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Optimization of blend formulation of agar/κ-carrageenan hydrogels for transdermal delivery of vitamin B12

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Abstract. Cancer patients require a new method of vitamin B delivery due to the ineffective and invasive methods currently practiced. A biopolymer hydrogel of agar/κ-carrageenan was proposed to transdermally deliver vitamin B12 to cancer patients. Varying mass ratios were considered for the agar/κ-carrageenan: 25/75, 50/50, 75/25, and 100/0. A reference of 1 mg vitamin B12 was used for the loading coefficients of 1x, 1.5x, and 2x. The percent release of vitamin B12 and time required to reach equilibrium release concentration were the response variables used to determine the optimal hydrogel blend. The vitamins were loaded into the biopolymer solution over a double boiler and the resulting gel was dried and sent for release analysis via colorimetry. The hydrogel blend of 50/50 at a loading coefficient of 1x was found to have the highest vitamin release of 314%, and shortest time to reach equilibrium release concentration at 337 minutes. Increasing loading coefficient increases the time to reach equilibrium response concentration. To further improve the study, other essential B-group vitamins may be added and other mass ratios and loading coefficients may be incorporated in the optimization.

1 Introduction

The B group vitamins, specifically B12 or cobalamin, are nutrients essential to many metabolic and physiological processes such as energy metabolism, cell reproduction, and protein synthesis. Despite their integral role in several biosynthesis processes in the body, humans are unable to synthesize these and are reliant on B-rich food to provide the necessary B-group vitamin intake [1, 2]. Deficiencies in the B-group vitamins lead to adverse health effects such as impaired physiological functions like megaloblastic anemia and neurologic symptoms, as well as impaired antioxidant defense and immune response that leave DNA and proteins vulnerable to tissue damage [2, 3].

Cancer patients have been found to be especially susceptible to vitamin B12 deficiency driven primarily by malnutrition, weight loss, and use of medication that interfere with vitamin B12 absorption [1-2]. Cancer-related surgical procedures have also been found to cause metabolic disturbances related to poor macronutrient absorption [4]. Vitamin B12 depletion has also been found to contribute to increased tumor cell proliferation and energy demands [1, 2].

With cancer being the third leading cause of death in the Philippines, providing affordable yet effective means to deliver vitamin B12 in B-deficient patients has become a necessity [5]. The most common delivery of the vitamin B complex for B-deficient patients is through oral supplements or intramuscular injection. Oral administration may not be effective for cancer patients due to the metabolic disturbances [4]. Meanwhile, intramuscular injection tends to cause significant pain. Injections may also pose risks to individuals who have autoimmune disorders or are taking anticoagulant medication [6]. A trending approach is the transdermal delivery of vitamin B12 such as using diffusion cells and via iontophoresis [7]. However, such technologies are too expensive to be accessible.

A viable alternative is the transdermal delivery of vitamin B12 via hydrogel dressings which are painless and non-invasive. Hydrogels are superabsorbent water-swollen materials that maintain a three-dimensional matrix [8]. Hydrogels used in biomedical applications can be produced from natural polymers as they are cheap, biocompatible, and biodegradable [9]. Notable biopolymers include alginate, agar, and κ-carrageenan which are seaweed-based.

To improve material performance, different polymers may be blended to form a hydrogel that exhibits all the advantageous properties of each component; however, several factors such as miscibility and crosslinking behavior must be considered when establishing the blend formulation. κ-carrageenan and agar exhibit desirable properties for this particular biomedical application. κ-carrageenan is mainly used as a gelling and thickening agent in the food industry [10]. It is capable of absorbing water 20 times its own weight as well as forming thermoreversible gels making it resistant to heat degradation [11]. Similarly, agar is also used as a gelling agent in the food industry. Powdered agar, the more commercially available form of agar, sets to a firm gel even at low concentrations [10]. Agar gels are not

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susceptible to heat degradation and can also hold a water content 20 times its own weight [12, 13]. Figure 1 shows the thermal crosslinking of κ-carrageenan and agar.

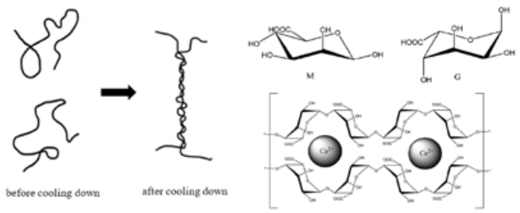

Fig. 1. Crosslinking of agar and κ-carrageenan (left) and alginate (right).

Currently, there is a need to explore the use of inexpensive and locally available materials in hydrogel technology for a non-invasive delivery of vitamin B12. This research aims to develop and contribute a simple and affordable method for vitamin delivery to make medical solutions more accessible. Specifically, this aims to determine the optimum hydrogel blend using seaweed-based biopolymers for the transdermal delivery of at least 1 mg of vitamin B12. The evaluation of this study involved identifying the effect that the agar/κ-carrageenan mass ratio and the vitamin loading coefficient have on the percentage of vitamin released and the time it took for the hydrogel to achieve its equilibrium release concentration. The optimum blend for this study was selected based on the highest percentage of release and quickest diffusion time.

2 Materials and methods

2.1 Chemicals

The bio-based polymers used were agar (Don Frank agar agar powder), alginate (Makintal high precision alginate), and κ-carrageenan (food grade). Vitamin B12 loaded into the hydrogel samples were sourced from Mason Natural.

2.2 Blend formulation and synthesis

Hydrogels of different biopolymers were synthesized. The individual gelling abilities of these biopolymers were investigated. As for the blended hydrogels, the formulation was derived from the study of Zhao et al. (2020) and Gupta et al. (2020) where they studied the physicochemical properties of agar/κ-carrageenan hydrogels along with their gelling properties [14, 15]. To determine the optimum blend, various mass ratios of agar and κ-carrageenan in the total biopolymer content were utilized. The ratios used for the agar/κ-carrageenan mixture were 100/0, 75/25, 50/50, 25/75, 0/100. Moreover, various loading coefficients of vitamin B12 were used to further determine the optimum blend formulation. For a target reference of 1 mg of vitamin B12, the loading coefficients used are 1x, 1.5x, and 2x.

The hydrogel formulation was made by mixing the total biopolymer components at 1.5wt% into 100 mL distilled water that was previously directly loaded with vitamin B12. The biopolymer-water-vitamin mixture was then heated to 90°C for thermal crosslinking while maintaining a constant stir. Once completely dissolved, the hot solutions were left to set at room temperature for 16 hours with a thickness of 1 mm in a 10-cm cylindrical silicone mold. Then, the firmed hydrogel samples were dried to a film for 3 hours in a turbo oven at 70°C. The same procedure was done for all mass ratios and loading coefficients in triplicate.

2.3 Vitamin B12 release

The dried vitamin-loaded hydrogels were subjected to release analysis by submerging in 0.9% brine solution. Through time, the solution changes its color as the pink-colored vitamin B12 diffuses from the hydrogels to the surrounding solution. To determine the amount of vitamin B12 released into the supernatant solution, samples of colored solutions were subjected to smartphone colorimetry via the Colorimeter® application. The generated absorbance results were then related to the mass of vitamin B12 released.

The response parameters were: (1) the percentage of vitamin B12 released, and (2) the time it took for the hydrogel to reach its equilibrium release concentration. The time was determined directly from the smartphone colorimetry procedure while percentage release was calculated from the ratio of vitamin B12 detected in solution and vitamin B12 loaded into the hydrogel. OFAT (one factor at a time) analysis was performed for each variable pair listed below to identify the effect each control parameter has on its corresponding response parameter.

- % vitamin B12 released vs mass ratio
- time it took to reach equilibrium concentrations vs mass ratio
- % vitamin B12 released vs loading coefficient
- time it took to reach equilibrium concentrations vs loading coefficient

For the blend optimization of the hydrogel blend, two independent variables (agar mass ratio, vitamin B12 loading coefficient) and two response variables (% vitamin B12 release, time to reach equilibrium release) were identified. Equation 1 was utilized to calculate the percent release for each trial.

$$\%\ vit.B12\ released = \frac{mass\ vit.released\ in\ supernatant}{mass\ vit.\ loaded\ into\ hydrogel} \times 100 \qquad (1)$$

Meanwhile, the optimum blend was determined through a central composite response surface generated using Design-Expert®.

3 Results and discussion

3.1 Biopolymer selection

All biopolymers underwent the same method as stated in the methodology for the unloaded hydrogels. The 100% κ-carrageenan mixture did not form a gel after it was set to rest. This is due to the lack of ability of the κ-carrageenan to form gels without chemical crosslinkers [10]. The same was observed for the 100% alginate mixture. The mixture remained liquid because of the lack of a chemical crosslinker. The 100% agar mixture was the only mixture that formed a gel since agar can form hydrogels at low concentrations even without any chemical crosslinkers.

To investigate the optimal biopolymer blend, the three biopolymers were blended at equal ratios. Table 1 shows the summary of gelling results of the three biopolymers [14]. For two-component mixtures, a ratio of 50:50 was used for each biopolymer component while three-component mixtures used a 33:33:33 ratio. All blends utilized thermal crosslinking and did not have any chemical crosslinkers.

Table 1. Synthesis of different hydrogel blends.

Blend	Result
alginate/κ-carrageenan	no gel formed
agar/κ-carrageenan	gel formed
agar/alginate/κ-carrageenan	gel formed

As shown in Figure 2, the alginate and κ-carrageenan blend remained liquid after setting time which is not ideal for a gel formulation. Both commonly require a chemical crosslinker to form rigid gels. κ-carrageenan is a polysaccharide composed of alternating copolymers of β-D-galactose and α-D-galactose bound by glycosidic linkages. It contains several OH groups and one sulfate group causing strong anionic behavior [16]. The most commonly used crosslinkers for κ-carrageenan are potassium and calcium salts [6, 14]. Since the scope of the experiment does not include the use of a chemical crosslinker, the alginate/κ-carrageenan blend was not optimal for the desired gelling conditions.

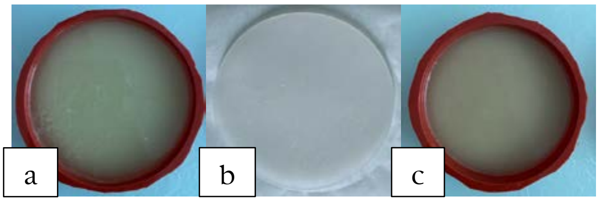

Fig. 2. Synthesized hydrogels of (a) alginate/κ-carrageenan, (b) agar/κ-carrageenan, and (c) agar/alginate/κ-carrageenan.

Using agar and κ-carrageenan as the components of the blend achieved a rigid gel. Agar is disaccharide composed of D-galactose and 3, 6-anhydro-L-galactopyranose with several OH functional groups [17]. Agar readily dissolves in hot water and forms a gel after cooling. It also does not require high concentrations of the polymer to form the gel. Ratios of 0.5% of agar with water are able to form rigid gels. The structure of agar contributes to the gel formation of the agar/κ-carrageenan blend. Agar gels are also thermoreversible making its property resistant to heat [13]. This achieved translucent gels which are ideal for the colorimetry method.

Typically, vitamin release from blended polymer matrices is dependent on several interactions such as polymer-polymer and vitamin-polymer [17]. Sol-gel experiments illustrated no associative interactions between the two biopolymers. This was likely due to their thermodynamic incompatibility which caused segregative phase separation within the mixture. Due to thermal crosslinking, the polymer matrix was likely formed by a continuous phase of agar. However, without the presence of a chemical crosslinker, the process yielded a discontinuous phase for κ-carrageenan. Since there were minimal polymer-polymer interactions within the matrix, the drug release mechanism can be attributed primarily to vitamin-polymer interactions instead.

The third blend analyzed in the experiment used the three biopolymers: agar, alginate, and κ-carrageenan. Similar to the agar/κ-carrageenan blend, using the three biopolymers formed a rigid gel. Although the components formed a gel, it was not visually fit for the method of determining the vitamin release. The gel formed using the three biopolymers formed a semi-green opaque hydrogel since the alginate component of the blend is green. This can disrupt the colorimetry method which is a limitation to this study.

With the firm and rigid gel that agar formed along with its blending with κ-carrageenan, the vitamin B12-loaded hydrogels did not experience any degradation and instability. Thus, among the three biopolymer blend variations, the agar/κ-carrageenan biopolymer blend showed the most desirable gelling conditions for this study.

3.2 Vitamin B12 release

To identify the effect that the mass ratio and loading coefficient have on the percentage of vitamin B12 released as well as the time it takes to reach equilibrium concentration, OFAT (one factor at a time) analysis was performed for each response-to-control variable pair.

3.2.1 Varying mass ratio

The experiment monitored the behavior of the response parameters following the variation of the agar/κ-carrageenan blend concentrations of each sample.

From visual inspection of hydrogel samples shown in Figure 3., it is evident that increasing the κ-carrageenan concentration also increases the opacity of the hydrogel sample. Meanwhile, physical handling of the samples illustrated that increasing agar concentrations result in firmer hydrogels.

Once dried, the hydrogels become thin yet pliable making them suitable for skin applications. No noticeable differences in thickness or texture were observed in any of the samples shown in Figure 4.

The percentage release data of the hydrogels with different mass ratios were plotted accordingly. It can be gathered from Figure 5 that the smartphone colorimetry experiment resulted in release values of over 100%. This was attributed to the development of turbidity in the solution. Potential sources of error can also be attributed to the use of the Colorimeter® application. Nevertheless, it was assumed that the driving force for the vitamin release was uniform across all samples and can thus be analyzed relative to each other rather than as individual magnitudes.

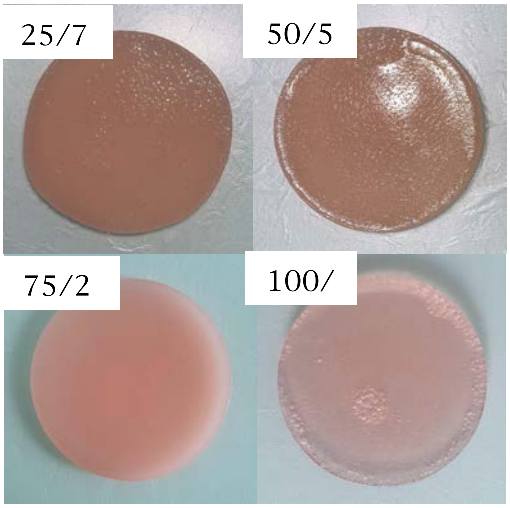

Fig. 3. Agar/κ-carrageenan samples before drying.

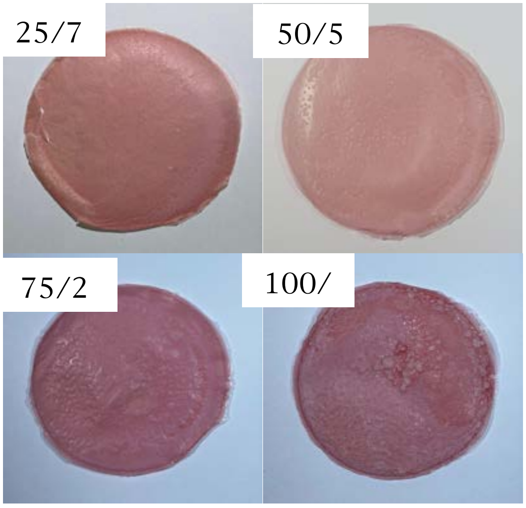

Fig. 4. Agar/κ-carrageenan samples after drying.

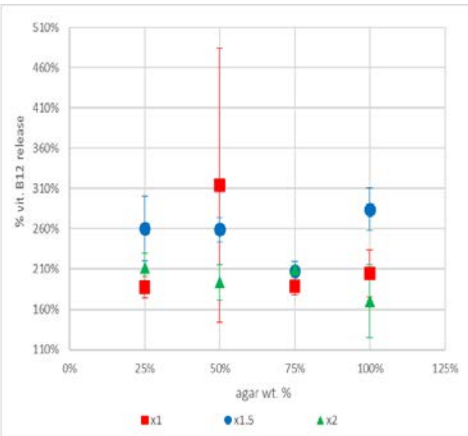

Fig. 5. % release of vitamin B12 against agar wt% at various vitamin loading coefficients.

From Figure 5, the hydrogel blend yielding the highest relative release was found to be the mass ratio 50/50 at the loading coefficient of 1. For most mass ratios, the highest release occurred at the loading coefficient of 1.5 however, this is not true for 50/50. Experimental data showed that one trial yielded an unusually high % release value of 498% compared to the other trials that reached only 281% and 163% released amounts. However, results of the Grubb's test validated the trial as not an outlier making 50/50 the mass ratio of highest average release. Meanwhile, the lowest vitamin B12 release occurred at the pure agar blend with a loading coefficient of 2. Fundamentally, no clear trend was observed between the biopolymer mass ratios to its % vitamin B12 released.

A shorter diffusion period to reach equilibrium concentration was considered a more desirable feature of the hydrogel. It was observed from Figure 6 that reaching this equilibrium concentration took longer as mass ratios exceeded 50% agar at loading coefficients of 1 and 2. Meanwhile, the opposite was true at a loading coefficient of 1.5 wherein diffusion time decreased with increasing agar concentration. Among all the mass ratios, the fastest diffusion occurred at 50/50 with a loading coefficient of 1.

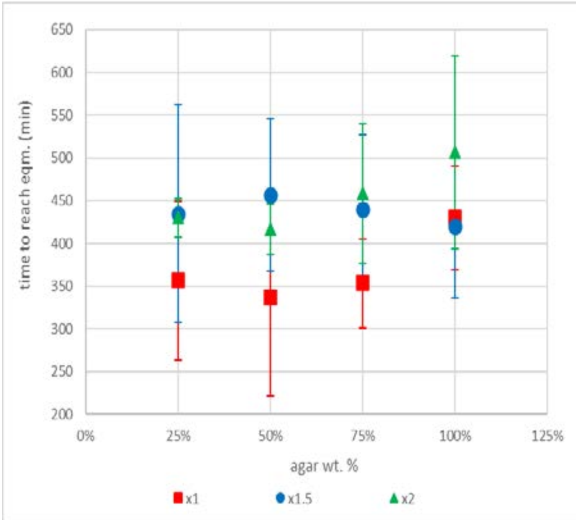

Fig. 6. Time to reach equilibrium release concentration against agar wt% at various vitamin loading coefficients.

3.2.2 Varying loading coefficient

The amount of vitamin B12 added to the hydrogel blend was manipulated to observe its effect on the response variables. As mentioned previously, the reference amount was 1 mg of vitamin B12.

As loading coefficient was manipulated, the most notable change in the physical properties of the hydrogel was the change in intensity of the color of the hydrogel brought by the increase in amount of vitamin B12. The procured vitamin B12 was a bright shade of pink which provides the distinctive color of the hydrogel formed, as shown in Figure 7. The color intensity was observable with the dried hydrogel compared to the pre-dried hydrogel. The change in shades of pink of the hydrogel can be seen in Figure 8, taken from the hydrogels with an agar wt% of 50/50 at varying loading coefficients.

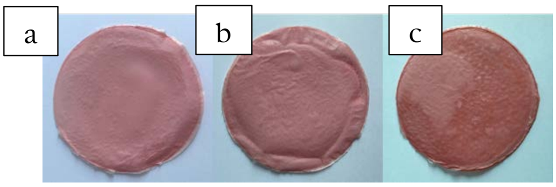

Fig. 7. Hydrogel color variation.

Fig. 8. Observable color change of (a) 1x, (b) 1.5x, and (c) 2x loading coefficients at a fixed 50/50 agar wt%.

As seen in Figure 9, the 1.5x loading coefficient had the highest average release, specifically, an average of 253% vitamin B12 released. The 1x loading and 2x loading coefficients had averages of 224% and 196% respectively. There were no significant trends on vitamin B12 release observed.

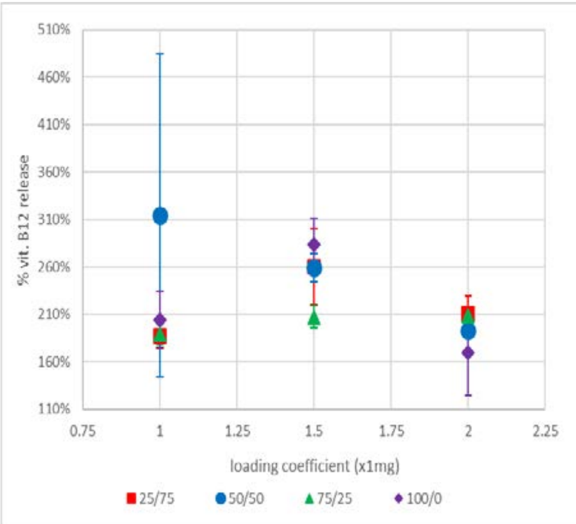

Fig. 9. % release of vitamin B12 against loading coefficient at various agar wt%.

In Figure 9, the highest relative release was found to be 314% which occurred at a vitamin loading coefficient of 1x with a biopolymer concentration of 50/50. This was 30 % higher than the % release of the hydrogel at a loading coefficient of 1.5% and a biopolymer concentration of 100/0, which was the second highest with a release of 284%.

As mentioned previously, a shorter time frame for the hydrogel to reach equilibrium release was preferred to reduce the probability of a bacterial infection in the wound. Based on Figure 10, the trend was evident that the time to reach its equilibrium release concentration increased as the loading coefficient increased. This trend was reasonable as more vitamin was loaded whenever the loading coefficient increased.

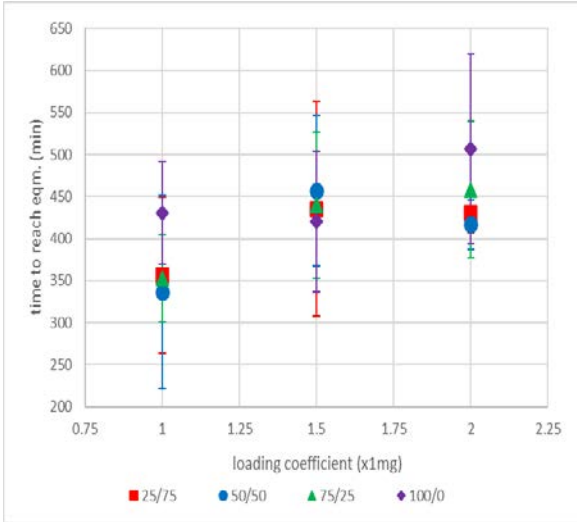

Fig. 10. Time to reach equilibrium release concentration against loading coefficient at various agar wt%.

The hydrogel at a loading coefficient of 2x and an agar wt% of 100/0 took the longest, which made it the least desirable hydrogel blend among all the blends. For the hydrogel at a loading coefficient of 1x and 50/50 agar wt.%, the equilibrium release was achieved after 337 minutes, making it the most desired hydrogel blend in terms of equilibrium release time as a function of loading coefficient.

3.3 Optimized blend selection

To select for the optimal blend, it is ideal that the hydrogel has a high vitamin release to maximize the amount of vitamins loaded. The desirable diffusion time of release needed to reach equilibrium optimal for the transdermal release of vitamins should have the shortest time.

3.3.1 Optimal release of vitamin B12

The effect of the amount of vitamin B12 loaded to a hydrogel and its mass ratio in terms of agar wt% was plotted against the % release of vitamin B12 through a central composite response surface. Through these plots, the optimal blend was determined. Figure 11 shows a contour and 3D plot of the response of the vitamin B12 in relation to the set parameters.

It is ideal that the hydrogel has a high release of the vitamin. This means that the amount of vitamins loaded was released at the maximum amount ensuring that less of the vitamins loaded were wasted.

The highest point of release can be seen at the red region of the plot in Figure 11 at the 50/50 mass ratio with a loading coefficient of 1. It can also be clearly seen in the 3D graph that the highest point is located at the 314% release. The hydrogel was able to release an average of 314% across three trials with the same loading coefficient and mass ratio.

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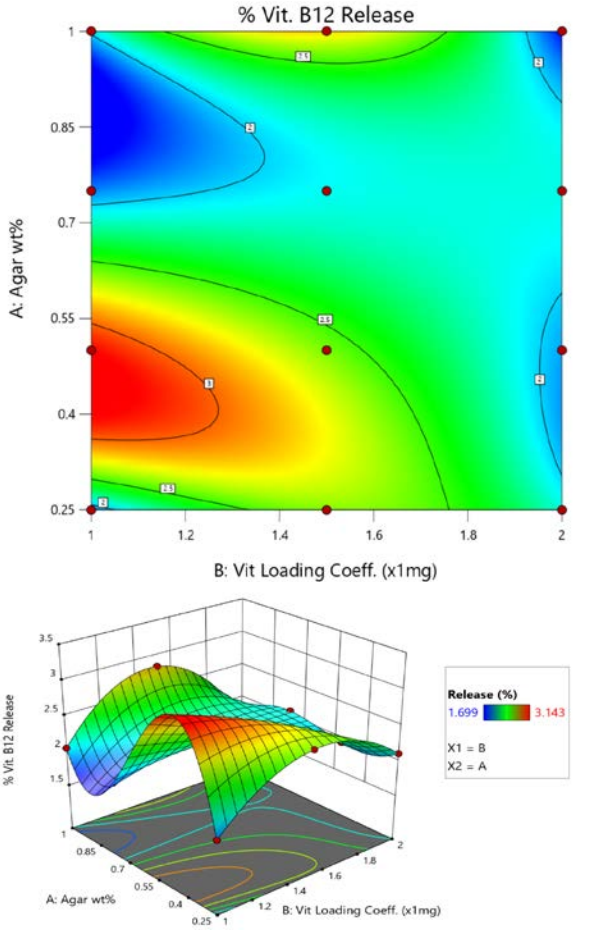


Fig. 11. Contour plot (top) and 3D plot (bottom) of the effect of loading coefficient and agar wt% to the % release of vitamin B12.

An earlier study on carrageenan-based drug delivery systems showed that agar and κ-carrageenan hydrogel blends used as metformin hydrochloride (MET) carriers displayed release profiles that are being dominated by electrostatic interactions between the pharmaceutical and polysaccharide [18]. It can be inferred that a similar release mechanism was present in the system evaluated in this study since the optimum agar/κ-carrageenan blend obtained was 50/50. Typically, for an agar and κ-carrageenan system, the loading efficiency for vitamin B12 is increased as κ-carrageenan concentrations increase as well. The anionic sulfate functional groups introduced by κ-carrageenan into the polymer matrix easily attracts the positively charged cobalamin molecules dispersed in solution. However, since no chemical crosslinker was used, once κ-carrageenan concentrations exceeded that of agar, the gel's structural integrity was compromised resulting in a poorer transport channel. Thus, it was gathered that insufficient agar concentrations resulted in ineffective transport media while insufficient κ-carrageenan concentrations exhibited inadequate release quantities. The blend formulation 50/50 struck a balance between structural integrity of the matrix and favorable electrostatic conditions for vitamin B12 delivery.

Using Design Expert®, the equation for optimization in terms of % release of vitamin B12 as a function of the wt% of agar and the loading coefficient was shown in Equation 2 where:

x – agar wt% of the hydrogel blend
y – loading coefficient of vitamin B12
z – total % release of vitamin B12

$$z = -27.07 + 137.17x - 103.14xy - 185.36x^2 - 5.94y^2 + 103.57x^2y + 15.00xy^2 + 69.92x^3 - 1.26x^2y^2 - 16.33x^3y - 11.66x^3y^2 \quad (2)$$

3.3.2 Optimal time to reach equilibrium release

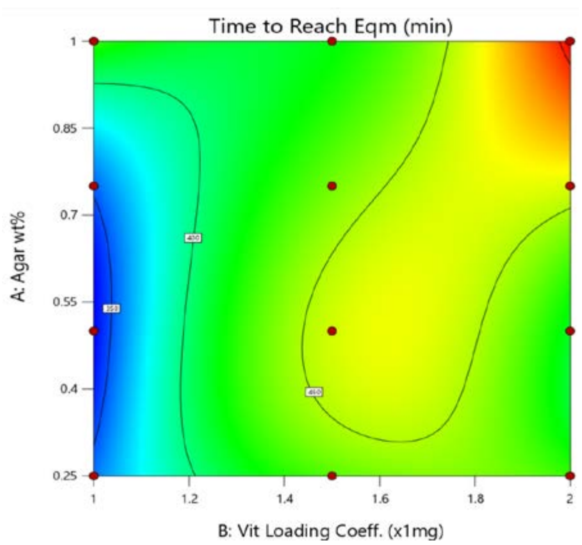
The effect of the amount of vitamin B loaded to a hydrogel as well as its mass ratio in terms of agar wt% was plotted against the time it takes for the release to reach equilibrium through a central composite response surface. The plots were used to determine the optimal blend in terms of the time needed to reach equilibrium release. Figure 12 shows a contour and 3D plot of the equilibrium release time in relation to the set parameters.

It is ideal that the time needed to reach equilibrium release is faster. Open wounds are exposed to more bacterial infection and other foreign materials that may cause wound complications. Faster release time reduces the hazards that the patients may experience given that the loaded hydrogel application is for open wounds.

Figure 12 shows the fastest and slowest release at equilibrium. It was observed that the time of equilibrium release at the loading coefficient of 1.5 has a similar range. The slowest release was observed at the 100/0 mass ratio and with a loading coefficient of 2. The fastest release time was at the 50/50 mass ratio and the loading coefficient of 1 at 337 minutes making it the optimal blend in terms of time of release to reach equilibrium.

Similar to the % release correlation in Equation 2, the time needed to reach equilibrium release for the hydrogel as a function of the agar wt% and the loading coefficient was obtained using Design Expert® where:

x – agar wt% of the hydrogel blend
y – loading coefficient of vitamin B12
z – time needed to reach equilibrium release



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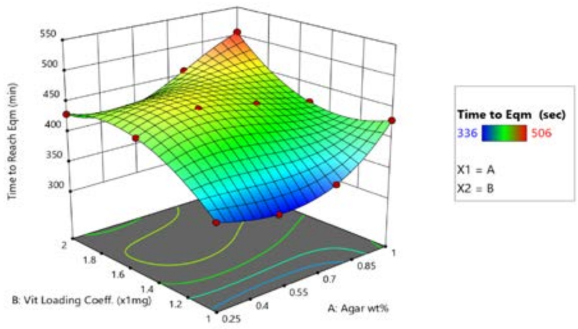

Fig. 12. Contour plot (top) and 3D plot (bottom) of the effect of loading coefficient and agar wt% to the time needed to reach equilibrium release.

$$\begin{aligned} z = 1238 - 7460x - 1352y + 11302.67xy \\ + 9904x^2 + 504y^2 \\ - 15624x^2y - 3976xy^2 \\ - 2944x^3 + 5648x^2y^2 \\ + 5173.33x^3y - 1948x^3y^2 \end{aligned} \quad (3)$$

4 Conclusion

In synthesizing a seaweed-based biopolymer hydrogel, the agar/κ-carrageenan hydrogel blend showed the most desirable characteristics of a vitamin B loaded hydrogel to be used for open wounds of cancer patients. Agar forms rigid gels at low concentrations even without a chemical crosslinker. On the other hand, κ-carrageenan was proven to have anti-cancer activity and increases the strength of the synthesized hydrogel.

Five mass ratios were performed at three different loading coefficients for each mass ratio. The mass ratio where the hydrogel contained 100% κ-carrageenan (0/100) did not form into a gel since it did not have any chemical crosslinker. The four other mass ratios (27/75, 50/50, 75/25, 100/0) all formed gels ranging from soft to rigid. The loading coefficients did not influence the rigidness of the hydrogels although as the loading increased, the color intensity also increased.

For the intended application, the optimal hydrogel blend should have a high % release, and a short amount of time needed for the release to reach equilibrium. The data shows limited trends between the parameters. The only observable trend based on the data shows that as the loading coefficient increased, the time of equilibrium release also increased. The highest % release of 314 % was observed at the 50/50 mass ratio with a loading coefficient of 1. The shortest amount of time needed for the release to reach equilibrium was found to be at 337 minutes and was also found at the same mass ratio and loading coefficient. Given these data, it was concluded that the optimal blend uses the agar and κ-carrageenan biopolymers, has a mass ratio of 50/50 agar/κ-carrageenan, and has a loading coefficient of 1.

For further studies, it is recommended to include other essential B-group vitamins such as B1 and B6 in future formulations. Other loading methods such as the post-loading method for vitamin loaded may also be studied for further experimentations. Smaller increments for mass ratios and other loading coefficients for the samples may also be investigated to add further insight on the hydrogel behavior. Additionally, a validation test may be included to further prove the validity of the optimal blend investigated.

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References

1. C. T. Peterson, D. A. Rodionov, A. L. Osterman, S. N. Peterson, B vitamins and their role in immune regulation and cancer. Nutrients **12**, 3380 (2020). https://doi.org/10.3390/nu12113380
2. C. Lin, M. Yin, B vitamins deficiency and decreased anti-oxidative state in patients with liver cancer. Eur J Nutr. **46**, 293–299 (2007). https://doi.org/10.1007/s00394-007-0665-8
3. E. C. R. de M. Viana *et al.*, Malnutrition and nutrition impact symptoms (NIS) in surgical patients with cancer. PLoS One **15**, e0241305 (2020). https://doi.org/10.1371/journal.pone.0241305
4. I.-H. Kim *et al.*, Korean practice guidelines for gastric cancer 2024: An evidence-based, multidisciplinary approach (Update of 2022 guideline). J Gastric Cancer. **25**, 5–114 (2025). https://doi.org/10.5230/jgc.2025.25.e11
5. 2024 Causes of Death in the Philippines. Accessed: Jul. **13**, (2025). [Online]. Available: https://psa.gov.ph/content/2024-causes-death-philippines-provisional-30-april-2025
6. H. Wang, L. Li, L. L. Qin, Y. Song, J. Vidal-Alaball, T. H. Liu, Oral vitamin B_{12} versus intramuscular vitamin B_{12} for vitamin B_{12} deficiency. Cochrane Database of Systematic Reviews **2018** (2018). https://doi.org/10.1002/14651858.CD004655.pub3
7. A. V. Sabiev, M. A. Korsten, W. A. Bauman, A comparison among three skin preparations for the transdermal delivery of vitamin B12 by iontophoresis: A novel approach to determine systemic absorption. (2021).
8. M. Rizwan, S. Naseem, S. R. Gilani, A. I. Durrani, Optimization of swelling and mechanical behavior of Acer platanoides cellulose combo hydrogel. Kuwait Journal of Science. **51**, 100177 (2024). https://doi.org/10.1016/j.kjs.2024.100177
9. Y. Ren *et al.*, Alginate-based hydrogels mediated biomedical applications: A review. International Journal of Biological Macromolecules. **279**, 135019 (2024). https://doi.org/10.1016/j.ijbiomac.2024.135019
10. D. J. McHugh, *A guide to the seaweed industry*. in FAO fisheries technical paper, no. 441. Rome: Food and Agriculture Organization of the United Nations (2003).
11. N. Jabeen *et al.*, Silymarin nanocrystals-laden chondroitin sulphate-based thermoreversible hydrogels; A promising approach for bioavailability

enhancement. International Journal of Biological Macromolecules. **218**, 456–472 (2022). https://doi.org/10.1016/j.ijbiomac.2022.07.114
12. R. C. Nissa *et al.*, The processability and properties of agar/gelatin film in a melt-mixing process. Green Materials. 1–9 (2024). https://doi.org/10.1680/jgrma.24.00002
13. Carrageenans, in *Carbohydrate Chemistry for Food Scientists*. Elsevier. 279–291 (2019) https://doi.org/10.1016/b978-0-12-812069-9.00013-3
14. J. Zhao, C. Sun, H. Li, X. Dong, X. Zhang, Studies on the physicochemical properties, gelling behavior and drug release performance of agar/κ-carrageenan mixed hydrogels. International Journal of Biological Macromolecules. **154**, 878–887 (2020). https://doi.org/10.1016/j.ijbiomac.2020.03.087
15. A. Gupta, J. Bhasarkar, M. R. Chandan, A. H. Shaik, B. Kiran, D. K. Bal, Diffusion kinetics of vitamin B_{12} from alginate and poly(vinyl acetate) based gel scaffolds for targeted drug delivery. Journal of Macromolecular Science, Part B. **59**, 713–730 (2020). https://doi.org/10.1080/00222348.2020.1800246
16. V. E. Santo *et al.*, Carrageenan-based hydrogels for the controlled delivery of PDGF-BB in bone tissue engineering applications. Biomacromolecules. **10**, 1392–1401 (2009). https://doi.org/10.1021/bm8014973
17. A. Saxena, A. Tahir, M. Kaloti, J. Ali, H. B. Bohidar, Effect of agar-gelatin compositions on the release of salbutamol tablets. Int J Pharm Investig. **1**, 93–98 (2011). https://doi.org/10.4103/2230-973X.82407
18. E.-M. Pacheco-Quito, R. Ruiz-Caro, M.-D. Veiga, Carrageenan: Drug Delivery Systems and Other Biomedical Applications. Marine Drugs. **18**, 583 (2020). https://doi.org/10.3390/md18110583