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RESEARCH ARTICLE

Design and optimization of a probiotic tablet for gastrointestinal tolerance by a simplex-centroid mixture

Mohd Akmal Azhar and Mimi Sakinah Abdul Munaim

Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, Kuantan, Malaysia

ABSTRACT

In this study, a simplex-centroid mixture design using design of experiment (DOE) software was implemented to evaluate the effect of biopolymers as excipients, which are hydroxypropyl methylcellulose, and alginate, on the gastrointestinal tolerance of probiotic tablet containing *Saccharomyces boulardii*. Microbial viability and dissolution time were used to evaluate the ideal formulation made using 39.01% carboxymethylcellulose and 60.99% alginate as excipients, which protected the probiotics from the acidic condition in the stomach with good dissolution time. The formulated probiotic tablet is more stable in terms of viability when stored at 4 °C compared to room temperature. However, the viability remains above 10^6 CFU/tablet after six months of storage at room temperature. This study shows that the simplex-centroid mixture design is valid and can be used to formulate probiotic tablets that possess gastrointestinal tolerance. This study can lead to the development of commercial production of probiotic yeast tablets with gastrointestinal tolerance.

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Gastric tolerance; probiotic; tablet; Saccharomyces boulardii; simplex-centroid mixture

Introduction

Probiotics are microorganisms such as bacteria and yeast that are orally introduced into the human digestive system and favorably respond to the digestive system to confer health benefits to the host. In the last decade, various studies regarding probiotic cells have determined them to benefit the human body. Probiotics function by maintaining a good intestinal microbial community, preventing the development of pathogenic microbes, activating the defending host system, relieving constipation, synthesizing vitamins and antimicrobials, and enhancing calcium absorption when there are sufficient probiotics in the colon [1,2].

One of the main issues for the oral probiotic formulation is probiotic microorganisms' viability because they are expected to be administered and transported viably to the target side. Some researchers suggested that the probiotics' viability must be maintained at 10^6 CFU/g or more to make it beneficial [3]. Others said that it must be maintained at 10^8 CFU/g [4] and 10^9 CFU/g to 10^{12} CFU/g [5]. This remains a significant problem for manufacturers and customers, as the probiotics can only be beneficial to the host if adequate viable cells are delivered to the target site after surviving the acidic condition in the gastrointestinal tract. Generally, various probiotic strains are sensitive to acidic conditions. They could not survive the stomach's gastric juice unless they possess acidic tolerance or have some form of protection such as coating or encapsulation [6]. Therefore, it is necessary to develop formulations that protect the probiotics from gastric pH and harsh conditions in the human stomach.

The probiotic formulation in a tablet matrix form or the monolithic matrix system is one of the ideal techniques to protect the probiotics from the harsh condition in the gastrointestinal tract, for instance, the extreme condition of the bile salt and acidic pH [7]. The matrix tablet formulation can be developed to improve the colonization of probiotic cells on the inner layer of the gastrointestinal tract. Additionally, the tablet dosage form offers some advantages compared to other dosage forms, such as enhancing microorganism stability at extreme temperatures, low cost for large-scale production, precise dosing, and ease of handling [8]. Moreover, with a suitable excipient formulation, a probiotic tablet can be formulated to regulate the release and delivery of probiotic cells to the target location in the gastrointestinal system [9].

In the tablet production process, biopolymers can be used as an excipient in the formulation. Alginate is one of the most common compounds integrated as an excipient for probiotic encapsulation because of its nontoxicity and biocompatibility. Moreover, this biopolymer is popular because of its low cost. It forms a gel when interacting with an aqueous medium and acts as a diffusion barrier for the probiotic cells as an active component in the formulation [10]. Hydroxypropyl methylcellulose (HPMC) is another essential biopolymer in the development of probiotic tablets. It forms a hydrogel layer by hydrating and swelling once it comes in contact with a liquid medium [11]. Therefore, it can regulate the diffusion of liquid into the tablet core, thus controlling the dissolution of the active ingredient inside the tablet. Carboxymethylcellulose (CMC) is another excellent biopolymer that improves the diffusion or dissolution system of tablet matrix design [12]. Generally, it facilitates the release of the active ingredient by hydrating the matrices, then diffusing the active ingredient into the bulk solution. It can achieve a constant delivery rate of active ingredient by partial dissolution or erosion. The chemical structures of alginate, HPMC, and CMC are shown in Figure 1.

One of the approaches to design and formulate pharmaceutical tablet dosage form is by using design of experiment (DOE) software. It is a type of statistical experimental design used in many fields, including pharmaceutical, engineering, and food industries.

CONTACT Mohd Akmal Azhar mohdakmalazhar@gmail.com Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, Gambang, Kuantan, Pahang 26300, Malaysia

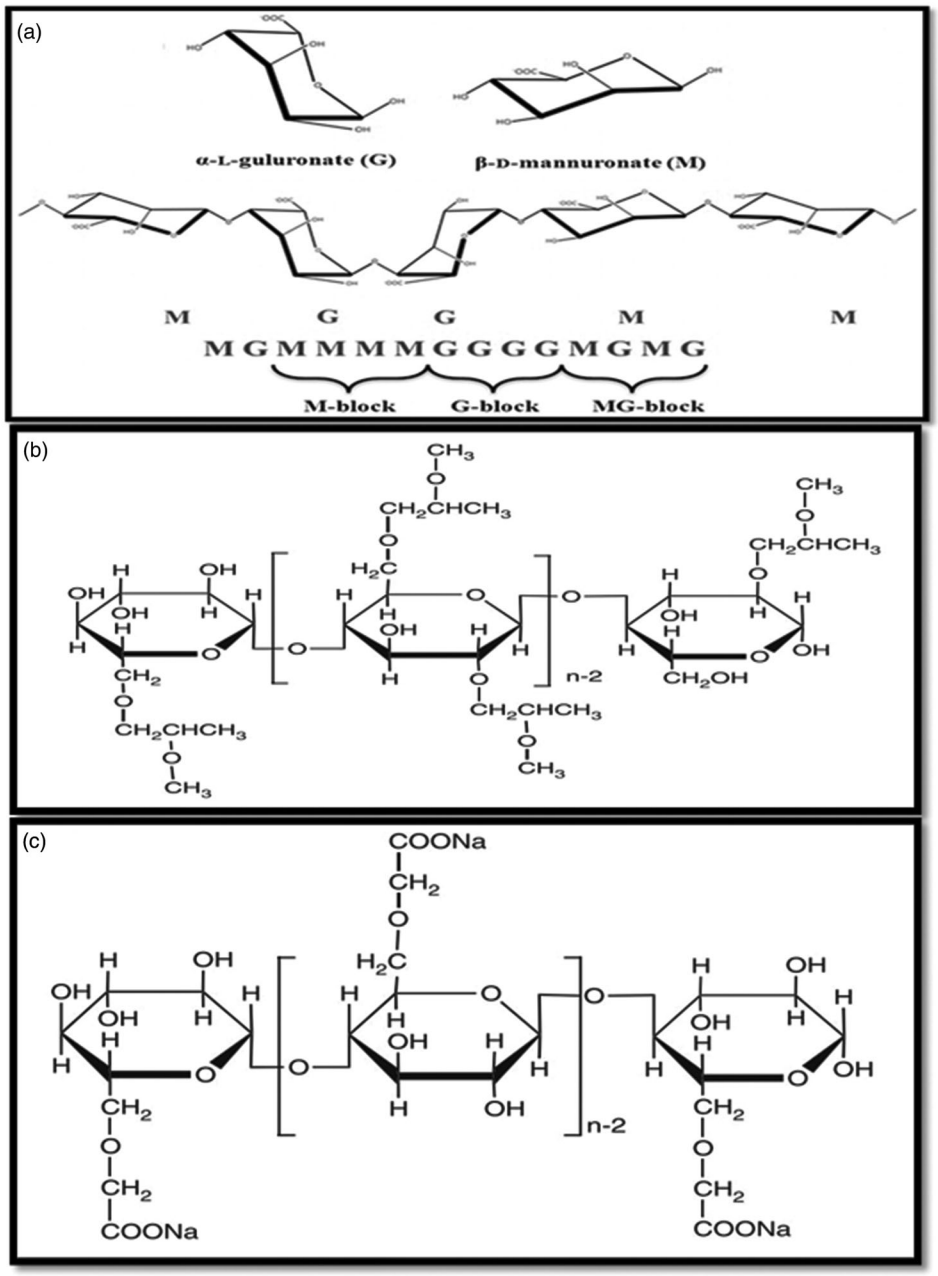

Figure 1. Chemical structures of (a) alginate, (b) HPMC, and (c) CMC.

This is a systematic approach to understand how the process and product parameters influence response variables such as processability, physical properties, or product output [13].

One of the DOE designs named the simplex-centroid mixture approach can be used to obtain the optimum formulation with the minimum number of experiments. It can be customized to select a suitable condition to obtain an optimized response through various specific components in the formulation [14]. Moreover, one of the advantages of using a mixture experiment design is the reduction of experiments required to assess multiple variables. This approach can also classify the interaction statistically, which can overcome the shortcoming of the traditional methods of the formulation [15].

Most of the previous studies for acidic tolerance are related to lactic acid probiotics. Few reports exist regarding probiotics from yeast compared to bacterial strains, although it has a good potential of probiotic ability that can benefit human health. Compared to bacterial probiotics, products containing yeast are not as widely available but are fast developing and becoming more common. In our previous study, a potential probiotic yeast was identified and evaluated for use in new probiotic yeast formulations or food supplements [16,17]. Therefore, the purpose of the current study is to achieve the ideal formulation for the probiotic tablets containing *Saccharomyces boulardii* with optimum dissolution time and high viable counts when coming in contact with low pH in the human stomach. Many studies regarding acidic tolerance used individual polymers or materials to encapsulate the probiotic strains. In this study, the optimal mixture design using Design-Expert software was implemented to optimize a combination of excipients from different functional polymers, which are CMC, HPMC, and alginate. In this study, the tablet matrix dosage form was formulated using the direct compression method, which has few manufacturing steps and is an economical process compared to other methods in the industry. This form can be used as a product in the

pharmaceutical field to ensure that the active cells are protected from the harsh condition and delivered into the intestinal tract.

Materials and methods

Preparation of probiotic powder

Saccharomyces boulardii that was isolated and screened as a probiotic strain from the previous study [17] was cultured and formed into powder by freeze-drying. The yeast was grown at 37 °C for 24 h in a YEPD medium. The cells were then harvested by a high-speed centrifuge (5810 R, Eppendorf) at 4000 rpm for 15 min [18]. An equal volume of 10% skim milk was added to the cells as a cryoprotectant. Then, a vacuum freeze dryer (BTP-8ZL00X, SP Scientific) was used to freeze-dry the mixture.

Preparation of tablets containing probiotic cells

A direct compression method was used to prepare the probiotic tablets. The process was started by mixing the freeze-dried probiotic powder containing *Saccharomyces boulardii* with other materials in the mixer VMD-AC (Shakti). The mixture was then compressed into tablet form using a rotary tablet machine (UET-6D, UEC Unity Equipment Co.), using a 12 mm oblong mold and compression force required to produce tablets with a hardness of about 25 N. The targeted tablet weight of 0.2 g was set to produce about 1000 tablets containing freeze-dried probiotic yeast, talc, magnesium stearate, CMC (Sigma), HPMC K4M pharmaceutical grade (Emory), and sodium alginate (R&M chemical) with different fractions based on the mixture design suggested by the DOE software.

Experimental design

The level of independent variables was based on the preliminary study (data not shown). Probiotic matrix tablets were formulated according to the design generated using Design Expert software version 11. A 13-run, three-factor, simplex centroid mixture design having three center points and three replicate runs were used to study the effect of formulation (independent) on microbial viability percentage and dissolution time (dependent variable). Polynomial models were generated for the responses Y1 (% microbial viability) and Y2 (dissolution time). The linear model was chosen based on the *p*-value for *F* value and lack of fit. The composition of the formulation was predicted by the model, which would provide the desired viability and dissolution time profile.

Simulated gastric fluid (SGF) preparation

The SGF was prepared according to a modified USP 35 method [19]. Two grams of sodium chloride and 3.2 g of purified pepsin were dissolved in sterile purified water. Next, 1 mL of concentrated hydrochloric acid was added to adjust the pH, and sterile water was added to make 1 L. The pH of the test solution was approximately 2.

Simulated intestinal fluid (SIF) preparation

The SIF was prepared according to USP 35 method [19]. First, 6.8 g of monobasic potassium phosphate was dissolved in 250 mL of purified water. Next, 77 mL of sodium hydroxide (0.2 N), 10 g of pancreatin, and 500 mL of purified water was added to the solution. The solution pH was adjusted to 6.8 ± 0.1 by using 0.2 N of sodium hydroxide or 0.2 N of hydrochloric acid. Lastly, purified water was added to make 1 L.

Treatment of probiotics in SGF

This procedure was modified from Jamilah et al. [20]. Samples were incubated for 3 h in 100 mL of SGF at 37 °C to mimic the human stomach condition with stirring at 200 rpm using an incubator shaker to mimic bowel movement. The undissolved samples were transferred to 100 mL of SIF after 3 h. If the samples dissolved in the SGF, 1 mL of SGF was collected and transferred to SIF. One milliliter of each suspension was removed and evaluated for the survivability of the probiotic cells.

Treatment of probiotics in SIF

This procedure was modified from Jamilah et al. [20]. After the gastric treatment using SGF, the undissolved samples or 1 mL of SGF samples were transferred into 100 mL of SIF and incubated at 37 °C and 200 rpm for 2 h. Then, 1 mL of each suspension was removed and evaluated for the survivability of the probiotic cells.

Assessment of cell survival

The evaluation of probiotics survival was based on Chiron et al. [21]. For each sample, 500 µL of cell suspension was collected. Next, 5 µL of thiazole orange (TO) solution and propidium iodide (PI) dye solution (BD cell viability kit) was added to the samples. The samples were then vortexed and incubated for at least 5 min at room temperature. The samples were passed through a flow cytometer (Accuri C6), and the data were collected using the FL1 versus FL3 dot plot. All experiments were conducted in triplicate. The percentage of microbial viability was calculated by using the formula [22]:

$$\text{Viability } (\%) = \frac{B}{A \times 100} \quad (1)$$

where A and B are viable counts before and after the gastric treatment, respectively.

Storage stability experiment

The tablets were stored at two different temperatures to validate the stability storage for the optimized tablet formulation. The tablets were sealed using plastic packaging and stored at 25 and 4 °C to verify the stability of the probiotic yeast tablets and examine the effect of different storage temperatures on them. The viability of the probiotic yeast inside the tablet was measured every two weeks.

Statistical evaluation

The data analysis including mixture and model, were conducted using Design Expert software version 11.

Results and discussions

Preparation of probiotic tablet

The tablet compression process resulted in tablet hardness around 25 N, with a total of 13 different formulations suggested by the software. The three functional polymers only comprise 67% excipient. Other static components in the tablet formulation are 30% probiotic yeast powder as the active ingredient, 2% talc as glidant, and 1% magnesium stearate as lubricant.

Table 1. The simplex-centroid mixture design and results of the experiment.

Formulation	X1	X2	X3	Y1 (%)	Y2 (H)
1	1.00	0.00	0.00	17.95	2.00
2	0.00	1.00	0.00	65.46	12.00
3	0.00	0.00	1.00	93.88	5.50
4	0.50	0.50	0.00	46.92	3.00
5	0.50	0.00	0.50	89.06	1.50
6	0.00	0.50	0.50	86.32	17.00
7	0.33	0.33	0.33	80.43	5.20
8	0.67	0.17	0.17	55.67	2.00
9	0.17	0.67	0.17	69.33	8.40
10	0.17	0.17	0.67	90.97	5.90
11	1.00	0.00	0.00	15.52	1.50
12	0.00	1.00	0.00	67.45	13.00
13	0.00	0.00	1.00	94.52	7.00

Mixture optimal design results and analysis of variance

The mixture design of the simplex-centroid approach was used with the help of Design-Experts to optimize the tablet formulation with three different functional polymers for gastrointestinal tolerance (X1: CMC, X2: HPMC, X3: Alginate). The design and the experimental results containing three replicates and ten model points are shown in Table 1. The evaluated response variables are Y1: microbial viability and Y2: dissolution time.

In this experiment, formulations 11, 12, and 13 are considered as replication models. A replication model is important to determine the precision of estimates effect and gives additional information on background process variation. A replication model can also be used to estimate pure error. It can provide information about the adequacy and efficiency of the specified model, known as a lack-of-fit test. The lack-of-fit tests and the comparison of the sequential model are summarized in Table 2. Statistically, the model properly explains the response based on the significant *p*-value (<.0001) of the response Y1 for the linear and quadratic models.

On the other hand, the special cubic and cubic models did not fit the data because of the large *p*-value (>.05). Similarly, the linear and quadratic models for Y2 indicate that it fits the data with a low *p*-value (<.05) of .0016 and .0014, respectively. As seen in Table 2, the large *p*-values (.2246 and .1649 for Y1 and Y2, respectively) of the lack-of-fit test indicate that the quadratic model can be accepted. A lack-of-fit test diagnoses whether a model sufficiently fits the data. It is based on the residual sum of squares calculation [23]. Hence, the regression model of both values of the response is valid and applicable for the analysis of microbial viability (Y1) and dissolution time (Y2).

The adjusted coefficients of determination (adjusted R^2) of the equations Y1 and Y2 for the quadratic model are 0.9954 and 0.9417, respectively, indicating that 99.54% and 94.17% of the results can be explained by the equations. The small difference (<0.2) between the adjusted coefficients of determination (adjusted $R^2 = 0.9954$) and predicted coefficients of determination (predicted $R^2 = 0.9920$) for Y1 indicates that Equation (2) is well fitted. Similarly, the other equations are also well fitted. A quadratic multinomial regression fitting was performed; the regression model was set by two indicators, and the following multiple regression equations were obtained:

$$Y1 = 16.89A + 66.32B + 93.83C + 20.92AB + 132.57AC + 20.46BC \quad (2)$$

$$Y2 = 2.01A + 12.42B + 6.18C - 20.12AB - 13.55AC + 24.93BC \quad (3)$$

Analysis of variance (ANOVA) in Table 3 shows that the linear mixture components for AC ($p_{AC} < .0001$) are significant model terms, indicating that the interaction between CMC and alginate has the greatest impact on Y1 (microbial viability) of probiotics tablets. In addition, the interaction between AB and BC also has a notable impact on the viable counts of probiotics, as their *p*-values are less than .05 ($p_{AB} = .0236$ and $p_{BC} = .0258$). Moreover, the linear components, AB, BC, and AC show a significant impact on the response value Y2 (dissolution time) because the *p*-value is lower than .05. Additionally, components AB and BC show the greatest impact on the response because of their lowest *p*-values.

Table 2. Model comparison for the sequential and lack of fit for the design model.

	Sequential *p*-value		Lack of fit *p*-value		Adjusted R^2		Predicted R^2	
Type of model	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2
Linear	<0.0001	0.0016	0.0014	0.0167	0.8239	0.6676	0.7520	0.5879
Quadratic	<0.0001	0.0014	0.2246	0.1649	0.9954	0.9417	0.9920	0.7858
Special cubic	0.9128	0.0728	0.1642	0.2690	0.9947	0.9619	0.9705	0.7848
Cubic	0.5670	0.2050	0.0746	0.3335	0.9940	0.9741	0.7539	0.5242

Effect of mixture component and formulation optimization

The contour map and the three-dimensional response surface of each element can visually demonstrate the relationship between the components of the mixing design of probiotic tablet formulation.

As shown in Figure 2(a), a high value of Y1 is observed at vertex C, where a higher percentage of C (alginate) is required for the maximum microbial viability percentage of probiotic tablets after acidic treatment. On the other hand, Figure 2(b) shows that a higher percentage of component A (CMC) and a low percentage of component B (HPMC) and component C (alginate) are required for the minimum dissolution time.

The coefficients (Table 4) of the equation to microbial viability (Y1) suggest that the linear terms present a high contribution of alginate (C), followed by HPMC (B) and CMC (A). There is an increase in viability when alginate or a binary mixture of CMC and alginate was used. In addition, for dissolution time (Y2), the linear term indicates that CMC provides the highest results for the minimum dissolution time, followed by alginate and HPMC. However, for the binary mixture, a combination of CMC and HPMC (AB) gives the highest value, followed by the combination of CMC and alginate (AC). On the other hand, the combination of HPMC and alginate (BC) has a negative effect on the response Y2.

The total time for food to move through the human stomach ranges around 2.5–4 h. Meanwhile, the pH in the gastric juice range around 2–3 after food consumption and can decrease to 1 during fasting [24]. Therefore, pH 2 was chosen as a significant value for this analysis of simulated gastric resistance, and 3 h was selected as the processing time. Probiotic microorganisms should be viable and reach their target of action alive. Thus, the probiotic product needs to have some protection from the gastric juice. The viability of the probiotics after acidic treatment varies depending on the ability of the matrix tablet to retain its form without being dissolved in the acidic medium. Based on the contour map and surface plot in Figure 2(a), alginate is an important component to protect the probiotics from gastric pH because of its ability to form a gel in acid if the pH is below its pKa (3.3–3.5) [25]. This ability is conferred by the presence of the carboxylic acid group in the alginate structure. This is one of the useful properties of alginate for enteric delivery vehicles.

Table 3. Analysis of variance (ANOVA) for the quadratic model of response Y1 and Y2.

	Y1					Y2				
Source	Sum of squares	df	Mean square	*F* value	*p*-value	Sum of squares	df	Mean square	*F* value	*p*-value
Model	8666.62	5	1733.32	520.84	<.0001	282.44	5	56.49	39.74	<.0001
Linear mixture	7414.78	2	3707.39	1114.02	<.0001	211.39	2	105.7	74.35	<.0001
AB	27.65	1	27.65	8.31	.0236	25.59	1	25.59	18	.0038
AC	1110.71	1	1110.71	333.75	<.0001	11.6	1	11.6	8.16	.0244
BC	26.44	1	26.44	7.95	.0258	39.29	1	39.29	27.64	.0012
Residual	23.3	7	3.33			9.95	7	1.42		
Lack of fit	18.16	4	4.54	2.65	.2246	8.2	4	2.05	3.51	.1649
Pure rrror	5.14	3	1.71			1.75	3	0.5833		
Correlation total	8689.91	12				292.39	12			

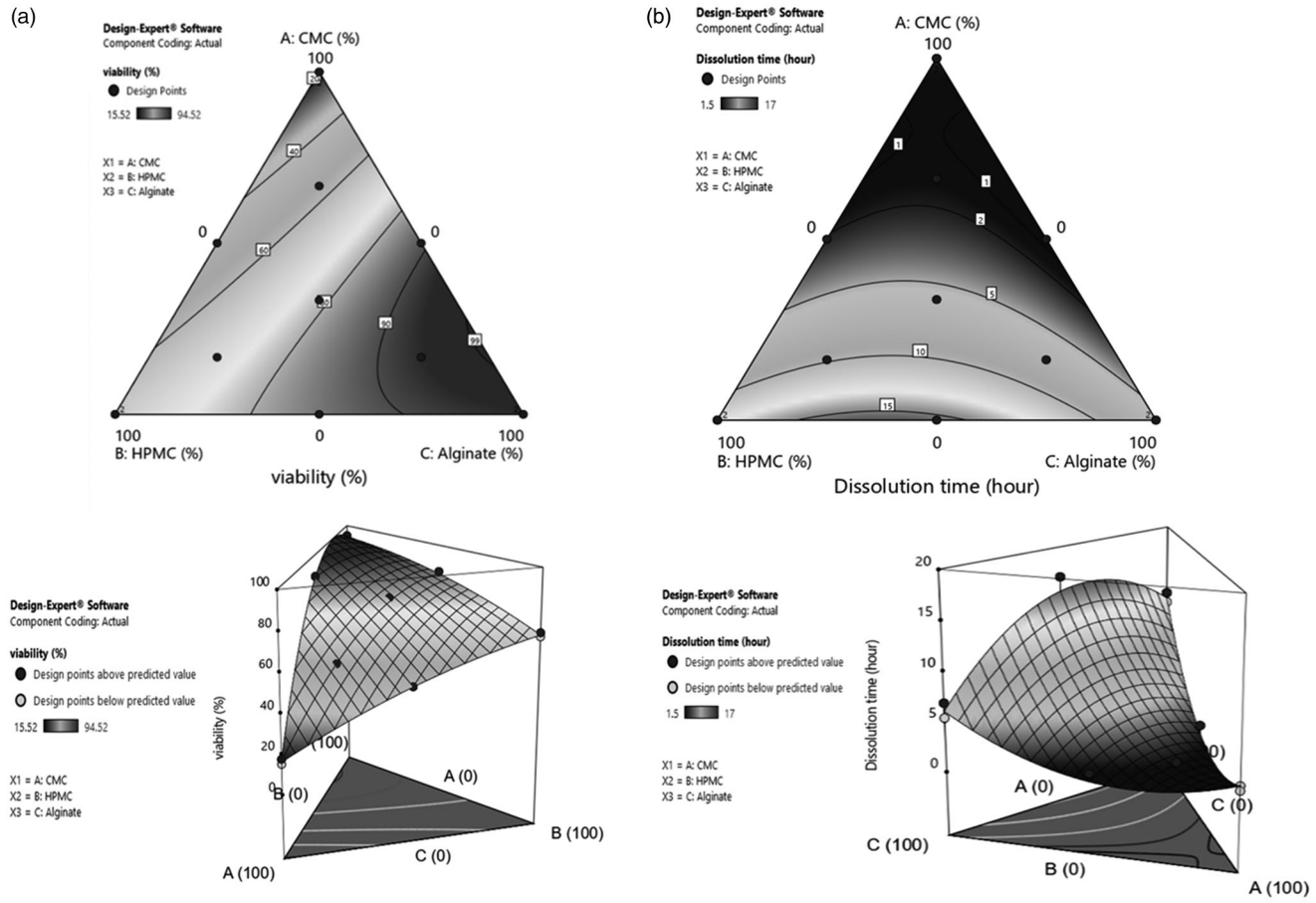

Figure 2. Surface and contour plot of mixture analysis for (a) viability and (b) dissolution time.

Table 4. Coefficients value for the mixture component for response Y1 and Y2.

Coefficient	Response function	
Component	Y1	Y2
A: CMC	16.89	2.01
B: HPMC	66.32	12.42
C: Alginate	93.83	6.18
AB	20.92	−20.12
AC	132.57	−13.55
BC	20.46	24.93

Alginate is a linear polysaccharide consisting of 1→4 linked β-(D)-glucuronic (G) and α-(L)-mannuronic (M) acids. It is commercially available and well suited for the encapsulation of microorganisms since it has generally recognized as safe (GRAS) status, is nontoxic, and has a mild gelling form [26]. It is a suitable compound that acts as a diffusion barrier for an active component, such as probiotics. Alginate particles are easily broken down in the intestines, but stable in gastric juice because the gels are stabilized by the intermolecular hydrogen bonding network if the pH is below the pKa of uronic acid [27].

In the pharmaceutical industry, alginate has been used in many applications in controlled release oral dosage form as a carrier in the hydrophilic matrix because of its ability to form viscous solution and gel when coming in contact with aqueous media. The potential application of alginate on the drug release properties in the matrix tablet has been widely described in the literature [28,29]. Huq et al. [10] also reported that alginate exhibits similar characteristics to tablets. The combination of alginate with HPMC and CMC (Figure 2) could form more viscous gels at gastric pH, representing an excellent matrix for protection of the probiotics. The combination of alginate and CMC is also a promising matrix for probiotics application as a dietary supplement [30].

CMC could be another important biopolymer for probiotic tablet development. CMC or cellulose gum is a cellulose derivative with carboxymethyl groups (–CH_2–COOH) bound to some of the

Table 5. The target range of the test factors and response values.

Factor/Response	Goal	Lower limit	Upper limit
A: CMC	In range	0	100
B: HPMC	In range	0	100
C: Alginate	In range	0	100
Y1: Microbial *via*bility	Maximum	15.52	94.52
Y2: Dissolution time	Minimum	1.5	17

hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone. As seen in Figure 2(b), the minimum dissolution time is affected by the higher percentage of CMC. This is due to the super disintegrant property of CMC when coming into contact with an aqueous medium, where it will swell immediately and become larger than its original volume. The minimum dissolution time is achieved when water reaches deep into the core of the tablet, resulting in the maximum water uptake in a short period [31]. This result is consistent with the literature [12,32,33] where many pharmaceutical preparations in the tablet dosage form use CMC in a sustained-release application. The role of CMC is important in synthesizing the probiotic tablet because, without the proper amount of disintegrant, it will be inappropriately dissolved or diffused. This may affect the delivery of the probiotics to the target side, thus reducing the effectiveness. Additionally, according to the FDA Select Committee on GRAS Substances, CMC is virtually unabsorbed and generally regarded as safe when used in reasonable quantities [34].

The study is based on the percentage of tablet dissolution time and microbial viability. The optimization function of the software was used to set the variation range of each component to acquire good probiotic tablets containing a minimum dissolution time and a high percentage of microbial viability. Then the expected response value was set. Response values for the viability were configured as maximum, while the dissolution time as minimum. All factors and responses with their restrictions are shown in Table 5.

After the software runs, the experimental prediction was carried out from the random combination until the target response value was obtained. At the same time, four groups of formulations close to the target response value were optimized by the Design-Expert software, as shown in Table 6. The first group was used for the verification of the optimal formula of probiotic tablets.

An interesting result was obtained when examining the prediction results after the optimization. Based on the software analysis, the HPMC alone or in combination with CMC has a significant impact on protecting the probiotic tablet because of the ability to form hydrogel surrounding the tablet. However, the best combination predicted by the software to obtain higher microbial viability and lower dissolution time only required 39.01% CMC and 60.99% alginate without HPMC for the excipient ratio. This may be because alginate and HPMC have a common characteristic of performing a diffusion barrier when coming in contact with an aqueous medium. On the other hand, when comparing the strength of these two components in terms of hydrogel layer forming, the alginate is a more significant and better excipient compared to HPMC. However, not all probiotic formulation tablet designs for gastrointestinal tolerance will have the same effect. Different combinations of polymers or materials might be good to produce higher viability depending on many factors. Elements such as the tablet encapsulation method, materials used, environmental conditions, and probiotic strain play an important role in the gastrointestinal tolerance formulation. For example, the *Lactobacillus* strains used in the study by Klayraung [35] showed higher viability in a low pH medium when a polymer combination of HPMC and alginate was used, while Graff [36] showed that individual polymer like HPMC produced better acidic tolerance for *Saccharomyces* strains.

Validation of the model

Based on the desirability criteria (maximum desirability = 1), the formulation with the highest desirability was chosen for confirmation and final optimization of the probiotic tablet. The optimal formula obtained by software optimization comprised 39.01% CMC, 0% HPMC, and 60.99% alginate. This consisted of 67% excipient in the formulation. Other static ingredients included 30.0% probiotic cell powder, 1.0% magnesium stearate, and 1.0% talc. The predicted response values under this formulation were expected to obtain microbial viability of 95.36% and dissolution time of 1.3 h. Three confirmatory experiments were carried out, and a microbial viability of 94.13 ± 2.0% and dissolution time of 1.6 ± 0.3 h for the tablets were obtained. A *t*-test was performed to confirm the concordance between the experimental and predicted values. There was no significant difference from the predicted values ($p > .05$), indicating that the optimal formulation of probiotic tablets can be accurately predicted using the simplex-centroid mixture design.

Storage stability

Cell viability over a storage period is an essential criterion to signify the effectiveness of the formulation in a stability test for long shelf life. The viability of the probiotic cells in different storage temperatures is shown in Figure 3. The tablets were stored at 4 and 25 °C to compare the effect of storage temperature toward the viability of probiotic yeast cells.

Initially, for both temperatures, the viable count of yeast probiotics was log 7.49 CFU/tablet. Afterwards, the number of viable cells obtained after 24 weeks of storage at 4 °C storage decreased to log 7.31 CFU/tablet, or about 0.18-log reduction. On the other hand, the storage temperature of 25 °C reduced the viable cell count to log 7.27 CFU/tablet by approximately 0.22-log reduction after two weeks of storage. Moreover, the viable cell decreased significantly by about 0.57-log over 24 weeks of storage time. This is because *Saccharomyces boulardii* is a mesophile, and its growth and metabolism occur naturally at room temperature. Therefore, the viability dropped when the tablets were stored at room temperature compared to cold storage, where the metabolic rate is slowed down, thus maintaining the viability and extending the shelf life [37]. These results are in line with a recent study [38] showing that probiotic tablet bacteria showed a significant decrease in viability when stored at room temperature compared to at 4 °C. These results show that storage temperature has a significant impact on microbial viability, and a high level of mortality is recorded when the temperature increased from 4 °C to room temperature, which is 25 °C. These findings suggest that the formulated tablets are stable when refrigerated, which is a common practice for probiotic products.

Although the probiotics viability when stored at 25 °C was lower than that of 4 °C after 24 weeks (8.3×10^6 CFU/tablet), it still achieved the minimum cell viability for the probiotics to confer the desired health benefits stated by FAO/WHO, which is at least 10^6 CFU/g or mL sample. This result indicates that lyophilization of this probiotic yeast using a freeze-drying process increases cell survival and protects the cells during storage periods. Several theories have been made regarding the maintaining of cell viability during storage by the addition of cryoprotectant in the freeze-drying process, including damage protection of the cell wall or cell

Table 6. The optimal excipient combination and prediction results for probiotic yeast tablet.

Number	A: CMC	B: HPMC	C: Alginate	Y1: Microbial *via*bility (%)	Y2: Dissolution time (H)	Desirability
1	0.39	0.00	0.61	95.53	1.36	1
2	0.40	0.00	0.60	94.82	1.26	1
3	0.37	0.00	0.63	96.21	1.47	1
4	0.37	0.63	0.00	52.79	3.84	0.63

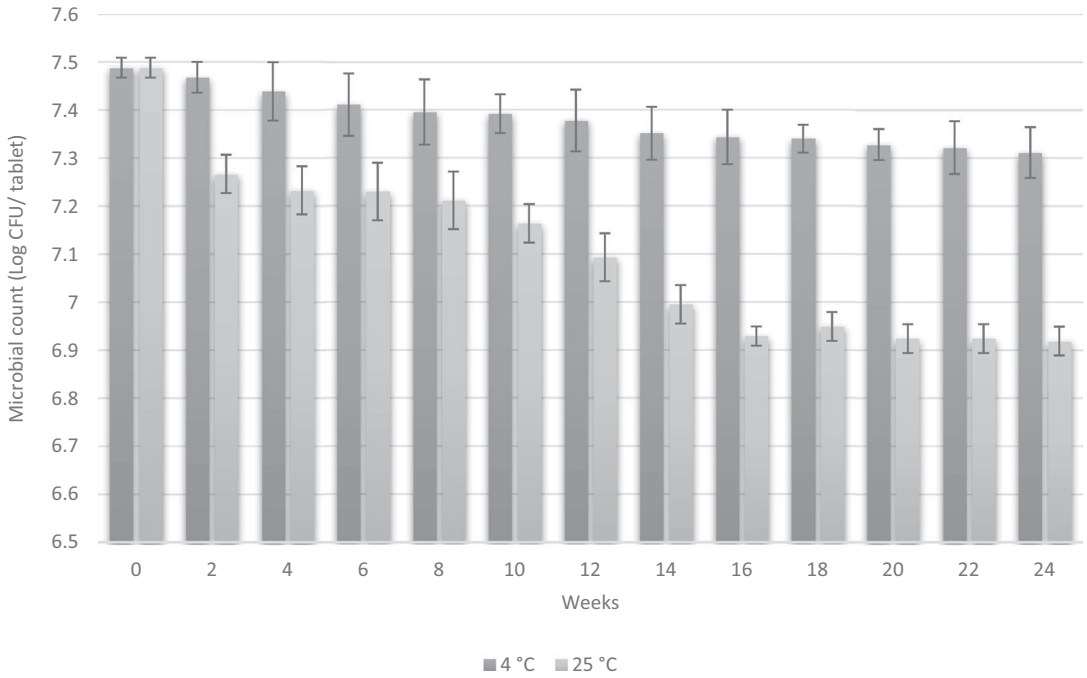

Figure 3. Microbial viability for storage stability test. Values are mean for three samples ± standard deviation.

membrane and reduction of cell death rate [39]. Moreover, different strains of the same species differ in their ability to maintain viability during the storage period. Freeze-drying is a well-known method of dehydration commonly used for microorganism preservation. It is also widely used in food preservation and for several medicinal uses, including protein-based medicines.

Conclusion

In this research, a monolithic tablet matrix formulation for probiotic yeast cell having gastrointestinal tolerance was obtained using a useful method which is simplex-centroid mixture based on statistical analysis. A ternary blend of CMC, HPMC, and alginate was evaluated by the centroid mixture design model. HPMC and alginate increased the viability in acidic conditions because of the viscous or gelling ability in the acidic medium, while CMC improved the diffusion or dissolution of the tablet, thus helping effective delivery of probiotic cells to the target site. According to the polynomial model simulated by the software, the optimized formulation is expected to have 95.36% viability after passing through the acidic gastrointestinal environment and take 1.33 h for dissolution using an excipient combination containing 39.01% CMC and 60.99% alginate.

In addition, the analysis indicates that the formulated probiotic yeast tablet is more stable when refrigerated in terms of viable cell count compared to being stored at room temperature for a six-month storage period. However, the viability after storage at room temperature was still maintained at the minimum value for commercial probiotic products as suggested by FAO/WHO, which is more than 10^6 CFU/g or mL of sample. Therefore, this study shows that the design using the mixture concept is reliable to formulate probiotic yeast tablets containing CMC, HPMC, and alginate that exhibit high viability even under acidic conditions and offer ideal stability for commercial purposes.

Disclosure statement

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ORCID

Mohd Akmal Azhar http://orcid.org/0000-0001-5195-9690
Mimi Sakinah Abdul Munaim http://orcid.org/0000-0003-4981-9802

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