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Original Research Article

Development and optimization of mouth-dissolving strips of L-methyl folate: A modern approach for patient compliance

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ABSTRACT

Background: The goal of this study is to create a mouth-melting strip of L-methyl folate calcium, which will dissolve and disintegrate more quickly when given orally for the onset of action.
Materials and Methods: Solvent casting was used to create a calcium L-methyl folate film that dissolves in the mouth. Polymer, plasticizer, and flavouring agents were chosen after early experiments. Once the excipients were chosen, 3^2 complete factorial designs were used to improve the formulation. Folding endurance (Y1), tensile strength (Y2), and disintegration time (Y3) were chosen as dependent variables, whereas HPMC E5 concentration (X1) and glycerine concentration (X2) were chosen as independent variables. The film's physicochemical parameters, including SEM, thickness, percent elongation, percent cumulative drug release, and palatability, were measured for the optimized batch and compared to those of the commercial product.
Results: The formulation of the mouth-dissolving strip took into account outcomes from the trial batch, including transparency, stickiness, and brittleness. The optimal batch has 55% HPMC E5 and 14.9% glycerine, with a folding endurance of 59±0.23, a tensile strength of 4.18±0.07 N/m^2, and a disintegration time of 40±0.12 seconds. The optimized film has dimensions of 0.08±0.01 mm in thickness, 60±1.58 mg in mass, 15.33±0.25% in elongation, and 98.33±0.27% in content uniformity. The SEM results validate the uniform film with uniform drug distribution. After 3 minutes, the L-methyl folate calcium mouth-dissolving strip had a %CDR of 99.42%. Findings of f2=52.85 and f1=12.5 for the similarity factor suggest that the medication release is clinically and commercially equivalent. One month of stability at 30°C and 65%RH was observed for the improved batch.
Conclusion: The results of the created mouth-dissolving strip test are satisfactory, indicating the intended medication release. When compared to pills that dissolve in the mouth, it is clear that it is more patient-friendly.

1. Background

When compared to the other available delivery methods, taking a drug orally has clear advantages. Due to pain avoidance and adaptability, more than 70% of medications on the market are accessible in the form of oral drug delivery systems. Although tablets and capsules have become the most common form of oral dosing, they present challenges for certain groups of patients, including the elderly, young children, and those with dysplasia caused by a wide range of medical conditions.[1] The fear of choking prevents many elderly and young patients from taking solid preparations. Tablets of any kind may cause anxiety of choking, even those that dissolve quickly. The size of the tablets was the

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most often mentioned issue, followed by their appearance and flavor. Elderly and pediatric patients, as well as those who are traveling and may not have access to water, were more likely to have difficulty swallowing pills.[2–4] In just a few seconds after being placed on the tongue or other oral mucosal tissue, the film rapidly hydrates and disintegrates to release the medication for oromucosal and intragastric absorption, thanks to the novel solid oral drug delivery system developed by scientists. Therefore, they provide significant benefits over tablets and capsules, including the elimination of swallowing and choking issues and the reduction of the need for hydration. The dissolving film takes less time to dissolve in the mouth than a pill would. This rapid disintegration and breakdown in the oral cavity occurs within seconds because of the film's enormous surface area, which wets rapidly when exposed to the moist oral environment. The medicine is promptly released from the polymer matrix once the film has been broken down, allowing it to reach the systemic circulation without being first-pass hepatically metabolized.[5] Each strip guarantees a more precise dosage administration than liquid formulations like drops or syrup. Dosing precision may also be enhanced with the use of films. The film's intuitive nature as a dose form and its natural simplicity of administration not only provides more precise administration of medications, but also helps enhance compliance. Since the first-pass action need not be taken into account, the dosage may be decreased, perhaps decreasing the molecules' negative effects. Many fast-dissolving tablets are soft, friable, or brittle and sometimes need specialized and costly packaging and processing since they are created by a very lengthy and expensive procedure like lyophilization. These tablets have an extremely short dissolution/disintegration time because they are either exceedingly porous or naturally soft-molded matrices. Compared to orally disintegrating tablets (ODTs), mouth-dissolving films (MDFs) provide a number of benefits. Solid dispersion extrusion, rolling, solvent casting, and hot melt extrusion are the most common methods of manufacturing MDF. The process of semisolid casting is more cost-effective than that of making mouth-dissolving tablets. The flexibility of MDF makes it less delicate than FDT and eliminates the need for special packaging for transporting or storing the material.[6,7] The MDFs can only be formulated with a low dosage. Therefore, for the medicine to be manufactured as MDF, the dosage can only be as high as 40 mg. Mouth-dissolving film is used since the recommended daily intake of L-methyl folate is between 800 mcg and 15 mg. L-methyl folate, often known as folic acid, is a form of vitamin B9 that is biologically active. Folic acid (vitamin B9) is a synthetic version of folate that is often used as a dietary supplement. Folic acid is another synthetic folate type. L-methyl folate, commonly known as levomefolate or l-methyl folate, is the active form of folic acid and folate. L-methylfolate, the active form of folic acid, is produced from regular folic acid through a four-step enzymatic conversion process. Folic acid is reduced by the enzyme dihydrofolate reductase (DHFR) into dihydrofolate (DHF). When DHF is methylated, it becomes Tetrahydrofolate (THF), and when THF is methylated, it becomes L-methyl folate (5-10 methylene THF). Methylene Tetrahydrofolate Reductase (MTHFR) is the enzyme responsible for converting 5-10 methylene THF to L-methyl folate. More than half of the population has a genetic mutation that prevents them from converting folic acid into L-methyl folate effectively.[8] Homozygous TT individuals make up around 10% of the population and are deficient in MTHFR enzymes, making them unable to benefit from folic acid. About 40% of the population seems to have a reduced ability to convert folic acid into L-methyl folate (heterozygous CT). The only type of folate that the body can absorb is L-methyl folate. Because of this poor absorption, the body is unable to store enough folic acid to prevent birth abnormalities, and its capacity to create and maintain adequate stores of folic acid is severely compromised.[8] L-methyl folate is readily absorbed and enters the bloodstream without being altered by MTHFR genotype differences or requiring an enzymatic conversion. When compared to folic acid, L-methyl folate is 700% more bioavailable. The quick beginning of action and improved oral bioavailability may result from the use of fast-releasing oral thin films of L-methyl folate, which is why such films have been created and evaluated.

2. Materials and Methods

L-methyl folate calcium was obtained as a gift sample from Panvo Chemicals Ltd, Chennai, India. HPMC 5 cps and HPMC E15 were purchased from Signet Chemicals, Bandra East, Mumbai, India. Maltodextrin was purchased from Richi Pharma, Ahmedabad, India. Sucralose was gifted from Suraxit Pharma, Kawarlal & Co. Ahmedabad, India, Ess. Pineapple supreme was purchased from Firmenich Ltd. Andheri East, Mumbai, India. Color quinolone yellow was purchased from Dynamic Drugs Ltd. Madras, India.

2.1. Preformulation studies

The process of creating an effective dosage form for a pharmacological substance begins with Preformulation research. Preformulation studies are conducted to collect data on the active pharmaceutical ingredient (API) that may be used later in the process of formulating various dosage forms. Preformulation is the study of a pharmacological substance's physicochemical qualities in combination with excipients. Preformulation studies are conducted to determine which of a drug's physicochemical features and excipients will have the most impact on the formulation's development, production strategy, and final pharmacokinetic-biopharmaceutical characteristics.[9]

2.2. Solubility

Quantitative analysis was used to determine solubility. Phosphate buffer with a pH of 6.8 and an abundance of L-methyl folate. The flasks were heated in the oven at 37 °C for 24 hours. The medication concentrations in the liquids were determined after 24 hours through spectrophotometric analysis.

2.3. Drug-excipients compatibility study

Compatibility issues between medications and excipients are a common source of instability in pharmaceutical formulations. The purpose of this research was to determine whether or not a certain excipient was compatible with a specific medicine. Here, FTIR (Fourier transform infrared spectroscopy) was used to get the job done. Pure drug and physical combination went through filter # 30 and were deposited in vials kept at 40°C±75% RH after being manufactured in a 1:1 ratio of drug and excipients. On days 0, 7, 15, and 30, we monitored all of the different combinations. After that, IR grade KBr was added to both the pure medication and the physical combination in their own distinct ways. The spectrum of this amalgam was then scanned from 4000 to 400 cm-1.[10]

2.4. Dose calculation of L-methyl folate Calcium

An oral dose of L-methyl folate is 1 mg, 3 mg, and 7.5 mg. Here the dose of L-MTHF Ca for each strip was selected at 3 mg because the same strength of L-MTHF containing conventional tablet are available in the market

Theoretical quantity of L-methyl folate calcium:

The label claim is = 3mg

The assay of the drug is = 97.8%

Quantity required per dosage unit = Lable claim * 100/ Assay =3.067 mg/strip A film 2 cm × 3 cm dimension (6 cm2) was planned to be prepared which should contain 3.067 mg of L-methyl folate calcium. A film 2 cm × 3 cm dimension (6 cm2) was planned to be prepared which should contain 3.067 mg of L-methyl folate calcium.

2.5. Preparation of mouth-dissolving strips of L-methyl folate calcium

Film preparation utilized solvent casting. Dissolve a reasonable quantity of polymers in distilled water in one beaker with constant stirring. Then add the appropriate plasticizer to the polymeric solution. Stir the medication with distilled water in another beaker. Continuously swirl the drug solution into the polymeric solution. Dissolve sweetener, color, and taste in water and mix into the film-forming polymeric solution for an hour. The aqueous solution remained undisturbed until the air bubbles were eliminated. As indicated in Figure 1, a film spreader cast the aqueous solution on a smooth glass plate. Room temperature dried the film in 24 hours. The dry film was carefully removed from the glass plate and trimmed to test size. Airtight plastic bags held the films until usage.[11,12]

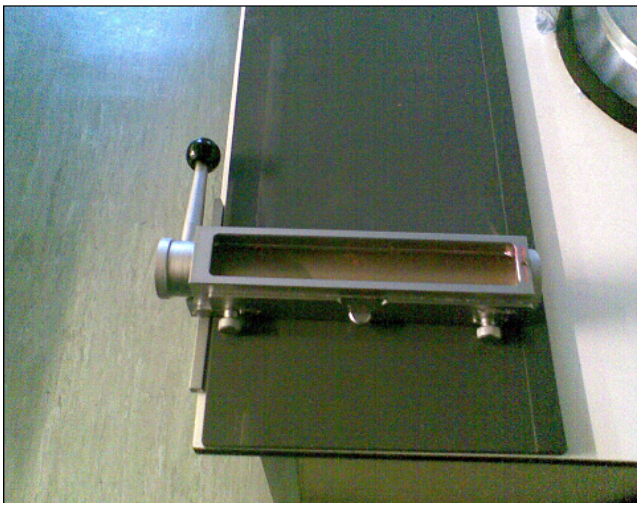

Fig. 1: Strip spreader

2.6. Preliminary trials for the Selection of polymers, plasticizers, and flavouring agents

Polymers, plasticizers, and flavouring agents were tested. The first trial used HPMC E15 and PVP K-30, the second used HPMC E15, the third used HPMC E15 and Glycerine as a plasticizer, the fourth used HPMC E15 and Maltodextrin, and the fifth used low viscosity grade polymer HPMC E5 and Maltodextrin. The sixth trial improved the strips' taste by adding pineapple or mango flavour as a flavouring agent. Based on physical appearance, film thickness, disintegration time, and Spreadability, acceptable excipients were chosen from the aforementioned experiments.[13,14] All trials with various compositions were shown in Table 1.

2.7. Optimization technique

Factorial designs are employed in experiments when the influence of multiple variables or circumstances on experiment outcomes needs to be explored. When you need to determine the impacts of several variables at once, including their interactions, you should use a factorial design. Qualitative and quantitative considerations are both valid. Combinations of all levels of all factors are represented by the levels of each factor. A factor's impacts are the modifications to the response that comes about as a result of changing the level(s) of that factor. One primary goal of a factorial experiment is to describe how changing one or more variables affects the outcome variable. Experiments conducted under cover of anonymity will provide more reliable findings for making inferences

Table 1: Composition of preliminary trials

Batch no	Drug	HPMC E15	PVP K-30	Glycerine	Sucralose	Quinilline yellow supra	Maltodextrin	Pineapple supreme	Mango flavour	Water
B1	3.067	27	11	12	1	0.05	-	-	-	Q.s.
B2	3.067	23	15	12	1	0.05	-	-	-	Q.s.
B3	3.067	19	19	12	1	0.05	-	-	-	Q.s.
B4	3.067	31	7	9	1	0.05	-	-	-	Q.s.
B5	3.067	31	7	12	1	0.05	-	-	-	Q.s.
B6	3.067	31	7	15	1	0.05	-	-	-	Q.s.
B7	3.067	50	-	-	1.5	0.05	-	-	-	Q.s.
B8	3.067	45	-	-	1.5	0.05	-	-	-	Q.s.
B9	3.067	40	-	-	1.5	0.05	-	-	-	Q.s.
B10	3.067	40	-	9	1.2	0.05	-	-	-	Q.s.
B11	3.067	38	-	12	1	0.05	-	-	-	Q.s.
B12	3.067	36	-	15	0.8	0.05	-	-	-	Q.s.
B13	3.067	30	-	9	0.7	0.05	4	-	-	Q.s.
B14	3.067	33	-	12	0.5	0.05	5	-	-	Q.s.
B15	3.067	36	-	15	0.3	0.05	6	-	-	Q.s.
B16	3.067	33	-	12	0.5	-	3	-	-	Q.s.
B17	3.067	33	-	12	0.5	0.05	4	-	-	Q.s.
B18	3.067	33	-	12	0.5	0.05	5	-	-	Q.s.
B19	3.067	33	-	12	0.5	0.05	6	-	-	Q.s.
B20	3.067	30	-	9	0.5	0.05	5	-	-	Q.s.
B21	3.067	36	-	15	0.8	0.05	5	-	-	Q.s.
B22	3.067	33	-	12	0.5	0.05	5	5	-	Q.s.
B23	3.067	33	-	12	0.5	0.05	5	-	5	Q.s.

than would be possible in a well-planned experiment using a specific factorial design. Creating an equation that characterizes the experimental outcomes as a function of the factor strengths simplifies the optimization process. The link between independent and dependent variables may be better understood with the use of an experimentally optimized model. The data from the experiments are broken down by factor.

2.8. Optimization of formulation using 3^2 full factorial design

The impact of film-forming polymer HPMC E5 (X1) and plasticizer glycerine (X2) on performance metrics including folding endurance (no.), tensile strength (N/m2), and disintegration time (sec.) was investigated using a 32-full factorial design. Two components were tested at three levels each, and nine different permutations were tested using experimental batches. Table 2 displays the variable values for 32 factorial designs. Table 3 shows the make-up of factorial design batches LMF1 through LMF9. For every 60 mg strip, the formulations include: 3.067 mg active ingredient, 5 mg maltodextrin, 0.5 mg sucralose, 5 mg pineapple supreme, and 0.06 mg quinolone yellow supra.

2.9. Evaluation of model / Check point analysis

In order to evaluate the model, checkpoint analysis was used. To evaluate the accuracy of the model and the usefulness of the determined contour plot and reduced polynomial equation in the production of mouth-dissolving strips, two checkpoint batches were produced and compared to the experimental and predicted values of responses. After producing each formulation three times, an average was calculated.

2.10. Preparation of Optimized formulation based on the desirability function

Optimization was used to find the optimal combination of independent variables (X1 and X2) to collect information for Y1, Y2, and Y3. The response was integrated during formulation development to build a product with the required quality. The fundamental function of desirability was to pool all answers into a single experiment and calculate the probability of making the most accurate prediction of the independent variables. After following the program's recommendations to the letter, an optimized formulation was developed and checked for accuracy against the program's expected values.

Table 2: Experimental design detail for optimization of mouth-dissolving strips

Independent factors	Coded value			Uncoded Value		
	Low	Medium	High	Low	Medium	High
Concentration of HPMC E5 (X1)	-1	0	1	50%	55%	60%
The concentration of Glycerine (X2)	-1	0	1	15%	20%	25%
Dependent factors						
Y1= Folding endurance						
Y2= Tensile strength (N/mm^2)						
Y3=Disintegration time (Sec.)						

Table 3: The composition of 32 full factorial design points

Formulation code	HPMC E5 (%)	Glycerine (%)
LMF 1	50	15
LMF 2	50	20
LMF 3	50	25
LMF 4	55	15
LMF 5	55	20
LMF 6	55	25
LMF 7	60	15
LMF 8	60	20
LMF 9	60	25

2.11. Evaluation of the mouth-dissolving strips

2.11.1. Mechanical properties of films

The mechanical property of the film gives an idea about to what extent the film can withstand the force or stress during processing, packaging, transport, and handling.

2.11.2. Thickness

The thickness of the patch was measured using a digital vernier Calliper with a least count of 0.01 mm at different spots of the film. The thickness was measured at three different spots of the patch an average was taken and SD was calculated.

2.11.3. Weight variation

From the cast footage, a piece of film measuring six centimeters square was taken out at three distinct locations. The weight of each film was measured, and the percentage of difference in weight was computed. Utilizing a computerized weighing scale allowed for the measurement of the variance in weight.

2.11.4. Folding endurance

Folding endurance was determined by repeated folding of the strip at the same place till the strip broke. The number of times the film was folded without breaking is computed as the folding endurance value.

2.11.5. Tensile strength

Tensile strength is the maximum stress applied to a point at which the strip specimen breaks. It is calculated by the applied load at rupture divided by the cross-sectional area of the strip as given in the equation below.

$$Tensile\ strength = \frac{load\ at\ breakage}{Strip\ thickness}\ X\ Strip\ width$$

2.11.6. Percentage elongation

When stress is applied, a strip sample stretches and this is referred to as strain. Strain is the deformation the of strip divided by the original dimension of the sample. Generally, the elongation of the strip increases as the plasticizer content increases

$$Elongation = \frac{increases\ in\ length}{original\ length}\ X100.$$

2.11.7. In-vitro Disintegration/dissolving time

In vitro disintegration time of l-methyl folate was measured in phosphate buffer (pH 6.8) in a 10 ml of beaker with gently shaking when the film was dissolved, time was noted. This was done on the three films of the same batch and the average of three measurements was taken into consideration.

2.11.8. In-vitro dissolution study

The dissolution of the mouth dissolving strip was carried out using USP paddle apparatus type II, using 300 ml of simulated saliva (pH 6.8) as a dissolution media. The bath temperature was 37°C ± 0.5°C and the paddle rotation speed was 50 RPM. Each film (2×3 cm^2) was placed in the vessel at the initial time. Care should be taken to sink the film that the film should not stick to the paddle. Sampling was done every 30 seconds. The sample was filtered through the Whatman filter paper. an equal volume of the fresh dissolution media, maintained at the same temperature was

added after withdrawing the sample to maintain the volume in the vessel. Samples were then analysed by UV-visible spectrophotometer.

2.12. Comparative dissolution of the optimized batch with MDT of L-MTHF Ca^{2+}

Mouth-dissolving tablets of L-methyl folate calcium were prepared by direct compression method. The optimized formulation of MDT (150 mg) contains L-MTHF Ca^{2+} (3.067 mg), CCS (7.5 mg), MCC 102 (38.5 mg), Mannitol (100mg) Magnesium stearate (1 mg). Dissolution was carried out with the same dissolution apparatus and dissolution condition as described in the above section.

2.13. Morphology study by SEM

The surface study of the film was done by using a scanning electron microscope (JEOL JSM 5610LV at M S University, Baroda). The strip was cut into 5×5 mm^2 and then it was put on the sample holder. The sample holder was put into SEM and starts the instrument. Microphotographs of the strip were taken by changing the magnification. Higher magnification was used for surface morphology. Uniform drug distribution, homogeneity of polymer, and texture analysis.

2.14. Evaluation of taste masking

L-methyl folate has a somewhat bitter or unpleasant flavor when used orally. The inclusion of a sweetener, such as sucralose or aspartame, might alleviate the issue of bitterness in a mouth-dissolving film of L-methyl folate, which is necessary for patient compliance. A tasting panel determined the level of acceptance of the flavor. The overall palatability level was recorded as A, B, C, or D grades in Table 4 after each formulation was delivered to a taste panel expert (healthy human volunteers) and kept in the mouth for 10-15 seconds before being thrown out. Before and after giving out the film samples, volunteers were instructed to gargle with distilled water.

2.15. Stability study

Films of optimized batches were subjected to a stability study. Each film was wrapped in butter paper and placed in a plastic zip bag. Films were exposed to 75±5% RH (saturated aqueous sodium chloride solution in desiccators), 40±2°C temperature, and ordinary room temperature and humidity (30±2°C AND 65±5% RH). The study was carried out for one month. The films were evaluated initially and every 10 days for their physical characteristics, in-vitro DT, drug content, and also for mechanical properties.[15,16]

3. Results

3.1. Preformulation study

The organoleptic properties of L-methyl folate calcium were performed and the observed data are shown in Table 5. The data shows its appearance, colour, odour, and feel.

3.2. Solubility

L-MTHF Ca^{2+} salt is easily soluble in water and phosphate buffer (6.8 pH). It is practically insoluble in alcohol. it was observed that in water it shows 12 ± 0.5 mg/ml and in 6.8 pH phosphate buffer 18 ± 0.7 mg/ml solubility. While in the case of methanol, it shows only 0.008 ± 0.45 mg/ml solubility.

3.3. Drug excipients compatibility study

Compatibility studies were performed using an FTIR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and polymer were studied by making a KBr disc. The characteristic absorption peaks of L-methyl folate Ca^{2+} were obtained at different wave numbers in different samples. As per fig. 2 and fig. 3 the FTIR of the pure drug was characterized by a peak of NH stretching at 3325, 3275, 3217, and 3174 cm^{-1}, for CH stretching at 2985, 2939 cm^{-1} and for C=O stretching at 1750 cm^{-1}. Now FTIR of the physical mixture was characterized by a peak of NH stretching 3325, 3298, 3217, 3174 cm^{-1}, for CH stretching at 2983.2938 cm^{-1} and for C=O stretching at 1750. The peaks obtained in the spectra of each physical mixture correlate with the peaks of the drug spectrum. So, all ingredients are compatible with the drugs.

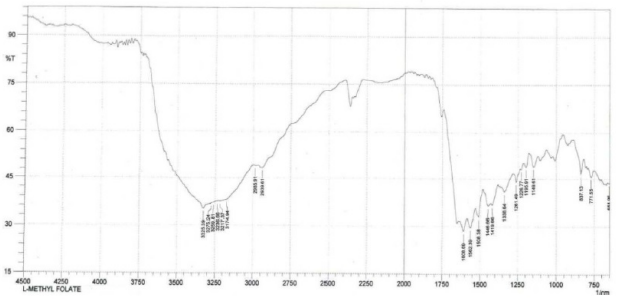

Fig. 2: FTIR spectra of L-methyl folate calcium

3.4. Experimental preliminary trials

As per the data shown in table 6 all the batches were prepared for the selection of polymers and plasticizers as well as flavouring agents. From the above results, based on the physical appearance like transparency, brittleness, stickiness, separability, folding endurance, and DT time

Table 4: Overall palatability taste grades

Grades	Overall Palatability
A	Very good
B	Good
C	Average
D	Poor

Table 5: Organoleptic properties of L-methyl folate

Properties	Observation
Physical appearance	Crystalline powder
Colour	Light yellowish colour
Odour	Odourless
Feel	Sticky

Table 6: Results of preliminary trials

Batch code	Physical Appearance	Thickness (mm)	Separability	Disintegration time (sec)	Folding Endurance
B1	Semi-Transparent, sticky, brittle	1.36	Poor	-	-
B2	Semi-Transparent, Sticky, brittle	1.20	Poor	-	-
B3	Semi-Transparent, very sticky, very brittle	-	Poor	-	-
B4	Semi-Transparent, Sticky, less brittle	1.36	Good	-	-
B5	Semi-Transparent, Sticky, less brittle	1.30	Good	-	-
B6	Semi-Transparent, very sticky, brittle	1.42	Good	-	-
B7	Semi-Transparent	1.59	Poor	-	-
B8	Semi-Transparent	1.46	Poor	-	-
B9	Semi-Transparent	1.39	Poor	-	-
B10	Semi-Transparent, Sticky	0.15	Good	80	-
B11	Semi-Transparent, Sticky	0.14	Good	76	-
B12	Semi-Transparent, Sticky	0.23	Good	82	-
B13	Semi-Transparent, Sticky	0.17	Good	44	50
B14	Semi-Transparent, Sticky	0.25	Good	48	70
B15	Semi-Transparent, Sticky	0.29	Good	66	85
B16	Transparent, non-sticky	1.20	Good	42	39
B17	Transparent, non-sticky	1.05	Very Good	41	42
B18	Transparent, non-sticky, good consistency	0.09	Very Good	36	51
B19	Transparent, non-sticky	0.08	Very Good	33	56
B20	Transparent, non-sticky	1.00	Good	42	62
B21	Transparent, non-sticky	1.33	Good	51	71
B22	Transparent	0.08	Very Good	34	-
B23	Transparent, non-sticky	0.08	Very Good	38	-

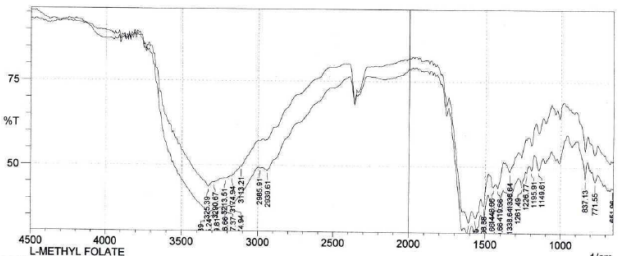

Fig. 3: FTIR of pure drug and physical mixture of drug and excipients

polymers and other excipients were chosen. The physical appearance was found to be in the range of transparent to semi-transparent, sticky to non-sticky. The thickness was found to be 0.08 to 1.59 mm, the disintegration time 33 to 82 seconds, and the folding endurance was 39 to 85 observed.

3.5. Optimization of formulation parameters

For all 9 batches, both the selected dependent variables (X1 and X2) showed a wide variation in folding endurance, tensile strength, and in-vitro disintegration time. The data indicated a strong influence of X1 and X2 on selected responses (Y1, Y2, and Y3). The polynomial equations can be used to conclude after considering the magnitude of the coefficient.

3.5.1. Effect of design factors X1 and X2 on Folding endurance Y1

Folding endurance = 884 + 43 X1 + 43X2 – 1.33 X1X2 - 0.36 $X1^2$ -61 $X2^2$ is the polynomial equation for response Y1.

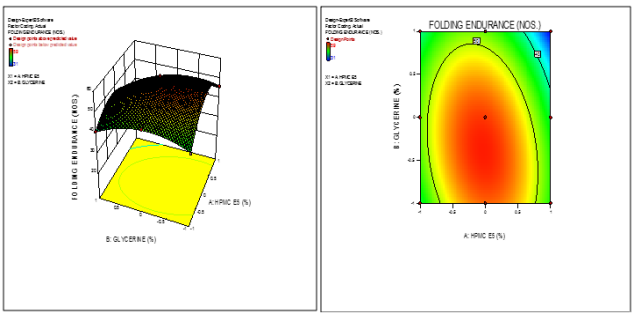

Fig. 4: Response surface plot and contour plot for folding endurance

The interaction of independent factors towards folding endurance indicates that as the concentration of HPMC E5 increases folding endurance increases up to some extent after that as the concentration of HPMC E5 increases decreases in folding endurance were observed. Figure 4 indicates the response surface plot and contour plot for response Y1

3.5.2. Effect of design factors X1 and X2 on Tensile strength (Y2)

Tensile strength =6.73+ 1.38X1 +0.066X2 X2 -0.01$X1^2$ +0.027 $X2^2$ is the polynomial equation for response Y2.

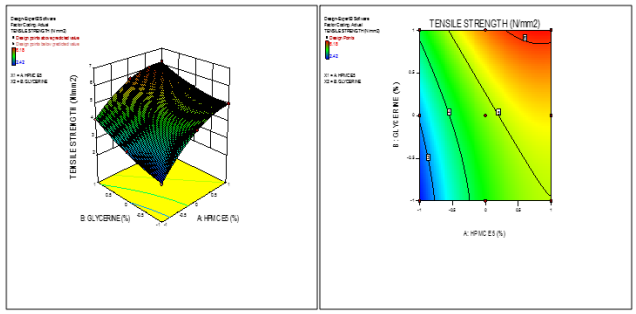

Fig. 5: Response surface plot and contour plot for tensile strength

3.5.3. Effect of design factors X1 and X2 on Disintegration time (Y3)

Disintegration time =559-26X1 -25X2 +1.0740 X1 +X2 +1.388$X1^2$ +0.407 $X2^2$ is the polynomial equation for response Y3.

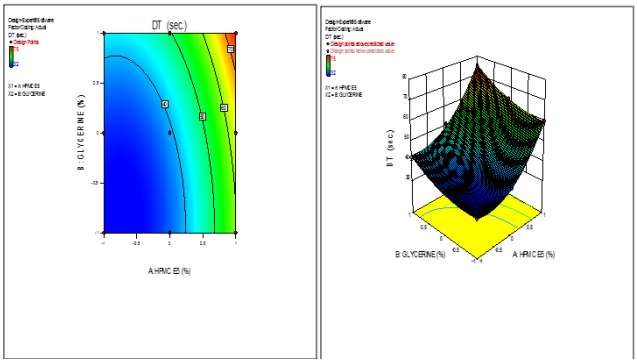

Fig. 6: Contour plot and response surface plot for DT

From fig. 6, it was observed that the contour plot and response surface plot provide the design space for the optimized product. As the concentration of HPMC E5 increases DT increases, and the concentration of glycerine decreases DT decreases.

3.5.4. Checkpoint study

As per the data shown in Table 8 it was observed that the observed value of all responses was quite nearer to the predicted value, which is obtained from the software.

3.5.5. Optimized batch

Based on the criteria like maximum folding endurance, high tensile strength, and less DT there were 20 solutions were suggested by software with different desirability. The optimized batch having a desirability of 0.792 was selected.

Table 7: 3^2 full factorial design layouts

Batch No.	Variables in Coded value		Folding Endurance (Nos.)	Tensile Strength (N/mm^2)	Disintegration Time (Sec.)
	X1	X2	Y1	Y2	Y3
LMF1	-1	-1	43±1.20	2.42±0.07	32±1.8
LMF2	-1	0	48±1.05	3.16±0.05	35±1.6
LMF3	-1	+1	41±0.82	4.11±0.06	42±2.4
LMF4	0	-1	56±0.95	4.36±0.08	36±1.8
LMF5	0	0	59±1.80	4.76±0.02	38±1.6
LMF6	0	+1	46±1.56	5.77±0.01	51±1.7
LMF7	+1	-1	46±0.87	5.02±0.09	60±1.3
LMF8	+1	0	41±0.65	5.27±0.04	64±1.1
LMF9	+1	+1	31±0.78	6.18±0.06	75±1.5

(Mean±SD, N=3)

Table 8: Checkpoint analysis

Batches	HPMC E5 (%)	Glycerine (%)	Folding endurance (Nos.) (Y1)		Tensile strength (N/mm^2) (Y2)		DT (Sec) (Y3)	
			Predicted	Actual	Predicted	Actual	Predicted	Actual
LMF 10	52.5	17.5	55	58±0.46	3.75	3.15±0.09	32	35±0.25
LMF 11	57.2	22.5	48	52±0.59	5.59	5.47±0.04	53	51±0.85

(Mean±SD, N=3)

The composition and results of the optimized batch are shown in Table 9.

3.6. Evaluation of optimized mouth-dissolving strip

3.6.1. Physicochemical evaluation

The batch of optimized formulation was then analyzed for physical appearance and mechanical properties shown in Table 10. Optimized batch showed good content uniformity as well as mechanical properties like % elongation and thickness values are as close as expected values.

3.7. In-vitro drug release study

In-vitro release studies of L-methyl folate calcium strips were carried out in simulated salivary fluid (pH 6.8). The graphical presentation shown in fig. 7. Cumulative drug release was calculated based on the drug content of L-MTHF Ca2+ present in the film. Rapid drug dissolution was observed in the optimized batch which release 99.42% at the end of 3 min.

3.8. Comparative dissolution of Optimized formulation and mouth-dissolving tablets of L-methyl folate calcium.

The %CDR of the optimized batch was compared with the marketed product (mouth-dissolving tablet of L-methyl folate). As per the graph shown in Figure 7 it was concluded that the Similarity factor analysis of the optimized batch showed an f2 value (f2=52.85) greater than 50 and an f1 value (f1=12.5) less than 15. So based on the similarity factor dissolution profile of the prepared film was quite similar to the marketed product.

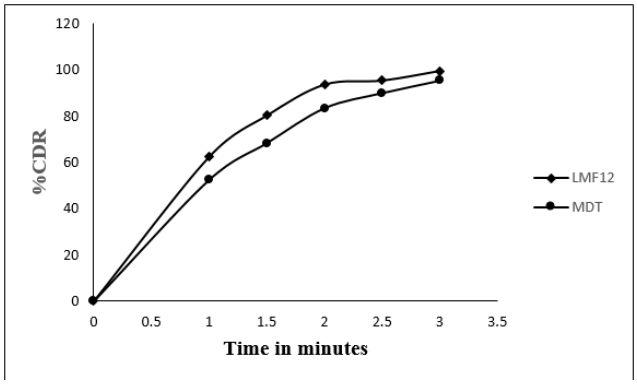

Fig. 7: %CDR of optimized Batch and MDTs of LMTHF

3.9. Overall palatability of optimized formulation

The optimized formulation was tested by five healthy volunteers and they were categorized according to the test of the formulation. The result obtained was compared with the grading system provided in Table 4. The formulation got a palatability grade of A, therefore it can be concluded that the final formulation has acceptable palatability.

3.10. Morphological study

The photographs of the film shown in Figure 8 indicate that the prepared film of L-MTHF Ca^{+2} shows a good homogeneous as well as transparent appearance and there

Table 9: Optimized batch based on the desirability

Optimized batch	HPMC E5 (%)	Glycerine (%)	Folding endurance (Nos.) (Y1)		Tensile strength (N/mm^2) (Y2)		DT (Sec) (Y3)	
			Predicted	Actual	Predicted	Actual	Predicted	Actual
LMF 12	55	19.4	57	59±0.23	4.72	4.18±0.07	38	40±0.12

(Mean±SD, N=3)

Table 10: Physicochemical properties of optimized strip

Optimized batch	Thickness (mm)	Weight variation (mg)	% Elongation	Content uniformity (%)	Physical appearance
LMF 12	0.08±0.01	60±1.58	15.33±0.25	98.33±0.27	A clear transparent and homogeneous film

(Mean±SD, N=3)

was no crystallization of drug or excipients. Scanning electron micrographs were taken on 500x and 1000x shown in Figure 9 which show a uniform distribution of the drug in the polymer matrix of the film.

Fig. 8: Photographs of the optimized L-MTHF Ca2+ film

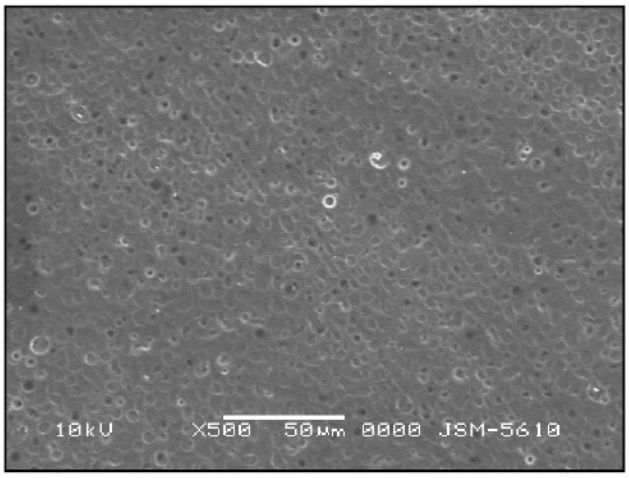

Fig. 9: SEM study of L-MTHF Ca^{2+} film on 500x

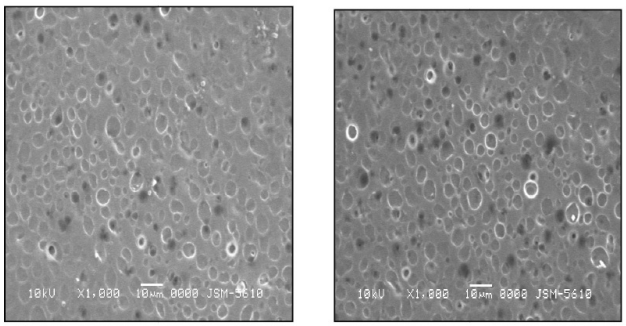

Fig. 10: SEM study of L-MTHF Ca2+ film on 1000x

3.11. Stability study

The stability study result shown in Table 11 proved that the films stored under two different conditions at 30±2°C and 65±5 %RH and 40±2°C and 75±5 %RH did not show major changes in the film.

4. Discussion

Better formulation might be achieved with the help of Preformulation research. L-methyl folate crystals are sticky, have no discernible odor, and have a pale-yellow color, as shown by the data. Evidence from the solubility test shows that the medication dissolves easily in both water and phosphate buffer. The purpose of the FTIR analysis was to verify the presence of a drug-excipient interaction. Pure drug and physical combination FTIR spectra indicate no large changes in the function group, indicating no interaction. In the first three batches of the pilot study, the plasticizer content is held constant while the polymer and solvent concentrations are varied. It was found that stickiness became more of a concern as PVP K-30 concentrations rose. The PVP K-30's hygroscopic nature made separation from the glass difficult, and the material's brittleness was the major issue. B3 formulation demonstrated the greatest stickiness, Formulations B4, B5, and B6 had less but the

Table 11: Result of stability data of optimized batch

Stability Condition	Sampling Time	Observation				
		Content Uniformity (%)	Folding Endurance (Nos.)	Tensile strength (N/mm^2)	DT (sec)	Visual Appearance
Room Temperature (30±2°C and 65±5 % RH)	Initial	98.33±1.25	59±0.45	4.18±0.07	40±0.12	Clear, Transparent and Homogeneous Film
	After 10 days	97.12±1.05	57±0.78	4.12±0.04	38±0.45	
	After 20 days	96.45±1.07	60±0.29	4.09±0.06	40±0.36	
	After 30 days	96.15±0.56	58±0.43	4.10±0.08	41±0.89	
Accelerated condition (40±2°C and 75±5 %RH)	Initial	98.33±1.25	59±0.23	4.18±0.02	40±0.12	Clear, Transparent and Homogeneous Film
	After 10 days	97.78±1.45	47±0.12	4.02±0.04	42±0.52	
	After 20 days	96.25±0.89	28±0.69	3.85±0.06	44±0.43	
	After 30 days	95.63±0.95	19±0.75	2.95±0.08	47±0.62	

(Mean±SD, N=3)

constant concentration of PVP k-30, and modifying the concentration of plasticizer could not alleviate the issue of stickiness and brittleness. The films cast on the glass plate using Formulations B7–B9 could not be peeled off. Film thickness decreased with decreasing polymer content; however, more experiments were conducted to increase both separability and film thickness. The addition of the plasticizer was shown to be beneficial for batches B10–B12. All of the formulations were moderately sticky, however, the plasticizer concentrations of 15%, 20%, and 25% w/w provided the best separation from the glass plate. The films' sweetness and flavour need to be enhanced. It was discovered that the films' disintegration time was longer than predicted. Disintegration times (DT) are longer for formulations B13–B15. Increasing the polymer amount was shown to boost DT. The films varied in appearance, and they were all a little sticky, but they all served the same purpose. It was found that employing HPMC E5 and maltodextrin resulted in uniform films during formulations B16–B21. Maltodextrin was utilized since it is a moderate sweetener and a film-forming polymer. The thickness dropped from batch B16 through B19 as the maltodextrin concentration rose, while the thickness rose as the polymer concentration rose in batch B21. Maltodextrin was shown to improve folding endurance at higher concentrations. Maltodextrin concentration was shown to be correlated with reduced in-vitro disintegration time. Both pineapple and mango flavours were used to enhance the strips' taste, however, the ones made with pineapple flavour were more popular with consumers.

Preliminary analyses of the process parameters showed that elements such as polymer HPMC E5 concentration and plasticizer concentration Folding endurance, tensile strength, and disintegration time of drug-loaded fast-dissolving film were all significantly impacted by the addition of glycerine. Therefore, 3^2 factorial designs were used to carry out additional optimization, with the HPMC E5 concentration (X1) and glycerine concentration (X2) serving as independent variables and the folding endurance, tensile strength, and disintegration time (Y1, Y2, and Y3, respectively) serving as dependent factors. There is a strong correlation (0.98) between the Y1 interaction effects investigated across all nine formulations. There is a significant relationship between X1 and Y1, with a p-value of 0.023 ($p<0.05$) and a p-value of 0.0033 ($p<0.05$) respectively. Here, the increasing concentration of polymer and plasticizer leads to greater folding endurance, as seen by the increasing X1 and X2 coefficients. The correlation coefficient for Y2 is 0.99 across all 9 models. X1 has a p-value of 0.0497 ($p<0.05$), and X2 has a p-value of 0.0015 ($p<0.05$); both of these variables have a significant effect on the dependent variable. Here, the increasing X1 and X2 coefficients suggest that the film's tensile strength improves with increasing polymer and plasticizer concentrations. For Y3, the correlation between the 9 different formulations is a respectable 0.99. Figure 6 shows that both independent variables, X1 and X2, have p-values less than 0.05 and so have a substantial impact on the dependent variable. Here, the shorter disintegration time is associated with lower polymer and plasticizer concentrations, as shown by the negative sign of the X1 and X2 coefficients. The checkpoint batches allowed us to draw the conclusion that the efficiency of the model is consistent with the amount of design space we were able to gain. Desirability function optimization results in a superior batch. Formulated films were found to have thicknesses of 0.08±0.01 mm. The numbers show that wall thickness grows progressively with polymer content. Weight variance was ±7.5% or less, which is well within pharmacopeial guidelines. Film tensile strength is the primary factor in determining percent elongation. Tensile strength and percent elongation are both influenced by the polymer and plasticizer types used. As the polymer and plasticizer concentrations were raised, the optimal batches' elongation percentage rose to 15.33±0.25%. Drug release patterns were found to be comparable between the mouth-dissolving film studied in vitro and commercially available mouth-dissolving tablets containing L-methyl folate. Patient compliance was higher with strips than with pills, perhaps

due to the fact that the film didn't need to be taken with water. The SEM picture confirms that the medication is evenly dispersed across the film, corroborating the results of the images. There was no visible change in the physical appearance of the films after 30 days of storage at room temperature; the films remained clear, transparent, and homogenous throughout storage. While the film's folding endurance was unaffected by room temperature storage, it was reduced under accelerated circumstances owing to the loss of water, which reduces the film-forming ability of the polymer. Under the high heat conditions, the film became rigid and fragile. When kept at normal temperatures, tensile strength is unaffected, but it declines when subjected to high temperatures. Accelerated circumstances increased the disintegration time. There were no noticeable changes in the film's content between the two temperature conditions. Therefore, unlike under accelerated circumstances, the optimized L-MTHF Ca^{2+} film was not unstable when kept at ambient temperature.

5. Conclusion

L-methyl folate Ca^{2+} mouth-dissolving strips were made using a solvent casting process, resulting in strips with good mechanical characteristics and adequate drug release. According to the results of this investigation, HPMC E5 has the potential to be used as a film-forming polymer in the production of a mouth-dissolving film containing L-methyl folate Ca2+.The polynomial equations are revealed by the results of a multiple regression analysis on the concentrations of HPMC E5 LV (a film-forming polymer) and glycerine (a plasticizer). The optimal batch has HPMC E5 at 55% w/w, Maltodextrin at 10% w/w, and glycerine at 19.4% w/w. When compared to other formulations, the best formulations dissolved in 40 seconds and displayed a maximum dissolving rate of 99.42% of drug release in 3 minutes. The medicine was released from the strips at a quicker rate than it was from the prepared mouth-dissolving tablets of L-methyl folate calcium in a dissolution trial of the optimized batch. The similarity between the dissolution profiles is indicated by an f2 score between 50 and 100. The mechanical qualities achieved were satisfactory. One month of stability at 30°C and 65%RH was observed for the improved batch.

6. List of Abbreviations

L-MTHF Ca: L- methyl folate calcium; MDFs: Mouth dissolving films; DHF: Dihydrofolate; THF: Tetrahydrofolate; THFR: Tetrahydrofolate reductase; HPMC: Hydroxypropyl methylcellulose; FTIR: Fourier transform infrared spectroscopy; SEM: Scanning electron microscopy; DT: Disintegration time; MDT: Mouth dissolving tablet.

7. Conflict of Interest

Author has no conflict of interest to declare.

8. Source of Funding

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