PJHRD
Philippine Journal of Health Research and Development
University of the Philippines Manila - The Health Sciences Center
Information, Publication and public affairs Office (IPPAO)
8/F Philippine General Hospital Complex, Taft Avenue, Manila 1000 Philippines
Online ISSN: 2738-042X
January-March
VOL. 28 NO. 1 2024
PAGE 32-36

ORIGINAL ARTICLE

Application of central composite experimental design for the formulation and optimization of meropenem loaded chitosan-alginate nanoparticles

Clinton B. Gomez*, Jan Vonrich M. Huna, Merrene Bright D. Judan, Carl Edward F. Pahuyo

[1]Department of Industrial Pharmacy, College of Pharmacy, University of the Philippines Manila

ABSTRACT

Background: Response surface methodology (RSM) is a cost-effective multivariate technique employed in optimization of pharmaceutical formulations. Central composite experiment design is one of the common designs under RSM used for determining optimum nanoparticle formulation parameters.
Objectives: To optimize a formulation for meropenem-loaded chitosan alginate nanoparticles using central composite experimental design.
Methodology: Meropenem loaded chitosan-alginate nanoparticles were fabricated using aqueous sodium alginate solution and ionotropic gelation with calcium chloride and chitosan, using an optimized formulation derived from a central composite design. The fabricated Mer-CS/Alg NPs were characterized for their particle size, zeta potential, encapsulation efficiency, and loading capacity. The central composite design has been used to adequately assess the influence of two factors namely meropenem concentration and Alg/CS mass ratio on the responses based on a limited number of 13 triplicate formulation runs.
Results: This study successfully formulated meropenem-loaded chitosan/alginate nanoparticles. The optimal formulation of the Mer-CS/Alg NPs was 1.7 mg/mL curcumin, and a Alg/CS mass ratio of 9.8:1. Based on the predicted values of the response variable, the optimal formulation would have a particle size of 490.64 nm, zeta potential of -28.59 mV and a loading capacity of 76.89%.
Conclusion: The central composite experimental design successfully optimized the nanoparticle formulation of meropenem and chitosan/alginate polymer solution. The optimum formulation produced nanoparticles with adequate size, high stability, and high drug load.

Introduction

Antimicrobial resistance (AMR) is an increasingly serious threat to global public health. AMR causes drugs to become ineffective, resulting in prolonged illness and increased risk of spreading infection and death [1]. In a report by the United Nations' Interagency Coordination Group (IACG) on Antimicrobial Resistance in 2019, AMR already caused at least 700,000 deaths globally a year, which are projected to increase to 10 million deaths a year by 2050 if no sustained efforts are taken to address the problem. Despite the wide availability of antimicrobials, treatment failures have been observed from first to last line antimicrobials, making infections untreatable.

In the scene of AMR, polymeric nanoparticles have gained attention since it has been shown to enhance the activity of antimicrobials, as well as prevent or eradicate biofilm formation [2]. Polymeric nanoparticles allow sustained release of the loaded drug, which results in prolonged residence time of the drug in the biofilm infection sites. The drug can also be released locally inside the biofilm due to the ability of nanoparticles to penetrate through the bacterial EPS matrix [3]. Furthermore, polymeric nanoparticles have shown to improve the physicochemical stability of the drug, increase drug penetration into cells and tissues, and protect the drug from biodegradation. All of which are deemed advantageous especially for antimicrobials with poor pharmacokinetic properties such as meropenem, which is a Biopharmaceutics Classification System (BCS) class IV drug.

Among polymeric nanoparticles, natural polymers such as chitosan and alginate are most popularly used [4]. Their unique properties such as nontoxicity, biodegradability, biocompatibility, high stability, low-cost processing, and high abundance in nature make them good drug carriers [5]. In this study two techniques will be utilized namely ionic gelation and polyelectrolyte interaction. Figure 1 details the mechanism of drug entrapment and formation of nanoparticle involved in these preparation techniques. The first polymeric layer is formed by the electrostatic interaction of negatively charged carboxylic group of alginate and a divalent cationic crosslinker such as calcium ions. This forms an egg-box structure that entraps the drug molecule. The second polymeric layer is formed by polyelectrostatic interaction of the positively charged amine groups of chitosan with the negatively charged carboxylic group of alginate [6].

Response surface methodology (RSM) is one of the most cost-effective multivariate techniques employed in formulation optimization. RSM refers to a collection of mathematical and statistical techniques utilized for creating empirical models. The primary purpose of this optimization tool is to establish a regression-based model and enhance a specific output variable (referred to as the "response"). The response is influenced by various independent input variables. Through a series of experiments, all the input variables are systematically altered to understand the factors impacting the output or response variable. In this study, the central composite design of RSM is applied to optimize a formulation for meropenem-loaded chitosan alginate nanoparticles. [7].

Methodology

Preparation and Optimization of Mer-CS/Alg NPs

Preparation of Solutions

Solutions of meropenem trihydrate (pharmaceutical grade), sodium alginate (medium viscosity), and calcium chloride will be prepared using distilled water, while solutions of chitosan (degree of deacetylation = 90%, MW = 22000 Da) will be prepared using dilute CH3COOH (1% v/v) The pH of sodium alginate solution will be adjusted to pH 4.9 using 1% v/v CH3COOH. On the other hand, the pH of chitosan solution will be adjusted to pH 4.6 using 4% w/v NaOH.

Preparation of Mer-CS/Alg NPs

A modified method of ionotropic gelation will be the main formulation technique employed in the fabrication Mer-CS/Alg NPs [8]. Varying concentrations of 1 mL meropenem trihydrate solution will be introduced dropwise (20 mL/hr) using a 26G needle into 20 mL of sodium alginate solution (0.6 mg/mL), followed by continuous mixing using a magnetic bar and stirrer at a speed of 1000 rpm for 10 minutes. To help break alginate polymers into shorter lengths, the mixture was sonicated for 15 minutes.

Corresponding author's email address:
cbgomez@up.edu.ph
Keywords: chitosan, alginate, nanoparticle, optimization, central composite

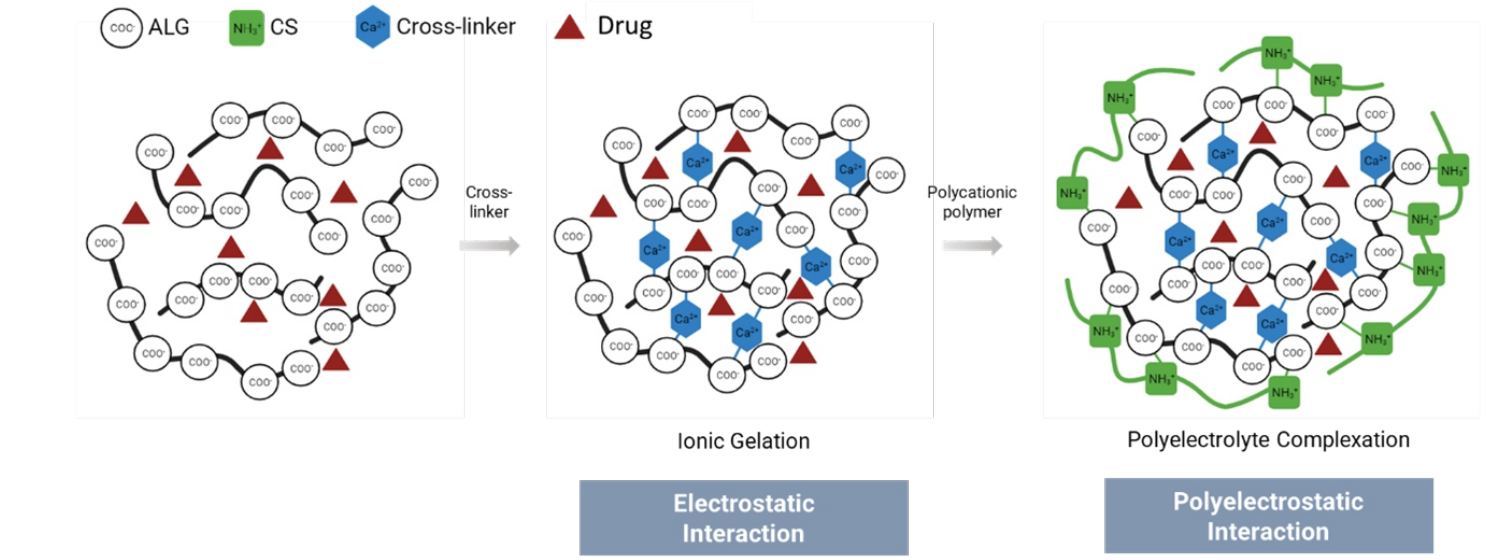

Figure 1. Mechanism of drug entrapment and formation of nanoparticle through the electrostatic interaction of alginate, chitosan, and calcium ions.

Four milliliters of CaCl2 solution (6 mM or 0.67 mg/mL) will be added dropwise with continuous stirring for another 30 minutes. In the same manner, varying concentrations of 4 mL chitosan solution will be incorporated to the resultant mixture. The obtained NP suspension will be left to equilibrate overnight prior to characterization.

Design and Optimization

Optimized Mer-CS/Alg NPs formulation will be determined by central composite design of response surface methodology (RSM) using Design-Expert® software 13.0.11.0 (Stat-Ease, Inc., Minneapolis, MN, USA) with the variables outlined in Tables 1 and 2.

Characterization of Mer-CS/Alg NPs

Particle Size and Zeta Potential

Particle size (specifically the hydrodynamic size) and zeta potential of the nanoparticles will be measured by dynamic light scattering using a Nano-ZS Zeta-sizer from Malvern Instruments. All samples will be sonicated for 15 minutes prior to testing. Dilution method and sample volume used for all measurements will be standardized for all formulation runs.

Encapsulation Efficiency and Loading Capacity

The encapsulation efficiency and loading capacity of Mer-CS/Alg NPs will be determined indirectly from the supernatant obtained after subjecting the suspensions of nanoparticles prepared to ultracentrifugation. Samples will be centrifuged at 14,000 rpm for 30 min at 25°C, and the amount of meropenem in the supernatant will be quantified using a modified UV–Vis spectrophotometric method (Agilent Cary 60) [9].

A calibration curve will be generated using standard solutions of meropenem trihydrate in concentrations of 5, 10, 15, 20, 25, 30, and 35 µg/mL read at 298 nm [10].

Encapsulation efficiency (EE, %) and loading capacity (LC, %) of the nanoparticles will be calculated using the following equations:

$$EE = \frac{Amount\ of\ drug\ initially\ added - Amount\ of\ drug\ in\ supernatant}{Amount\ of\ drug\ initially\ added} \times 100\% \quad (1)$$

$$LC = \frac{Amount\ of\ drug\ in\ the\ formulation - A\ mount\ of\ drug\ in\ supernatant}{Weighed\ of\ dried\ nanoparticles\ recovered} \times 100\% \quad (2)$$

A central composite design of response surface methodology was conducted for the optimization of Mer-Alg/CS nanoparticles. This design has been used to adequately assess the influence of two factors on the responses based on a limited number of 13 formulation runs. The observed responses of the 13 nanoparticle formulations are summarized in Table 4. All responses varied and ranged from 327 to 750 nm for particle size (Y1), –30.6 to –27.5 mV for zeta potential (Y2), 0.23 to 1.17 mg for amount of meropenem in nanoparticle (Y3), and 5.8% to 76.8% for loading capacity (Y4).

The responses were simultaneously fit into linear, two-factor interaction (2FI), and quadratic models using Design Expert® software. Table 5 shows a summary of the sequential model sum of squares, lack of fit, and model summary statistics.

From each response, the most suitable model was identified. A suitable model is preferred to have a significant sequential model of sum squares ($p < 0.05$), a non-significant lack of fit ($p > 0.05$), and an adjusted and predicted R2 close to 1. The polynomial equation for particle size (Y1), zeta potential (Y2), amount of meropenem in nanoparticles (Y3), and loading capacity (Y4) is shown in Equations (3)-(6), whereas a positive value signifies a direct relationship wherein the dependent variable Y increases proportional to the independent variable X, while a negative value signifies an inverse relationship between the dependent and independent variables:

$$Y_1 = 519.49 + 15.43X_1 - 74.35X_2 + 46.36X_1X_2 - 42.33{X_1}^2 + 66.42{X_2}^2 \quad (3)$$

$$Y_2 = -29.22 - 0.0139X_1 - 0.0795X_2 - 0.9975X_1X_2 \quad (4)$$

$$Y_3 = 0.6249 + 0.3141X_1 + 0.1185X_2 \quad (5)$$

$$Y_4 = 19.18 + 5.02X_1 + 22.41X_2 - 5.39X_1X_2 + 0.6306{X_1}^2 + 12.84{X_2}^2 \quad (6)$$

Table 1. Independent variables or Factors in the formulation optimization

Independent Variables or Factors	Levels	
	Low	High
Meropenem Concentration (mg/mL)	1.0	4.0
Alginate/Chitosan mass ratio	3:1	7:1

Table 2. Dependent Variables or Responses in the formulation optimization.

Dependent Variables or Responses	Constraints
Particle Size	200 nm to 500 nm
Dispersity	Minimize
Zeta Potential	-20 mV to -40 mV
Encapsulation Efficiency	Maximize
Loading Capacity	Maximize

Table 3. Factors used in the optimization of Mer-Alg/CS nanoparticles

Factors	Levels		α
	Low	High	
X_1 = Meropenem concentration (mg/mL)	1	4	0.6
X_2 = Alg/CS mass ratio	5:1	9:1	0.8

Table 4. Summary of factors and responses of the different formulations

Run	Factors		Responses			
	X_1 mg/mL	X_2	Y_1 nm	Y_2 mV	Y_3 mg	Y_4 %
1	1	5:1	616.72	-29.9	0.2505	5.76
2	2.5	9.8:1	611.18	-29.8	0.8913	76.84
3	2.5	7:1	466.3	-29.4	0.5644	17.69
4	2.5	7:1	487.7	-29.93	0.5604	16.8
5	0.4	7:1	483.07	-29.63	0.2293	7.53
6	4.6	7:1	451.9	-28.03	1.1749	31.16
7	2.5	7:1	525.68	-29.07	0.5637	16.2
8	2.5	7:1	609.25	-29.87	0.6715	18.52
9	4	5:1	606.93	-29.08	0.7202	19.87
10	2.5	7:1	506.05	-28.4	0.621	26.77
11	4	9:1	502.47	-30.62	1.032	50.84
12	2.5	4.2:1	750.1	-28.7	0.5063	9.7
13	1	9:1	326.82	-27.45	0.3382	58.31

Table 5. Regression analysis summary of the responses

Response	Model	Sequential p-Value	Lack of Fit p-Value	Adjusted R^2	Predicted R^2	Remarks
Y_1	Linear	0.1180	0.0986	0.2173	-0.3301	
	2FI	0.3425	0.0917	0.2177	-0.3624	
	Quadratic	**0.0362**	**0.2631**	**0.6102**	**-0.1054**	**Suggested**
Y_2	Linear	0.9729	0.1361	-0.1934	-1.0204	
	2FI	**0.0298**	**0.2747**	**0.2373**	**-0.6997**	**Suggested**
	Quadratic	0.8320	0.1611	0.0696	-1.9243	
Y_3	Linear	**< 0.0001**	**0.1147**	**0.9213**	**0.8703**	**Suggested**
	2FI	0.1672	0.1321	0.9301	0.8642	
	Quadratic	0.6257	0.0839	0.9214	0.7301	
Y_4	Linear	0.0011	0.0153	0.6940	0.5138	
	2FI	0.3950	0.0134	0.6877	0.4293	
	Quadratic	**0.0012**	**0.2404**	**0.9408**	**0.8282**	**Suggested**

Table 6. Significance ($p < 0.05$) of the factors in the generated models (ANOVA)

Factors	Y_1 Particle size	Y_2 Zeta potential	Y_3 Meropenem in nanoparticle	Y_4 Loading capacity
X_1 (Meropenem concentration)	0.5274	0.9608	< 0.0001	0.0314
X_2 (Alg/CS mass ratio)	0.0150	0.7789	0.0018	< 0.0001

Results are also summarized using a response surface plot (Figures 1a-1d) which shows the interrelationships of factors and responses. A summary of the significance of each independent variable per model generated is shown in Table 6.

Previous studies showed that particle size mainly depends on the polymer's mass ratio and molecular weight [12,13]. This is reflected in the results of the study as Eq. (1) showed an inverse relationship between particle size (Y1) and Alg/CS mass ratio (X2). An increase in particle size as Alg/CS mass ratio decreases may be due to increased viscosity of the dispersed phase and formation of a compact membrane of chitosan on the surface of alginate nanoparticles [14]. Meropenem concentration (X1) does not have any significant effect on the particle size. Because the pH of the formulation is between the pKa values of the carboxylic (pKa = 2.9) and amine (pKa = 7.4) functional groups of meropenem, it is expected that the net charge of meropenem is close to zero [15]. This may cause meropenem to be encapsulated within the center of the nanoparticle and not on the surface, that is why changing its amounts does not affect the particle size [16].

Zeta potential determines the net surface charge and in turn, the stability of the nanoparticles in a colloidal suspension. Results were found to range from -30.6 mV to -27.5 mV, indicative of good physical stability. Zeta potential is expected to be negative because alginate is the core material of the nanoparticles. Results from ANOVA showed that both meropenem concentration and Alg/CS mass ratio had no significant effects on zeta potential. As mentioned, meropenem has a neutral charge in the system, which makes the zeta potential unaffected by variations in meropenem concentration. In addition, the amount of meropenem used in the formulation relative to alginate and chitosan is relatively low to influence changes in the zeta potential. Furthermore, meropenem is expected to be encapsulated within the nanoparticle covered by layers of alginate and chitosan which makes it unlikely to contribute to the surface charge during zeta potential measurement. It is expected that an increase in Alg/CS mass ratio will result to a decrease in zeta potential because there will be more negative charges from alginate on the nanoparticle surface to neutralize positively charged chitosan molecules [17]. However, for all the tested formulations, the zeta potentials measured were consistently around -30 mV. No decrease in the zeta potential was observed upon increasing the Alg/CS mass ratio from 5:1 to 9:1. A possible reason explanation for this is that the amount of alginate incorporated into and consequently the negative charge measured on the nanoparticle was limited by the amount of crosslinker, CaCl2 which was added at a fixed volume only. Any excess alginate will dissolve into supernatant solution and not be able to form a layer on the particle due to the calcium ions already being used up.

The amount of meropenem in nanoparticle and loading capacity had significant synergistic relationship with meropenem concentration. This is expected since the amount of drug incorporated is the main determinant of drug encapsulation [18]. Similarly, higher Alg/CS mass ratios were observed to produce nanoparticles with significantly higher amount of meropenem and loading

Table 7. Responses and their constraints

Responses	Constraints
Y_1 = Particle Size (nm)	326 to 500
Y_2 = Zeta Potential (mV)	-20 to -30
Y_3 = Amount of Meropenem in Nanoparticles (mg)	0.6 to 1.17
Y_4 = Loading Capacity (%)	Maximize

Table 8. Predicted values for the optimized formulation variables.

Variables	Optimized Formulation Predicted Values
X_1 = Meropenem concentration (mg/mL)	1.7
X_2 = Alg/CS mass ratio	9.8:1
Y_1 = Particle Size (nm)	490.64
Y_2 = Zeta Potential (mV)	-28.59
Y_3 = Amount of Meropenem in Nanoparticles (mg)	.6237
Y_4 = Loading Capacity (%)	76.89

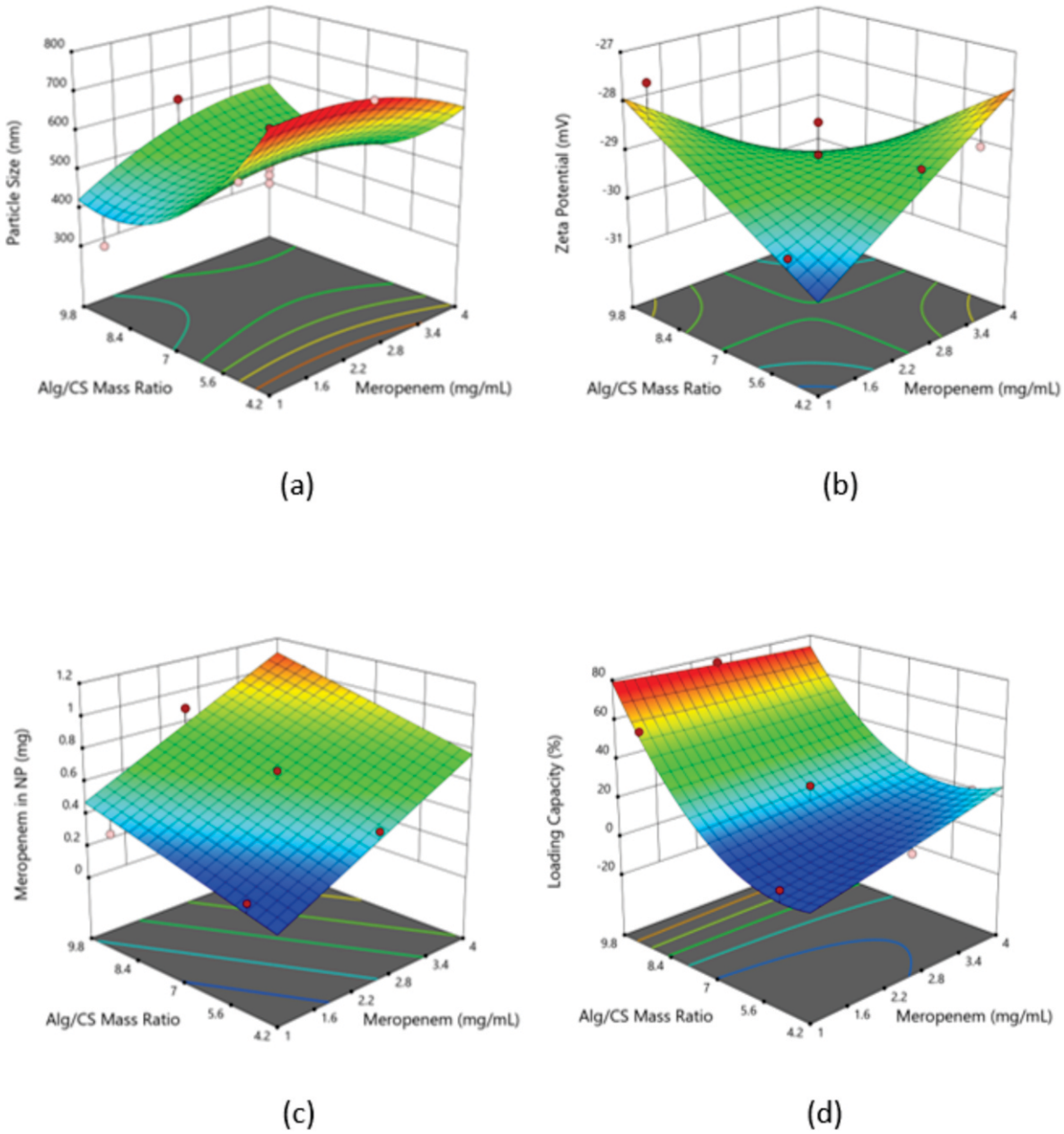

Figure 2. Three-dimensional response surface plots showing interaction effects of meropenem concentration and Alg/CS mass ratio on (a) particle size, (b) zeta potential, (c) amount of meropenem in nanoparticles (NP), and (d) loading capacity

capacity. In higher chitosan concentrations, the interaction of the drug entrapped in the alginate matrix may be reduced because the interaction between the amino groups of chitosan and the carboxylic acid groups of alginate is more favored. This effect results to leakage of the drug to the external environment, which may explain why lower amount of meropenem in nanoparticle and loading capacity were observed in formulations with low Alg/CS mass ratio [19].

The responses were optimized simultaneously in Design Expert® software using a desirability function (δ) to yield a response that fits the constraints set in Table 7. A high desirability ($0 \leq \delta \leq 1$) indicates that a formulation can produce acceptable results. The predicted values ($\delta = 1.000$) of the optimized formulation are shown in Table 8.

Conclusion

This study successfully formulated meropenem-loaded chitosan/alginate nanoparticles. The value of the factors that produced the optimum formulation of the Mer-CS/Alg NPs was 1.7 mg/mL curcumin, and a Alg/CS mass ratio of 9.8:1. Based on the predicted values of the response variable, the optimal formulation would have a particle size of 490.64 nm, zeta potential of -28.59 mV and a loading capacity of 76.89%. These set of characteristics indicate that adequately sized, stable, and high drug containing nanoparticle can be prepared using meropenem and chitosan/alginate polymer solution.

References

1. World Health Organization. (2017) WHO publishes list of bacteria for which new antibiotics are urgently needed.
2. Shaaban MI, Shaker MA, Mady FM. (2017) Imipenem/cilastatin encapsulated polymeric nanoparticles for destroying carbapenem-resistant bacterial isolates. Journal of Nanobiotechnology 15(1). doi: 10.1186/s12951-017-0262-9.
3. Kiew YT, Cheow WS, Hadinoto K. (2014) Importance of biofilm age and growth medium on the viability of probiotic capsules containing Lactobacillus rhamnosus GG biofilm. LWT - Food Science and Technology 59(22): 956–963. doi: 10.1016/j.lwt.2014.07.053.
4. Liu Z, Jiao Y, Wang Y *et al.* (2008) Polysaccharides-based nanoparticles as drug delivery systems. Advanced Drug Delivery

Reviews 60(15): 1650–1662. doi: 10.1016/j.addr.2008.09.001.
5. Katuwavila NP, Chandani Perrera ADL, Samarakoon SR, *et al.* (2016) Chitosan-Alginate Nanoparticle System Efficiently Delivers Doxorubicin to MCF-7 Cells. Journal of Nanomaterials 2016. doi: 10.1155/2016/3178904.
6. Untari B, Wijaya DP, Mardiyanto M, *et al.* (2019) Physical Interaction Of Chitosan-Alginate Entrapping Extract Of Papaya Leaf And Formation Of Submicron Particles Dosage Form: New Dosage Form to Inhibit The Dengue Diseases. Science & Technology Indonesia 4(3): 64-69. doi: 10.26554/sti.2019.4.3.64-69.
7. Gomez CB, Muangnoi C, Sorasitthiyanukarn F, *et al.* (2019) Synergistic Effects of Photo-Irradiation and Curcumin-Chitosan/Alginate Nanoparticles on Tumor Necrosis Factor-Alpha-Induced Psoriasis-Like Proliferation of Keratinocytes. Molecules 24(7): 1388. doi: 10.3390/molecules24071388.
8. Biswas S, Bal M, Behera S, *et al.* (2019) Process Optimization Study of Zn2+ Adsorption on Biochar-Alginate Composite Adsorbent by Response Surface Methodology (RSM). Water 11(2): 325. doi: 10.3390/w11020325.
9. Amini Y, Amel Jamehdar S, Sadri K, *et al.* (2017) Different methods to determine the encapsulation efficiency of protein in PLGA nanoparticles. Bio-Medical Materials and Engineering 28(6): 613–620. doi: 10.3233/bme-171705.
10. Abdelkader A, El-Mokhtar MA, Abdelkader O, *et al.* (2017) Ultrahigh antibacterial efficacy of meropenem-loaded chitosan nanoparticles in a septic animal model Carbohydrate Polymers 174: 1041–1050. doi: 10.1016/j.carbpol.2017.07.030.
11. Bhunchu S, Rojsitthisak P, Rojsitthisak P. (2015) Effects of preparation parameters on the characteristics of chitosan–alginate nanoparticles containing curcumin diethyl disuccinate. Journal of Drug Delivery Science and Technology 28: 64–72. doi: 10.1016/j.jddst.2015.05.010.
12. He C, Hu Y, Yin L, *et al.* (2010) Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles. Biomaterials 31(13): 3657–3666. doi: 10.1016/j.biomaterials.2010.01.065.
13. Makraduli L, Crcarevska MS, Geskovski N, *et al.* (2012) Factorial design analysis and optimisation of alginate-Ca-chitosan microspheres. Journal of Microencapsulation 30(1):81–92. doi:10.3109/02652048.2012.700957.
14. Sorasitthiyanukarn FN, Ratnatilaka Na Bhuket P, Muangnoi C, *et al.* (2019) Chitosan/alginate nanoparticles as a promising carrier of novel curcumin diethyl diglutarate. International Journal of Biological Macromolecules 131: 1125–1136. doi: 10.1016/j.ijbiomac.2019.03.120.
15. Tomasello C, Leggieri A, Cavalli C, *et al.* (2015) In Vitro Stability Evaluation of Different Pharmaceutical Products Containing Meropenem. Hospital Pharmacy 50(4):296–303. doi: 10.1310/hpj5004-296.
16. Honary S, Zahir F. (2013) Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems - A Review (Part 2). Tropical Journal of Pharmaceutical Research 12(2). doi: 10.4314/tjpr.v12i2.20.
17. Sorasitthiyanukarn FN, Muangnoi C, Ratnatilaka Na Bhuket P, e*t al*. (2018) Chitosan/alginate nanoparticles as a promising approach for oral delivery of curcumin diglutaric acid for cancer treatment. Materials Science and Engineering: C 93: 178–190. doi: 10.1016/j.msec.2018.07.069.
18. Martínez Rivas CJ, Tarhini M, Badri W, *et al*. (2017) Nanoprecipitation process: From encapsulation to drug delivery. International Journal of Pharmaceutics 532(1):66–81. doi: 10.1016/j.ijpharm.2017.08.064.
19. Carmona-Ribeiro AM, de Melo Carrasco LD. (2013) Cationic Antimicrobial Polymers and Their Assemblies. International Journal of Molecular Sciences 14(5):9906–9946. doi: 10.3390/ijms14059906.