 350 rpm for 40 minutes until two layers were formed in a test tube. The upper layer of the supernatant containing unencapsulated phenolics was separated for analysis. Entrapment efficiency was evaluated based on the concentration of unencapsulated phenolics in the supernatant. Unencapsulated phenolics in the supernatant that had previously been dissolved in ethanol before the centrifugation process were then analyzed using a UV-Vis spectrophotometer at a wavelength of 755 nm.

2.6. Determination of Antioxidant Activity

Each nanoparticle stock solution was added with 1 ml of DPPH solution, the volume was made up with methanol to the limit mark and homogenized. Incubated then the absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength.

3. Results and Discussion

The nanoparticle preparation that has been made as many as 8 runs was then characterized including particle size, polydispersity index, zeta potential and entrapment efficiency. The following data are the results of the characteristic test of the nanoparticle preparation:

Table 3. Characteristic test results of *Aloe vera* extract nanoparticles

Run	Chitosan: TPP (%)	Particle Size (nm)	Polydispersity index	Zeta potential (mV)	Adsorption Efficiency (%)
1	0.2: 0.2	842.8	0.8475	-29.53	72.13
2	0.3: 0.1	718.6	0.8589	-30.52	74.66
3	0.25: 0.15	818.7	0.8867	-31.14	73.80
4	0.2: 0.2	851.7	0.7791	-29.57	72.16
5	0.225: 0.175	885.1	0.7704	-30.08	78.83
6	0.3: 0.1	756.3	0.7922	-30.78	76.03
7	0.275: 0.125	674.0	0.8277	-36.94	71.66
8	0.25: 0.15	811.3	0.8322	-30.95	74.39

Particle size plays an important role in determining drug distribution and release, targeting ability of nanoparticle systems, and toxicity. The smaller the particle size, the greater the surface area of the particles so that the desired activity of the active substance will be achieved[13], [14]. Differences in particle size in each formula can be caused by differences in the concentration of the carrier system, namely chitosan and sodium tripolyphosphate used in each formula. The results of particle size testing that have been carried out, the particle size of 8 formulas ranges from 674.0 - 885.1. Where the smallest

particle size is in run 7 with a comparison of the chitosan and sodium tripolyphosphate carrier systems (0.275%:0.125%), while for the largest particle size in run 5 with a ratio of chitosan and sodium tripolyphosphate (0.225%: 0.175%).

The polydispersity index value is used to see the distribution of particle sizes that occur in the formulated nanoparticles. The range of polydispersity index values is between 0 and 1. Polydispersity index values approaching 0 indicate homogeneous dispersion, while polydispersity index values exceeding 0.7 indicate high heterogeneity [15], [16]. The results of measuring the polydispersity index of 8 formulations have high polydispersity index values for each formula, ranging from 0.7704-0.8867

The zeta potential of a nanoparticle is usually used to characterize the surface charge properties related to the electrostatic interactions of nanoparticles. Zeta potential is a measure of the surface charge of particles that are spread in relation to the dispersing medium. Particles must have a high charge or zeta potential compared to the dispersing medium to prevent aggregation. The repulsive force carried by similar ion charges on surface particles will prevent the attractive force determined by hydrogen bonds. By controlling zeta, optimal conditions are obtained to prevent aggregation [17]–[19]. Based on the results of the research test, the zeta potential value of 8 formulas ranged from -29.53 to -36.94. Nanoparticles with a zeta potential value less than -30 mV and greater than +30 mV have higher stability. Particles that have a large negative or positive zeta potential value tend to repel each other. However, if it has a low zeta potential value, approaching zero), the attractive force between particles becomes dominant and the particles experience aggregation and grafting. Dispersion systems with low zeta potential values are easier to form aggregates along with the Van der Waals force in particle interactions.

Entrapment efficiency is a determination that aims to determine how much active compound is trapped in the encapsulation process and determine the level of success of the encapsulation process. Entrapment efficiency is an important parameter to evaluate the success of a drug delivery system. This value indicates the percentage of encapsulated material (eg, active ingredients, drugs, etc.) that is successfully trapped into the drug carrier after the encapsulation process for protection, absorption, delivery into the body, and controlled release [20]. The percentage value of entrapment efficiency is one of the parameters of encapsulation success where the higher the entrapment efficiency value, the greater the success value of the encapsulation stage. The entrapment efficiency with the highest value in this study was in formula 5 at 78.83%.

The antioxidant activity test was carried out using the DPPH method. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a synthetic radical that is sensitive to light, oxygen, and pH but is stable at room temperature and soluble in polar solvents[21]. The results obtained are in the form of an IC50 value which determines the effectiveness of aloe vera extract nanoparticles in counteracting free radicals by 50%. A sample is stated to have very strong antioxidant activity if the IC50 value is less than 50 ppm. The examination of antioxidant activity in aloe vera extract nanoparticles aims to determine the antioxidant activity contained in the aloe vera extract nanoparticles that have been made. In this test, vitamin C is used as a positive control. Testing antioxidant activity by soaking in DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals is carried out by making a sample solution and a positive control of vitamin C in several concentration series, namely 10, 20, 30, 40 and 50

ppm. Then the absorbance per concentration of the sample solution and the positive control is measured at a maximum wavelength of 518 nm. In testing the antioxidant activity of aloe vera nanoparticles, 3 replications were carried out and the IC₅₀ values were obtained as follows:

Table 4. IC₅₀ value of *Aloe vera* extract nanoparticles formula

	Average IC ₅₀ (ppm)
1	106.011 ± 2.826
2	87.886 ± 11.047
3	95.342 ± 5.489
4	109.122 ± 5.695
5	85.718 ± 3.811
6	93.833 ± 7.350
7	123.226 ± 10.611
8	89.066 ± 1.904
Vit C	18.041 ± 2.948

The results of the antioxidant activity test on the aloe vera nanoparticle preparation above show that the aloe vera nanoparticle preparation has an IC₅₀ value ranging from 85.718-123.226 ppm and is included in the strong-medium antioxidant group. Where according to Mardawati.[22], strong antioxidant activity ranges between 50-100 pm and moderate antioxidants range between 101-150 ppm. Based on the results of the calculation of the percentage of vitamin C inhibition, the IC₅₀ value of vitamin C is 18.041 ppm. This means that the antioxidant activity of vitamin C is very strong. It can be seen from the results obtained, it is known that the concentration affects the absorbance value. The greater the concentration of aloe vera extract nanoparticles and positive control (vitamin C), the smaller the absorbance value and the greater the percentage inhibition value obtained. The lowest IC₅₀ value of aloe vera extract nanoparticles was in run 7 with a value of 123.226 ppm. These results are much lower when compared to the positive control, namely vitamin C. This means that the positive control has very strong antioxidant activity, because it is a pure compound. The difference in nanoparticle activity in the sample formulation run is influenced by the concentration of the carrier system used, namely chitosan and tripolyphosphate. Nanoparticles that have the highest antioxidant activity are in formula run 5 with a chitosan:tpp ratio of 0.225:0.175 with the smallest IC₅₀ value of 87.718 ppm. Because the smaller the IC₅₀ value, the more effective the antioxidant activity. This shows that reducing the color of free radicals to half its concentration in run 5 is more effective than the other runs.

Determination of the optimum area of *aloe vera* extract nanoparticles using simplex lattice design software in this study used a numerical approach to determine the optimum formula. The data entered as parameters are the characteristics of the nano preparation including particle size, polydispersity index, zeta potential and entrapment efficiency and antioxidant activity. The most optimal formula is the formula with the maximum desirability value. The desirability value is the value of the optimization objective function that shows the program's ability to meet what is desired based on the criteria specified in the final product. The desirability value ranges from 0 to 1. Where when the desirability value increases and approaches 1, it indicates that the results are increasingly optimal in

producing the desired product. The optimization score itself is not always considered good to achieve a value of 1, but to find the best conditions in uniting all functions [23]–[25].

Table 5. Optimum formula with simplex lattice design

Chitosan	Tripolyphosphate	Particle Size	Polydispersity index	Zeta potential	Adsorption Efficiency	Antioxidant activity	Desirability
0,300	0,100	728,81	0,784	-30,675	73,538	90,103	0,697

The models for each response are all significant as indicated by a P-value of less than 0.05, so it can be concluded that the response is in accordance with the model. In addition, the lack of fit of the five responses is all not significant as indicated by a P-value of more than 0.05, so it can be concluded that the model is in accordance with the data. A good regression model can be seen from the R2 value. The five responses have a fairly high R2 value, indicating a good correlation between the independent variables and the response. The optimum formula obtained was then made into aloe vera extract nanoparticles and tested on each parameter.

Table 6. Optimum formula characteristic testing results

Response	Predicted Mean	Predicted Median	Data Mean	95% PI high
Ukuran partikel Indeks	728,81	728,82	742,3	779,638
Polidispersitas	0,78375	0,78375	0,7829	0,859719
Zeta Potensial	-30,6746	-30,6746	-30,08	-29,9637
Efisiensi Penjerapan	73,5384	73,5384	74,7	74,7611
Aktivitas Antioksidan	90,1029	90,1029	95	101,19

Predicted response characteristics of aloe vera extract nanoparticles optimum formula obtained from analysis using Design Expert software version 12, then compared with the response characteristics of the optimum formula obtained in the test using IBM® SPSS® Statistics 25 software

4. Conclusion

The optimum proportion of chitosan and sodium tripolyphosphate was obtained from the results of tests that had been carried out on the characteristics of aloe vera nanoparticle preparations and antioxidant activity tests of aloe vera nanoparticles with variations in the concentration of chitosan: sodium tripolyphosphate in runs 2 & 6 using the simplex lattice design method had the most optimum formula with a ratio of chitosan: sodium tripolyphosphate = (0.3:0.1).

REFERENCES

- [1] V. Saritha, K. Anilakumar, dan F. Khanum, "Antioxidant and Antibacterial Activity of Aloe vera Gel Extracts," *Int. J. Pharm. Biol. Arch.*, vol. 1, hal. 376–384, Jan 2010.
- [2] E. Christaki dan P. Florou-Paneri, "Aloe vera: A Plant for Many Uses," *J. Food, Agric. Environ.*, vol. 8, Apr 2010.
- [3] K. S. Ahlawat dan B. S. Khatkar, "Processing, Food Applications and Safety of Aloe vera Products: A Review," *J. Food Sci. Technol.*, vol. 48, no. 5, hal. 525–533, 2011, doi:

- 10.1007/s13197-011-0229-z.
- [4] K. Salawu, E. Ajaiyeoba, O. Ogbale, A. Adeniji, T. Faleye, dan A. Agunu, "Antioxidant, Brine Shrimp Lethality, and Antiproliferative Properties of Gel and Leaf Extracts of Aloe schweinfurthii and Aloe vera," *J. Herbs. Spices Med. Plants*, vol. 23, hal. 1–9, Mei 2017, doi: 10.1080/10496475.2017.1318328.
- [5] M. Y. Rohiyati, Yohanes Juliantoni, dan A. Hakim, "Formula dan Uji Aktivitas Antioksidan Sediaan Masker Peel Off Ekstrak Daun Lidah Buaya (Aloe vera Linn.)," *Unram Med. J.*, vol. 9, no. 4, hal. 317–322, 2021, doi: 10.29303/jku.v9i4.426.
- [6] J. Madan dan R. Singh, "Formulation and Evaluation of Aloe vera Topical Gels," *Int. J. Pharm. Sci.*, vol. 2, hal. 551–555, Mei 2010.
- [7] R. Schellekens *et al.*, "Film Coated Tablets (ColoPulse technology) for Targeted Delivery in the Lower Intestinal Tract: Influence of the Core Composition on Release Characteristics," *Pharm. Dev. Technol.*, vol. 17, hal. 40–47, Okt 2010, doi: 10.3109/10837450.2010.513986.
- [8] I. Khan, K. Saeed, dan I. Khan, "Nanoparticles: Properties, Applications and Toxicities," *Arab. J. Chem.*, vol. 12, no. 7, hal. 908–931, 2019, doi: 10.1016/j.arabjc.2017.05.011.
- [9] R. Salama, H. Osman, dan H. M. Ibrahim, "Preparation of Biocompatible Chitosan Nanoparticles Loaded with Aloe vera Extract for Use as a Novel Drug Delivery Mechanism to Improve the Antibacterial Characteristics of Cellulose-based Fabrics," *Egypt. J. Chem.*, vol. 65, no. 3, hal. 581–595, 2022, doi: 10.21608/ejchem.2021.96434.4512.
- [10] E. S. Kim, Y. Baek, H. J. Yoo, J. S. Lee, dan H. G. Lee, "Chitosan-Tripolyphosphate Nanoparticles Prepared by Ionic Gelation Improve the Antioxidant Activities of Astaxanthin in the In Vitro and In Vivo Model," *Antioxidants*, vol. 11, no. 3, 2022, doi: 10.3390/antiox11030479.
- [11] A. Setiawan, D. R. Widiana, dan P. N. A. Nugroho, "Sintesis dan Karakterisasi Kitosan Mikropartikel dengan Modifikasi Gelasi Ionik," *J. Perikan. Univ. Gadjah Mada*, vol. 17, no. 2, hal. 90–95, 2015.
- [12] Suryani *et al.*, "The Self-nanoemulsifying Drug Delivery System Formulation of Mefenamic Acid," *Asian J. Pharm.*, vol. 13, no. 4, hal. 287, Jul 2019.
- [13] S. M. Jafari, "An Overview of Nanoencapsulation Techniques and Their Classification," S. M. B. T.-N. T. for the F. and N. I. Jafari, Ed. Academic Press, 2017, hal. 1–34.
- [14] Y. Amgaonkar, N. Kochar, A. Chandewar, S. Bute, K. Wadher, dan M. Umekar, "Overview of Emulgel as Emergent Topical Delivery: Recent Applications and Advancement," *J. Pharm. Res. Int.*, vol. 33, hal. 258–268, 2021, doi: 10.9734/jpri/2021/v33i62b35565.
- [15] Y. Anggraeni, "Formulasi Sediaan Gel Transdermal Glukosamin HCl untuk Terapi Osteoarthritis," *Pusat Penelitian dan Penerbitan LP2M Uin Syarif Hidayatullah*, 2017.
- [16] M. R. Avadi *et al.*, "Preparation and characterization of insulin nanoparticles using chitosan and Arabic gum with ionic gelation method," *Nanomedicine Nanotechnology, Biol. Med.*, vol. 6, no. 1, hal. 58–63, 2010, doi: <https://doi.org/10.1016/j.nano.2009.04.007>.
- [17] D. P. Wijaya, "Preparasi Nanoartikel Sambung Silang Kitosan_Tripolifosfat Yang Mengandung Ginsenosida," 2013.
- [18] R. Anggraini, S. Surini, dan F. C. Saputri, "Formulation and Characterization of Bitter Melon (Momordica charantia Linn.) Fruit fFraction Loaded Solid Lipid Nanoparticles," *Pharmacogn. J.*, vol. 13, no. 6, hal. 1347–1354, 2021, doi: 10.5530/PJ.2021.13.170.
- [19] N. Kathe, B. Henriksen, dan H. Chauhan, "ChemInform Abstract: Physicochemical Characterization Techniques for Solid Lipid Nanoparticles: Principles and Limitations," *Drug Dev. Ind. Pharm.*, vol. 40, Apr 2014, doi: 10.3109/03639045.2014.909840.
- [20] D. Hudiayanti, M. F. Al Khafiz, K. Anam, P. Siahaan, dan S. M. Christa, "In Vitro Evaluation of Curcumin Encapsulation in Gum Arabic Dispersions under Different Environments," *Molecules*, vol. 27, no. 12, hal. 1–14, 2022, doi: 10.3390/molecules27123855.
- [21] S. Mahadi *et al.*, "Antioxidant and Anti-Tyrosinase Activities of Aloe vera Rind and Gel Extracts," *Glob. Med. Heal. Commun.*, vol. 7, Jan 2020, doi: 10.29313/gmhcv7i3.4453.
- [22] E. Mardawati, F. Filianty, dan H. Marta, "Kajian Aktivitas Antioksidan Ekstrak Kulit Manggis (Garcinia mangostana L) dalam Rangka Pemanfaatan Limbah Kulit Manggis Di Kecamatan Puspahiang Kabupaten Tasikmalaya," *J. Ind. Teknol. Pertan.*, vol. 2, no. 3, 2008.
- [23] F. Crendhuty, S. Sriwidodo, dan Y. Wardhana, "Sistem Penghantaran Obat Berbasis

- Biopolimer Kitosan sebagai Film Forming System," *Maj. Farmasetika*, vol. 6, Okt 2020, doi: 10.24198/mfarmasetika.v6i1.27457.
- [24] S. Raissi dan R. E. Farsani, "Statistical Process Optimization Through Multi-response Surface Methodology," *World Acad. Sci. Eng. Technol.*, vol. 39, no. 3, hal. 280–284, 2009.
- [25] U. Sadiq, H. Gill, dan J. Chandrapala, "Ultrasound-Assisted Encapsulation of Anthraquinones Extracted from Aloe-Vera Plant into Casein Micelles," *Gels*, vol. 8, no. 9, 2022, doi: 10.3390/gels8090597.