SEMARAK ILMU PUBLISHING

Journal of Advanced Research in Micro and Nano Engineering

Journal homepage: https://semarakilmu.com.my/journals/index.php/micro_nano_engineering/index
ISSN: 2756-8210

Efficient Screening of Biopolymers for Tablet Dosage Form using the Mixture Screening Design to Enhance Probiotic Viability and Gastric Resistance

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ARTICLE INFO

Article history:
Received 1 April 2025
Received in revised form 7 May 2025
Accepted 11 June 2025
Available online 16 July 2025

Keywords:
Probiotics; mixture design; biopolymers; acid resistance; viability

ABSTRACT

It is widely acknowledged that probiotics are beneficial for health, but harsh gastric conditions can destroy them. This condition highlights the need for protective delivery mechanisms in the stomach. Probiotics must be protected from the harsh gastric environment and provided with the right delivery mechanism for maximum effectiveness. This study aimed to identify significant biopolymer excipients that can be used to produce acid-resistant probiotic tablets using the mixture screening design. In this study, six biopolymers, namely, carboxymethyl cellulose (CMC), pectin, carbopol, hydroxypropyl methylcellulose (HPMC), alginate, and xanthan were screened at different ratios to evaluate their effects on probiotic viability and tablet disintegration time in simulated gastric/intestinal fluids. A 21-run design with three replicates was implemented using a mixture screening approach. Statistical analysis showed that CMC, HPMC, and alginate have significantly improved the viability and dissolution time of the tablets in the gastric environment. These polymers were selected for further optimisation studies to develop protective probiotic tablets. The mixture screening design allowed the efficient screening of polymer combinations to identify key excipients for maximising viability and gastric resistance.

1. Introduction

The term “probiotic” refers to live microorganisms that are beneficial to health once consumed. However, their efficacy depends on their viability during gastrointestinal (GI) transit. Probiotics can be damaged by harsh acidic gastric conditions and bile secretions, thus limiting their delivery to target sites [1]. Developing protective delivery systems is critical for shielding probiotics from the hostile GI environment. Tablets offer a convenient probiotic dosage form with several advantages, such as

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https://doi.org/10.37934/armne.40.1.2737

precise dosing, ease of use, and handling [2]. However, tablets require suitable excipients to increase acid resistance and gastric retention time of the probiotics [3].

Biopolymers are widely used as excipients to modify tablet properties. They can form protective matrices, control hydration and swelling, prolong disintegration, and modulate drug release [4]. Identifying optimal combinations of biopolymers is a crucial step but challenging due to potential excipient interactions. Although individual polymers have been extensively studied for probiotic delivery, there is limited understanding of how combined polymers may interact and affect probiotic protection. However, to test all possible polymer combinations would be incredibly time-consuming and resource-intensive. Mixture experimental design is an efficient approach for screening multiple polymers simultaneously and assess their synergistic effects at different ratios. This approach allows the identification of optimal polymer combinations for maximising probiotic gastric tolerance rather than just the effects of individual polymers. The rationale behind using a mixture design is that polymeric interactions may provide enhanced protection compared to single polymers alone.

This study aimed to utilise the mixture screening design to identify suitable biopolymer excipients for developing acid-resistant probiotic tablets. A total of six biopolymers, namely, CMC, pectin, carbopol, HPMC, alginate, and xanthan gum, were screened for their effects on probiotic viability and tablet disintegration time under simulated GI conditions. The collected data were then statistically analysed to determine key biopolymers that could significantly improve gastric tolerance. Biopolymers with the best results were selected to undergo further optimisation studies to formulate protective probiotic tablets.

2. Methodology

2.1 Powder Containing Yeast Probiotic

The probiotic yeast strain, *Saccharomyces boulardii*, was obtained from a previous study [5]. The strains were grown in yeast extract peptone dextrose (YEPD) broth for 24 hrs at 37 °C, and then, the cells were harvested via centrifugation at 10,000 rpm for 10 minutes [6]. This strain was then homogeneously suspended in the 10% skim milk as a cryoprotectant before proceeding to freeze-drying for powder preparation.

2.1 The Preparation of Tablets

A rotary tablet machine was used to prepare tablets using the direct compression method. Tablets containing probiotic powder and different biopolymers at different fractions have been prepared according to the formulations given by the mixture software, as shown in Table 1. These tablets contained freeze-dried probiotic yeast, talc, magnesium stearate, and different biopolymers (CMC, pectin, carbopol, HPMC, alginate, and xanthan) at different fractions, all weighing approximately 0.2 g.

2.2 The Preparation of Tablets

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2.3 Experimental Design for Biopolymer Screening

CMC, pectin, carbopol, HPMC, alginate, and xanthan were screened using a simplex mixture screening design to develop acid-resistant probiotic tablets. Each tablet is composed of 67% of functional polymers, with the remaining components being 30% of probiotic yeast powder as the active ingredient, 2% of talc as glidant, and 1% of magnesium stearate as the lubricant. The biopolymers for the tablet excipient were screened using the Design of Expert (DOE) statistical software, version 13. The effect of different biopolymers (independent variables) on microbial viability and duration of dissolution in the test system was investigated using a 21-run design. Table 1 lists the 21 formulations to be experimented, with six factors in three replicate runs. R1 (microbial viability percentage) and R2 (time taken by the tablet to dissolve) were generated as polynomial models. The linear model was selected based on the p-values of lack-of-fit and the F-values. This model was used to predict the most significant component of the formulation that will be used in the optimisation design.

Table 1
Experimental runs for the mixture screening design

Formulation	CMC	Pectin	Carbopol	HPMC	Alginate	Xanthan	R1 (%)	R2 (h)
1	1.000	0.000	0.000	0.000	0.000	0.000		
2	0.000	1.000	0.000	0.000	0.000	0.000		
3	0.000	0.000	1.000	0.000	0.000	0.000		
4	0.000	0.000	0.000	1.000	0.000	0.000		
5	0.000	0.000	0.000	0.000	1.000	0.000		
6	0.000	0.000	0.000	0.000	0.000	1.000		
7	0.583	0.083	0.083	0.083	0.083	0.083		
8	0.083	0.583	0.083	0.083	0.083	0.083		
9	0.083	0.083	0.583	0.083	0.083	0.083		
10	0.083	0.083	0.083	0.583	0.083	0.083		
11	0.083	0.083	0.083	0.083	0.583	0.083		
12	0.083	0.083	0.083	0.083	0.083	0.583		
13	0.000	0.200	0.200	0.200	0.200	0.200		
14	0.200	0.000	0.200	0.200	0.200	0.200		
15	0.200	0.200	0.000	0.200	0.200	0.200		
16	0.200	0.200	0.200	0.000	0.200	0.200		
17	0.200	0.200	0.200	0.200	0.000	0.200		
18	0.200	0.200	0.200	0.200	0.200	0.000		
19	0.167	0.167	0.167	0.167	0.167	0.167		
20	0.167	0.167	0.167	0.167	0.167	0.167		
21	0.167	0.167	0.167	0.167	0.167	0.167		

2.4 Simulated Gastric Fluid (SGF)

A modified approach was followed in the preparation of the SGF based on the USP 35 guidelines [7]. In this procedure, 2 g of sodium chloride was dissolved in sterile purified water. Subsequently, pH adjustment was conducted by introducing hydrochloric acid (HCl, 1 ml). Additional sterile water was added to achieve a total volume of 1 l. The resultant test solution was standardised to a pH of 2 ± 0.1.

2.5 Simulated Intestinal Fluid (SIF)

USP 35 protocol was followed when preparing the SIF [7]. Initially, 6.8 g of potassium dihydrogen phosphate (KH_2PO_4) was dissolved in 250 mL of water. This solution was then combined with 77 mL of 0.2N sodium hydroxide (NaOH) and 500 mL of water. Adjustments to the pH were made by adding either 0.2N NaOH or 0.2N HCl to reach a pH of 6.8 ± 0.1. Next, the volume was brought to 1,000 mL by adding water.

2.6 Treatment of Tablets in SGF

This method was adapted from Jamilah et.al, 2018 [8], and it involved incubating samples in 100 mL of SGF at 37 °C for 3 hrs to replicate gastrointestinal conditions. An incubator shaker was set at 200 rpm to simulate the movement of the bowels during the incubation process. Following the 3-hr period, the undissolved samples were transferred to 100 mL of SIF. Then, 1 mL of SGF was collected from the samples that were dissolved in SGF and transferred to SIF. Further assessment of the viability of probiotic cells was conducted using 1 mL of each suspension.

2.7 Treatment of Tablets in SIF

Following the treatment of tablets in SGF, approximately 1 mL of undissolved sample or 1 mL of SGF treated sample was transferred into 100 mL of SIF and incubated at 37 °C for 2 hrs while being stirred at 200 rpm. Following the incubation period, 1 mL of each suspension was extracted and subjected to an assessment of probiotic cell survivability.

2.8 Assessment of Cell Survivability using Flow Cytometer

The viability of the probiotic cells was evaluated using flow cytometry following fluorescent staining based on the protocol by [9] with modifications. Briefly, 500 µL aliquots of cells were obtained from each tablet sample. Propidium iodide (PI) and thiazole orange (TO) dyes from BD BioSciences were added to stain live and dead cells differentially. After vortexing and 5 min of incubation, flow cytometry was performed on the stained samples using an Accuri C6 detector with FL1 and FL3 channels. Instrument thresholds were set on forward scatter to exclude debris. A fluidic rate of 2,500 events/sec ensured good data resolution. The viability percentage was calculated by comparing the viable counts before and after the samples underwent simulated gastric/intestinal conditions using Eq. (1).

$$\%\,Viability = \frac{Post-treatment\ viable\ count}{Initial\ viable\ count}\ X\ 100 \tag{1}$$

3. Results and Discussion

A mixture design experiment is an experiment that does not rely on the total amount of mixtures but is based on the proportion of the components in each mixture. Mixture experiments in some fields, for example, the pharmaceutical and chemical industries, would normally involve large numbers of components, including six or more potential components that can be considered potential candidates [10]. It is important to identify the most significant component from all potential candidates in these experiments. In this case, the screening process was important before the actual experiment could be conducted, for example, the optimisation process.

The functional polymers, namely, CMC, pectin, carbopol, HPMC, alginate, and xanthan, were screened to determine the most effective combination of biopolymers as the excipient for the probiotic tablet dosage and to evaluate the responses, which were R1 (microbial viability) and R2 (dissolution time). By using the DOE software, a total of 21 formulations were generated by combining the six biopolymers as components in a mixture. The proportion of each component ranged from 0 to 1. The functional polymers were added at 67%, with the static component of 30% of probiotic yeast powder as an active ingredient, 2% of talc as a glidant, and 1% of magnesium stearate as a lubricant. The design and the experimental results obtained using three replications and 18 model points are shown in Table 2.

Table 2
Mixture screening design

Running	R1 (%)	R2 (h)
1	78.00	2.00
2	49.30	5.50
3	23.00	35.0
4	86.00	3.00
5	93.00	6.00
6	94.60	48.0
7	77.00	4.00
8	42.40	9.75
9	35.00	25.0
10	81.00	5.00
11	96.00	8.50
12	80.20	35.0
13	60.10	19.0
14	68.20	14.0
15	82.70	7.00
16	65.00	13.0
17	77.10	16.5
18	81.10	11.0
19	73.90	12.0
20	77.10	13.5
21	70.60	11.0

An overview of the lack-of-fit test and the sequential model is provided in Table 3. In terms of statistics, the linear model could adequately explain the response based on the significant p-values < 0.0001 for R1 and R2. In this experiment, an assumption was made whereby the linear effect was enough to decide which component truly mattered to the screening process. As seen in this table, the large p-values of $p = 0.1182$ and $p = 0.1469$ of the lack-of-fit test for R1 and R2, respectively, indicate that this model can be accepted.

Table 3
Sequential and lack-of-fit results for the linear model

Type of model	Sequential p-value		Lack-of-fit p-value		Adjusted R2		Predicted R2	
	R1	R2	R1	R2	R1	R2	R1	R2
Linear	< 0.0001	< 0.0001	0.1182	0.1469	0.8050	0.9395	0.6946	0.8348

An analysis was conducted to estimate the linear gradients and effects of the individual components. Figure 1 illustrates the trace plots for both R1 and R2 responses for the six biopolymer mixture screening experiments. These trace plots would be a helpful visual representation of the relative effects of each component. These plots can illustrate the effects of altering the proportion of the biopolymers from the reference blend on viability (see Figure 1(a)) and dissolution time (see Figure 1(b)). If the other biopolymers were held at a constant ratio to each other, the trace plot could be a very useful tool in tracking the movement of any given biopolymer. This study focused on the dissolution time and microbial viability values since a good probiotic tablet is expected to have minimum dissolution time and a high percentage of probiotic viability.

As shown in Figure 1(a), only components that are higher than the horizontal reference line is significant, which are E, F, D, and A, while components B and C do not show any significant to the viability. Based on the dissolution time analysis, as shown in Figure 1(b), only components A, D, B, and E that are below the horizontal reference line show significant, while components F and C are not significant. Therefore, based on the Cox trace plot analysis, only components E (alginate), D (HPMC), and A (CMC) were chosen to proceed to the optimisation process.

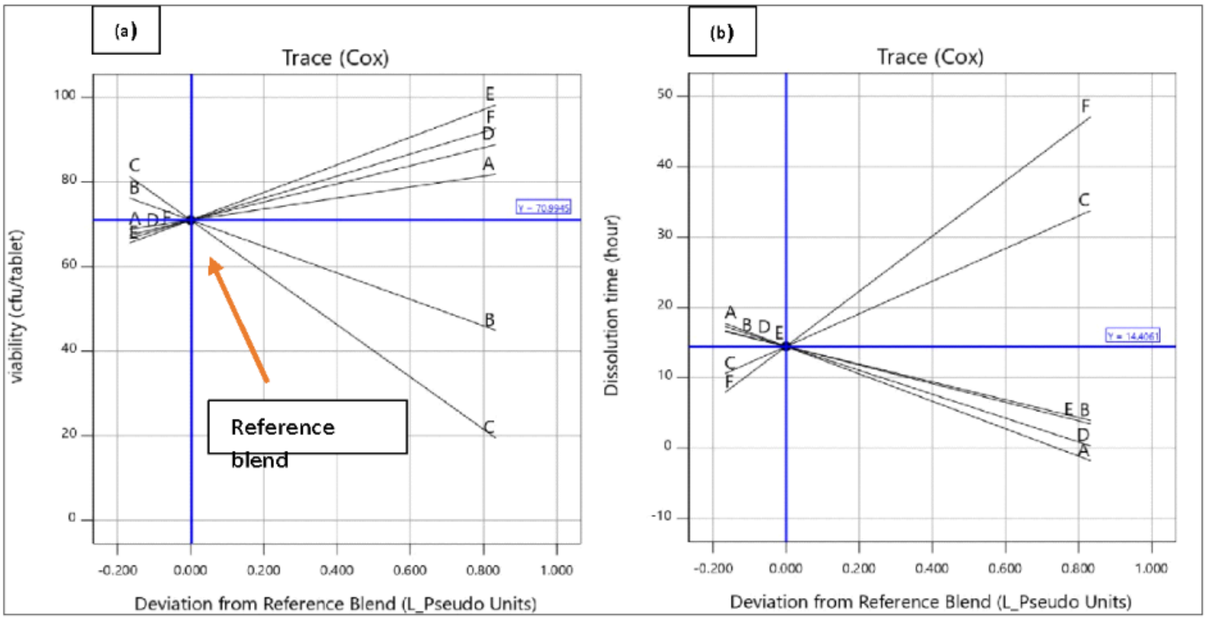

Fig. 1. Trace plots of the mixture screening experiments of six biopolymers: a) effect of individual component towards viability and b) effect of individual components towards dissolution time

Based on the predicted optimisation function, the desirability criteria were also used to select biopolymer screenings. The assessment of this study is to determine a good probiotic tablet that was based on the indicator of higher viability and minimum dissolution time. Thus, a value was set for the expected response. All factors and responses with their restrictions are provided in Table 4. Meanwhile, response values for viability were configured as “maximum” and the dissolution time was “minimum.” The top three highest desirability was used as a reference for the selection of components for the optimisation process. From this analysis, only three components have shown significance towards the response, which were CMC, HPMC, and alginate.

Table 4
Optimisation predictions by the software

Number	1	2	3
CMC	4.10	6.14	7.33
Pectin	0	0	0
Carbopol	0	0	0
HPMC	8.77	5.21	16.16
Alginate	54.13	55.65	43.51
Xanthan	0	0	0
Cell	30.0	30.0	30.0
Talc	2.00	2.00	2.00
Magnesium Stearate	1.00	1.00	1.00
Viability	96.00	96.00	96.00
Dissolution time	2.71	2.72	2.11
Desirability	0.96	0.97	0.98

Based on the desirability criteria, the formulation with the highest desirability (Formulation no 3) was chosen for confirmation and validation of the model. The optimal formula obtained by software optimization comprised 7.33% CMC, 16.16% HPMC, and 43.51% alginate. The predicted response values under this formulation were expected to obtain microbial viability of 96.00% and dissolution time of 2.11 h. Three confirmatory experiments were carried out to validate the predicted values obtained from the mixture design approach. The results of the three confirmatory experiments align with the predictions made using the mixture design approach. The obtained microbial viability of 94.25 ± 3.0% and dissolution time of 2.6 ± 0.4 h for the probiotic tablets were statistically comparable to the predicted values, as confirmed by the t-test ($p > 0.05$). The t-test confirmed that these differences are within an acceptable range, indicating that the experimental results align well with the predictions from the mixture design approach. This concordance between the experimental and predicted outcomes validates the effectiveness of the mixture design in accurately screening and identifying the optimal biopolymer combinations for developing acid-resistant probiotic tablets. The mixture design approach proved to be a reliable and efficient method for evaluating the synergistic effects of multiple biopolymers on probiotic viability and tablet dissolution time under simulated gastrointestinal conditions.

Alginate showed the most significant positive effect on the maintenance of high probiotic viability following exposure to simulated gastric and intestinal fluids. Alginate can form a gel network that can encapsulate and protect probiotic cells from harsh acidic conditions. The carboxyl groups in alginate interacted with divalent cations like Ca^{2+} to form egg-box junctions, which created a hydrogel matrix that provided a physical barrier against low pH and enzymes [11].

Probiotic yeasts encapsulated in sodium alginate beads or chitosan-coated beads have demonstrated greater survival in simulated gastrointestinal conditions than unencapsulated cells [12]. The addition of carrageenan or nanocrystalline cellulose to alginate beads has also been proven to provide excellent protection for probiotic bacteria during both storage and in vitro simulation of gastrointestinal tract function [13]. The morphology of alginate beads can affect the release profile and stability of encapsulated probiotics, further emphasising the importance of encapsulation materials [14]. The choice of encapsulating materials, such as alginate, has been shown to play a crucial role in improving probiotic survivability. Based on the previous literatures, it is well established that the encapsulation of probiotics in alginate beads or gels could significantly improve their viability and survival under various conditions.

HPMC similarly improved probiotic survival in gastrointestinal conditions. As a non-ionic cellulose derivative, HPMC hydrated rapidly to form a thick gel layer on the tablet surface. This viscous gel layer helped prevent rapid tablet disintegration and release of probiotics in the stomach. HPMC was able to prolong gastric retention time and reduce probiotic exposure to destructive acidic pH [15]. At gastric pH, alginate combined with HPMC can form more viscous gels, which can provide an excellent matrix for proteobacterial protection [16]. Probiotics could also be applied to this combination as a dietary supplement with promising results.

CMC could be an ideal component of probiotic tablets due to its beneficial properties, such as the ability to absorb water and high solubility. The higher percentage of CMC affected the minimum dissolution time due to its ability to disintegrate rapidly in aqueous medium, leading to immediate swelling and volume increase [17]. These conditions could help the probiotic tablets to dissolve quickly in the stomach, allowing for better absorption of the probiotics. CMC is also non-toxic and non-sensitising, making it a safe choice for use in probiotic tablets. The use of CMC in sustained-release tablet formulations is well-documented in the literature [18], [19], [20]. This cellulose derivative has been widely employed in pharmaceutical preparations to achieve sustained drug release. Additionally, the use of methyl cellulose in gel formulations has been noted due to its sustained-release action. Alginate, HPMC, and CMC have polymeric networks and gel-forming properties that can protect the probiotic cells from acid and, thus enhance probiotic viability in gastrointestinal conditions.

To obtain information concerning the interaction of biopolymer components in tablet formulations on probiotic viability, contour plots were created. Figure 1(a)–d illustrates how different components interact to influence probiotic viability and dissolution time. For probiotic viability, plots show that increasing concentrations of CMC and HPMC significantly enhance viability, indicated by the yellow regions near their vertices in Figure 1(a). Similarly, alginate and xanthan positively impact viability in Figure 1(b). Significant effects on decreasing dissolution time are seen with pectin, CMC, alginate, and HPMC, reflected by the blue regions near their vertices in Figure 1(c) and Figure 1(d).

These findings highlight the crucial role of CMC, alginate, and HPMC in optimizing tablet formulations for enhanced probiotic viability and controlled dissolution. CMC and HPMC appear to form protective matrices around probiotic cells, enhancing their viability during tablet formulation. Alginate’s gel-forming ability likely offers similar protective effects, while xanthan gum's thickening properties help maintain cell integrity. On the other hand, the blue regions indicating shorter dissolution times for these biopolymers suggest they can also facilitate faster release of probiotics in the gastrointestinal environment, which is beneficial for effective delivery. These results support the trace plot analysis, which further corroborates the positive influence of CMC, alginate, and HPMC on probiotic viability and the controlled dissolution time of the tablets. The alignment between the contour plots and trace plot analysis strengthens the conclusion that these biopolymers are essential for developing protective probiotic tablets with enhanced gastric tolerance and viability.

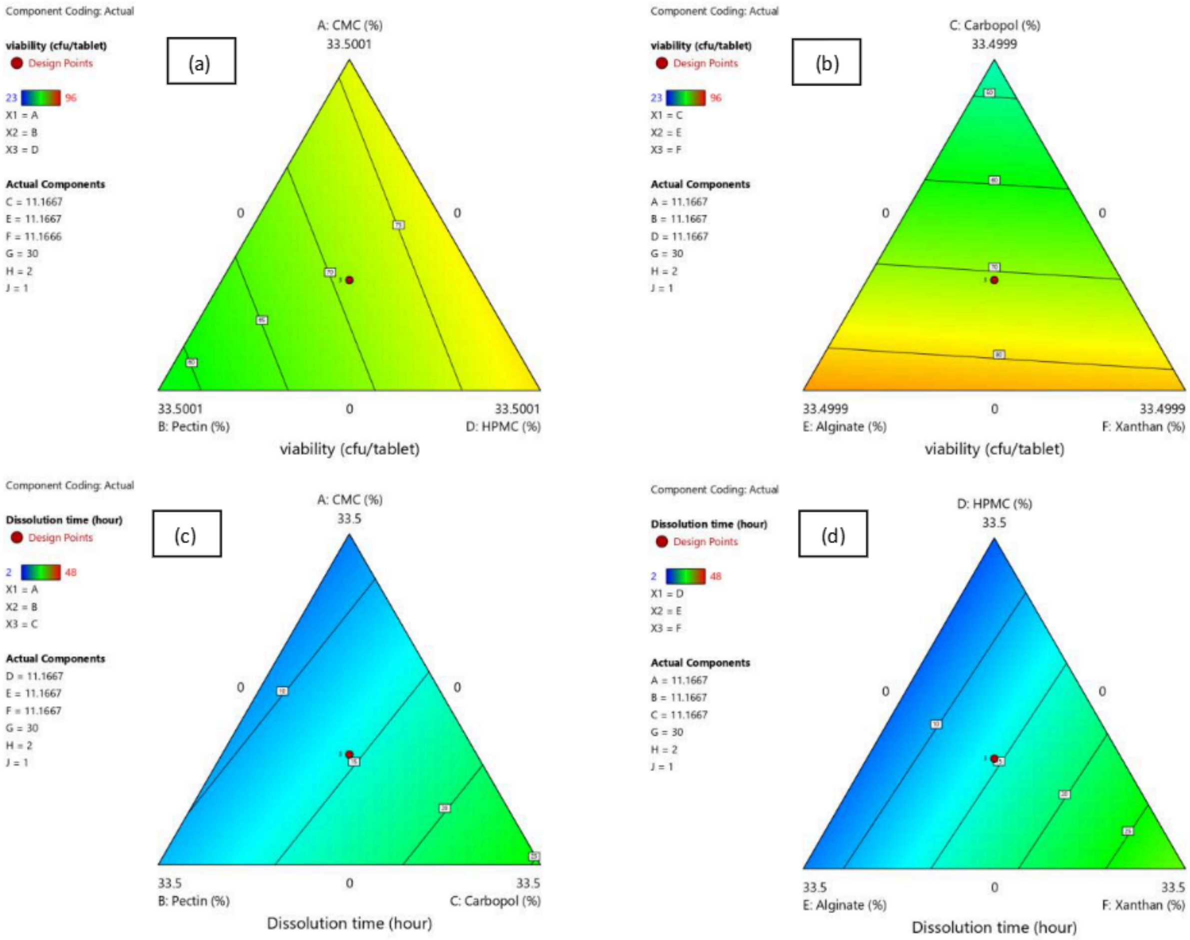

Fig. 2. Contour plot of mixture analysis for (a), (b) probiotic viability and (c), (d) tablet dissolution time

4. Conclusions

This study has demonstrated the application of a simplex mixture design to efficiently screen and identify optimal biopolymer excipients for developing acid-resistant probiotic tablets. The effects of six biopolymers were evaluated on probiotic viability and tablet disintegration time under simulated gastrointestinal conditions. Statistical analysis of the experimental data showed that CMC, HPMC, and alginate produced the most significant positive effects on maintaining high probiotic viability while minimising disintegration time in acidic gastric fluids. These key biopolymers were selected to undergo further optimisation studies to determine the ideal ratios to formulate a protective probiotic tablet dosage form. The mixture design allowed the screening of multiple formulation components simultaneously, which saved time and resources compared to testing all possible combinations individually. The generated data provided insight into the roles of specific biopolymers in improving gastric tolerance and oral delivery efficiency of probiotics. This rational formulation approach can serve as a model for developing optimised probiotic dosage forms with maximal stability under gastrointestinal conditions.

Acknowledgement

The funding for this work was obtained from the internal grant of Universiti Malaysia Pahang Al-Sultan Abdullah (RDU220345) and Tabung Persidangan Luar Negara (TPLN) UMPSA.

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