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FORMULATION DEVELOPMENT AND OPTIMIZATION OF FAST DISSOLVING FILM OF NARATRIPTAN HYDROCHLORIDE USING BOX BEHNKEN DESIGN

MANJULA BP*, LIKHITHA TN AND GEETHA M

Department of Pharmaceutics, Government College of Pharmacy, Rajiv Gandhi University of Health Sciences, Bengaluru -560027, Karnataka, India

***Corresponding Author: Manjula B P: E Mail:** karthikamogh@gmail.com

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ABSTRACT

Naratriptan hydrochloride, a bitter-tasting drug was formulated as fast-dissolving, palatable oral films to mitigate migraine and provide quick relief to migraineurs. Fast dissolving oral films were prepared by solvent casting method and optimized by applying Box-Behnken design. HPMC E15, stevia extract powder, and strawberry flavour were selected as independent variables whereas tensile strength, percent elongation, surface pH, and film thickness served as response parameters respectively. The prepared films were evaluated for appearance, tensile strength, percent elongation, folding endurance, thickness, disintegration time, surface pH, and *in vitro* drug release profile. A double-blind cross-over-study in human volunteers was used to assess the extent of taste masking and bitterness index of the optimized formulation. The optimized film had a smooth, elegant, and translucent surface with good mechanical properties as indicated by tensile strength 0.6125 kg cm^{-2}, percent elongation 29.22%, surface pH 3.73, and thickness 0.124mm respectively. 100 % *in vitro* release was achieved within 20 min; the Higuchi model being indicated as a diffusion-controlled release mechanism. Quick *in vivo* disintegration was achieved in 17.5 sec. Taste masking and in *vivo* bitterness evaluation study indicated that the optimized formulation had a slight but tolerable bitter taste. Thus palatable, fast dissolving oral films of naratriptan hydrochloride is a potential pharmaceutical approach to alleviate migraine and provide quick relief from excruciating pain.

Keywords: Box-Behnken design, Fast dissolving oral film, Migraine, Naratriptan hydrochloride, Solvent casting method, Taste masking

INTRODUCTION

Migraine is a chronic neurological disorder characterized by recurrent, moderate to severe headache often in association with several autonomic nervous system-related symptoms. Typically, it affects one-half of the head, is pulsating in nature, and lasts for about 2 to 72 hours **[1]**. About 33% of patients suffering from migraine perceive an aura: a transient visual, sensory, language, or motor disturbance that signals that the headache will occur soon. Symptoms are disturbances such as altered mood, irritability, depression or euphoria, fatigue, craving for certain foods like sweets, stiff muscles especially the neck, constipation or diarrhoea, and sensitivity to smell or noise **[2]**. Also associated symptoms may include nausea, vomiting, and sensitivity to light, sound, or smell. The pain worsens with physical activity. Worldwide, migraine is the fifth leading cause of disability and is three times more common among females, often occurring between early and middle adulthood **[3]**.

Fast dissolving oral film (FDOF), an innovative drug delivery system offers several advantages such as ease of self-administration, fast onset of action, and instant relief since a very thin oral strip is just placed on the patient's tongue, which instantly gets wet and hydrated by saliva, rapidly disintegrates and dissolves to release the medication within seconds into the oral cavity **[4]**.

Naratriptan hydrochloride (NH), a triptan drug used for the treatment of migraine, is a selective 5-hydroxytryptamine receptor 1 (5HT1) subtype agonist **[5]**. Orally administered naratriptan reaches its peak time in 2 to 3 h and peak concentration is 10.8ng/ml and 16.6 ng/ml in males and females respectively with an average half-life of 6h. The oral bioavailability of NH is 74% and the daily dose which should not exceed 5 mg in 24 h may be administered in divided doses twice or thrice a day. 50% of an administered dose of naratriptan is excreted unchanged in the urine and about 30% is excreted as metabolic products of cytochrome P450 oxidation **[6]**. NH has a lower headache recurrence rate and is less likely to cause drug interaction; however, the main drawback of any triptan drug is its bitter taste. Hence it is prudent to mask the unpalatable taste to improve product acceptance as well as patient compliance **[7-8]**.

Bitterness masking of an active pharmaceutical ingredient (API) is a great challenge in the formulation of oral products. Stevia, a natural sweetening agent, intended to be used in the formulation, is about 300 times sweeter than sucrose. The acceptable daily intake

(ADI) of stevia is 4mg/kg as per FDA regulation **[9-10]**.

Hence, an attempt was made to formulate taste-masked FDOFs of NH with quick onset of action to provide instant relief from migraine and possibly reduce the side effects.

MATERIALS AND METHODS

Materials

Naratriptan hydrochloride and HPMC E15 were gifted by APOTEX Pharmaceuticals Pvt Ltd. Stevia extract powder and strawberry flavour were purchased from LB Consumer Goods Pvt Ltd and DAWN Foods and Flavours respectively. Croscarmellose sodium, citric acid, ethanol, and propylene glycol were of analytical grade.

Methods

Formulation of fast dissolving oral films containing NH

Formulation design

The Box-Behnken design was chosen to get different experimental runs using design expert software version 11. The independent variables selected were concentration of polymer, HPMC E15, stevia extract, and strawberry flavour respectively. The factors and levels are mentioned in **Table 1**.

Table 1: Box-Behnken design

Sl. No.	Independent factors	Units	Low level	High level
1	Polymer concentration	mg	2.5	3.0
2	Stevia extract powder	mg	75	100
3	Flavour	µl	10	20

Method of preparation

FDOF was prepared by solvent casting method. HPMC E15 was dissolved in 10ml distilled water. NH and stevia extract powder were dissolved separately in 5ml of 50% ethanol. The drug solution was added to the polymer solution and mixed well. Initially stirring was carried out at low speed and later at high speed. Citric acid, a saliva stimulating agent, and propylene glycol, a plasticizer were added followed by the addition of flavour and colour to confer good taste and appearance. Stirring was continued to aid uniform dispersion of all the components and then poured into a Petri plate (area 64 cm^2) pre-coated with glycerine followed by drying overnight in a hot air oven at 35°C. The cast film was peeled carefully and cut into six strips each measuring 4 cm^2. The film was wrapped in an aluminium foil-lined with butter paper and stored in a desiccator until further use.

EVALUATION PARAMETERS

Appearance

The prepared films were physically examined for smooth finish and elegance.

Transparency

The transparency of the film was determined using the Shimadzu UV-1800 UV-Visible spectrophotometer. A 4cm^2

strip was placed on the inner side of the cuvette. The transmittance (%T) of the film was read at 514nm.

Thickness uniformity

The thickness of the film was measured at three specific points chosen at random using Absolute Digimatic digital verniercallipers to determine uniformity.

Tensile strength and percentage elongation

A strip from each formulation measuring 6 x 2 cm was placed between the fixed upper and movable lower jaw of the Hounsfield tensile strength apparatus. The lower jaw was moved gradually to increase the applied force necessary to break the film. The percent elongation was calculated by measuring the increase in length of the film after tensile strength measurement by using the following formula. Three strips from each formulation were used to determine tensile strength and percent elongation **[11]**.

$$\textbf{Percent elongation} = \frac{\mathbf{L - L0}}{\mathbf{L0}} \times \mathbf{10}$$

Where L = Final length

L0 = Initial length

Drug content

A film measuring 4 cm^2 was transferred to a 100 ml volumetric flask and dissolved in simulated saliva of pH 6.8. 1 ml of the resulting solution was diluted to 10 ml using simulated saliva; a final dilution was made by diluting 1 ml of the previous solution to 10 ml with simulated saliva. A 20µl aliquot of the final dilution was used to determine the drug content by a validated HPLC method. All determinations were carried out in triplicate **[12]**.

Folding endurance

A folding endurance test was performed on three strips for each formulation. Folding endurance value is the number of times, a film can be folded at the same place without breaking **[13]**.

Percentage moisture content

Three films measuring 4 cm^2 of the optimized formulation were weighed individually and stored in a desiccator containing fused calcium chloride at room temperature. Individual films were weighed to constant weight at 24h intervals. The percentage of moisture content was calculated using the formula:

$$\textbf{Percentage moisture content} = \frac{\textbf{Initial weight} - \textbf{Final weight}}{\textbf{Initial weight}} \times \mathbf{100}$$

Percentage moisture uptake

The percentage moisture uptake test was carried out to check the physical stability and integrity of the films in highly humid conditions. The moisture absorption capacity of the films was determined by placing three 4 cm^2 films of optimized formulation in the desiccator containing a saturated solution of potassium chloride, to maintain a high RH of 84%. After 24h the films were taken out and weighed to

determine the percentage moisture uptake **[14]**.

$$\text{Percentage moisture uptake} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Surface pH

The film was moistened with 0.5 ml of distilled water and kept for 1h in a petri dish. Surface pH was noted after stabilization; approximately one minute after the electrode of the pH meter comes in contact with the surface of the moistened formulation **[15]**.

***In vitro* drug release study**

In vitro, drug release studies were carried out using Franz diffusion cell with a receptor compartment volume of 7 ml and an effective diffusion area of 0.64 cm^2. Simulated salivary fluid was taken as the diffusion medium to determine the drug release. Release studies were carried out using rat mucosal membrane. A 4cm^2 strip of the optimized formulation was placed between the donor compartment and the receptor compartment containing simulated saliva stirred continuously using a magnetic stirrer at 50rpm. A 0.3ml sample was withdrawn immediately and after every 2 min for a total period of 20 min from the receptor compartment and replaced with an equal volume of fresh medium. The collected samples were diluted to 10 ml and the absorbance was measured at 223 nm using a UV visible spectrophotometer. The percent cumulative drug release was calculated using the standard calibration curve.

Statistical optimization

All the response variables were analysed by ANOVA and subjected to regression analysis. A numerical optimization technique based on the desirability approach was used to optimize the formulation. Optimum settings for dependent and independent variables were defined. All the response variables were subjected to regression analysis to determine the regression coefficients

Evaluation of optimized formulation

The optimized formulation **(Table 2**) was prepared following the predicted model and evaluated for the responses.

Table 2: Composition of optimized FDOF

Ingredients	Quantity
Naratriptan hydrochloride	2.5 mg
HPMC E15	2.9 mg
Stevia extract powder	86 mg
Cross carmellose sodium	10 mg
Citric acid	10 mg
Propylene glycol	114 mg
Flavour	16 mcg

Fourier Transform Infrared Spectroscopy (FT-IR)

Drug-excipient interaction was studied using Shimadzu FT-IR 8400 spectrophotometer. Potassium bromide pellet technique was used to prepare the sample and a scan was run in the range of 400-4000 cm $^{-1}$ for the pure drug and the optimized formulation

Differential scanning calorimetry (DSC)

Differential scanning calorimeter [Shimadzu DSC -60] was used to record thermograms for pure drug, optimized formulation, and physical mixture of drug and excipients. The thermal behaviour of the sample was investigated using nitrogen as purging gas at a scanning rate of 10°C/min, covering the temperature range of 100-300°C.

***In vivo* bitterness evaluation**

A simple cross-over study was designed to evaluate the bitterness level and overall acceptability of the optimized film in comparison with a placebo film. A taste panel consisting of six healthy human volunteers aged 18-20 years of either sex were divided into two groups, I and II (three volunteers in each group), and asked to assess the bitterness and overall acceptability of the coded sample (optimized film or placebo film). Volunteers were asked to perceive the taste of the coded sample after allowing the film to dissolve in the mouth, which took about 30 sec. They were also asked to gargle their mouth with a glass of water after spitting and immediately report the intensity of bitterness and overall acceptability according to the respective numerical score **[16]**.

RESULTS AND DISCUSSION

Formulation of fast dissolving oral film.

Experimental design

Different formulations were prepared by applying Box-Behnken design to study the influence of various independent variables, such as HPMC E15, stevia extract powder, and flavour. Dependent variables selected were tensile strength, percent elongation, thickness, and surface pH.

Characterization of NH fast dissolving oral films:

Appearance

All the formulated FDOFs were elegant and had a smooth uniform surface.

Transparency

The optimized film was found to be translucent as indicated by percent transmittance, 75.2%.

Thickness and uniformity of films

Thickness measurements at three different points were found to be 0.0833 - 0.1667mm. The thickness of the film decreases with an increase in polymer concentration (HPMC E15), as it is a low viscosity grade polymer.

Folding endurance

The folding endurance of the films ranged between 198±07.51 - 265± 2.57.

Formulation, F10 showed maximum folding endurance, 265± 2.57and exhibited good physical and mechanical properties.

Drug content uniformity

The drug content of NH fast dissolving films was 2.42 - 2.48mg implying uniform distribution of the drug in the films.

***In vitro* disintegration time**

All the formulations of fast dissolving films were found to disintegrate in the range of 12-25 sec. *In vitro* disintegration time was found to decrease with an increase in the concentration of HPMC E15 in formulations. It was observed that the disintegration time of the film decreased from 28 sec to 18 sec, a prerequisite for quick onset of action.

OPTIMIZATION

Response 1: Tensile strength

The tensile strength of the films ranged from 0.1407kg/cm^2 to 1.4762 kg/cm^2. Tensile strength increases with an increase in the concentration of HPMC E15 and flavour and decreases with an increase in the concentration of stevia extract powder, which is explained by the polynomial equation.

Tensile strength = 21.04297+0.729450 A -0.502379 B +0.002794 B*B

The quadratic model was used for the analysis of tensile strength and was found to be significant with F value (P-value) of 5.82 (0.0124).

This may be due to an increased concentration of stevia extract powder exerting a reducing effect on the mechanical properties of films. An increase in the concentration of HPMC E15 and flavour showed a slight increase in tensile strength.

Response 2: Percent elongation

The percent elongation of all the formulations was found to be between 23.44% and 37.11%. Percent elongation increases with an increase in the concentration of HPMC E15 and flavour and decreases with an increase in the concentration of stevia extract powder, which is explained by the polynomial equation.

Percent elongation = +74.94567+7.27500 A -0.729800 B-3.85775C +0.042280 B*C.

The response was analysed by a 2FI model. The Model F value of 1.46 and P-value of 0.2861 implies that the model is not significant.

Response 3: Surface pH

The surface pH of all the formulations ranged from 3.71 to 4.09. Low pH values can be attributed to the presence of citric acid in the formulation which gives a tart taste, which in turn induces salivation causing the film to dissolve rapidly.

Surface pH increases with an increase in the concentration of flavour and stevia extract

powder but decreases with the increase in the concentration of HPMC E15 which was explained by the polynomial equation.

Surface pH = +10.78429-0.340000 A -0.161600 B +0.12900 C -0.001360 B*C +0.001029 B*B

The response was analysed by a reduced quadratic model with a P value of 0.0172 and F value of 5.09 indicating a significant response.

Response 4: Thickness

An increase in the concentration of stevia extract powder and flavour increased the thickness of the film. Whereas an increase in the concentration of HPMC E15 decreased the thickness of film which is explained by the polynomial equation.

Thickness = -0.241507 - 0.061600 A + 0.006098 B + 0.039905 C - 0.000455 B*C

The response was analysed by a reduced 2FI model. The Model F value (P-value) of 15.04 (0.0003) implies that the model is significant.

Regression analysis

All the dependent variables were found to be significant except percent elongation. The desirability value of the optimized formulation was found to be 1. The results indicated that all the dependent factors played an important role in the preparation of FDOFs containing NH.

Evaluation of optimized formulation

The actual and predicted values of optimized FDOF formulation of NH were compared **(Table 3)** and found to be in close agreement with each other, indicating the relevance of optimization procedure in the successful development of FDOFs formulation.

The optimized film formulation had moisture content of 2.16%, moisture uptake was 4.09% and *in vitro* disintegration time of 20sec. It exhibited an *in vitro* diffusion profile of 100% within 20 min in the mucosal membrane **(Figure 1)**.

A diffusion-controlled drug release mechanism was observed which was indicated by the Higuchi model.

Fourier Transform Infrared Spectroscopy (FT-IR)

Comparison of FT-IR spectra of NH and optimized formulation suggested compatibility of NH with excipients, as no major changes were observed concerning standard peaks of the drug in both the spectra **(Figure 2)**.

Differential scanning calorimetry (DSC)

Interpretation of DSC thermograms of pure NH and optimized FDOF **(Figure 3)** indicate the presence of

amorphous form NH in the formulation and probably entrapped within the polymer as observed by an endothermic peak at 251°C for NH and its disappearance in FDOF respectively **(Figure 5, 6)**.

Scanning Electron Microscopy (SEM)

The surface morphology of FDOFs was investigated by SEM. As shown in the photographs **(Figure 4)**, the results of optimized formulation of FDOFs depicted a slightly rough outer surface which might be due to the crystalline form of NH and the possible presence of an amorphous form when cast into a film, which showed network like structures indicating that the drug particles are embedded within the polymer matrix.

***In vivo* bitterness evaluation by the human panel**

Taste evaluation was carried out by a panel consisting of six volunteers. This study was done for both placebo and optimized film. The data was analysed by one-way ANOVA and P values were found to be 0.0007 and <0.0001 for placebo and optimized film respectively **(Figure 5)**. Significant P values were obtained for disintegration time, bitterness index, and overall acceptability for both placebo and optimized oral film.

Table 3: Predicted and actual values of optimized formulation

Variables	Predicted	Actual
Tensile strength (kg/cm^2)	0.592	0.6125
Percent elongation (%)	29.132	29.22
Surface pH	3.73	3.73
Thickness (mm)	0.121	0.124

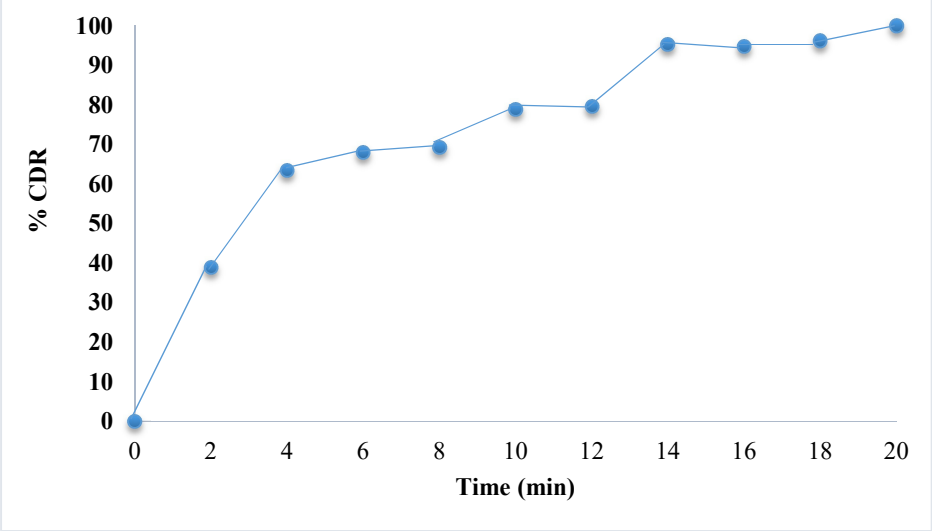

Figure 1: *In vitro* % CDR profile of optimised formulation using mucosal membrane

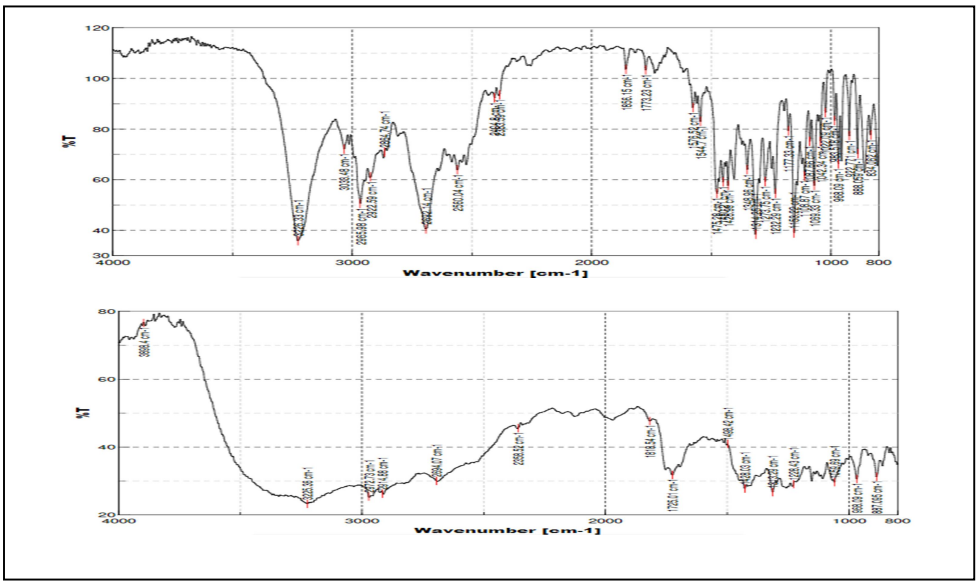

Figure 2: FTIR Spectra of NH and optimized formulation

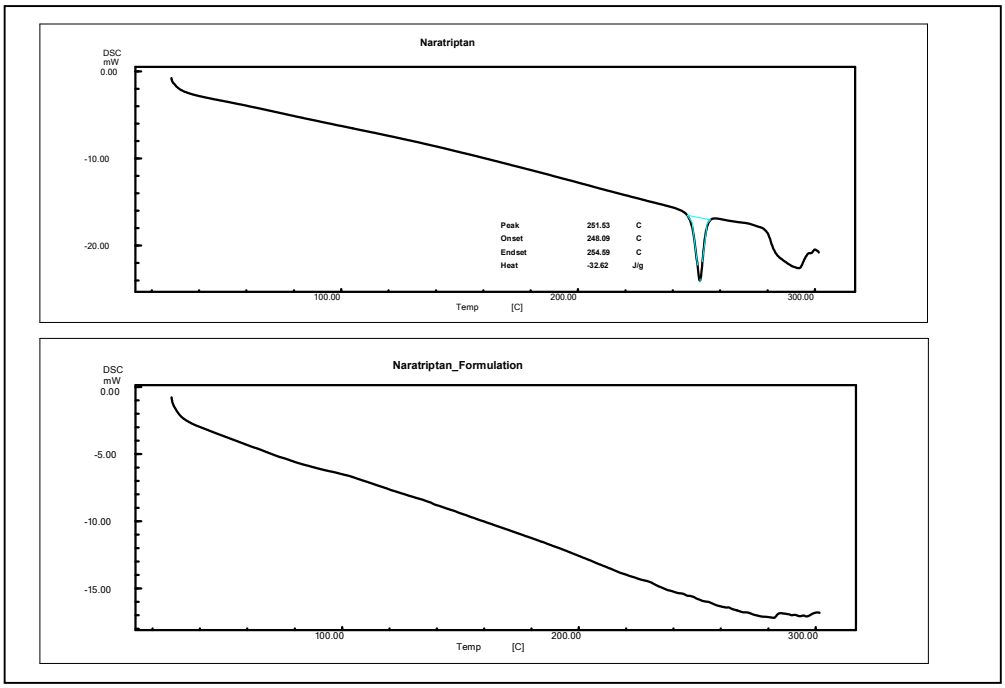

Figure 3: DSC thermograms of NH and optimized formulation

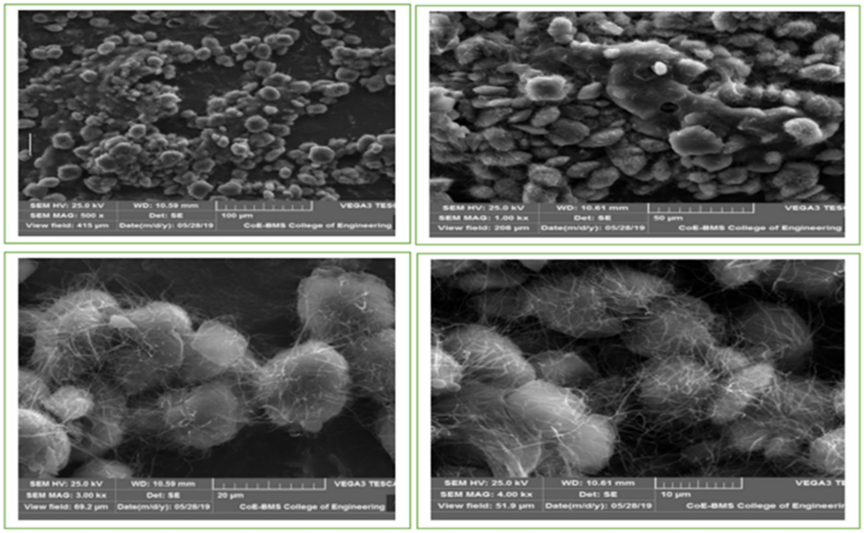

Figure 4: SEM images of optimised formulation

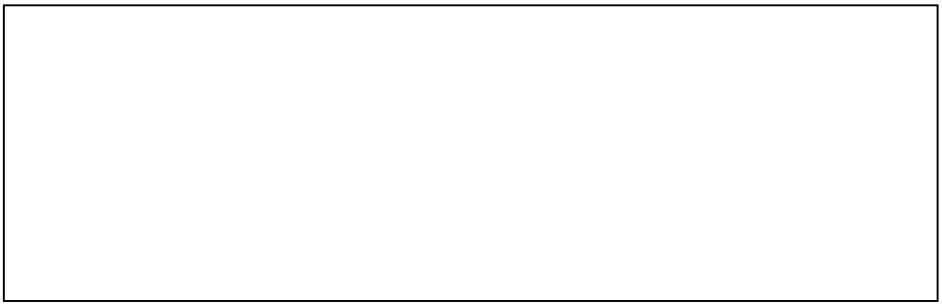

Figure 5: One way ANOVA analysis of taste evaluation

An average disintegration time of 10 and 17.5 sec (score 1 and 3) was obtained for placebo and optimized film respectively which indicated that placebo disintegrated faster than the optimized formulation. However, the disintegration time for placebo and optimized film complies with the generally acceptable criterion of 30 sec or less.

A score of 0 and 1 were assigned for placebo and optimized formulation respectively for bitterness and overall acceptability, which indicated that the optimized film was acceptable sincebitterness of the drug has been considerably masked with stevia extract powder and strawberry flavour.

CONCLUSION

FDOFs of NH were successfully prepared by applying Box Behnken design and optimized using numerical optimization technique to obtain an acceptable film. Thus, FDOFs with adequate taste masking of the bitter tasting drug is a promising approach to mitigate migraine due to quick onset of action as demonstrated by rapid *in vivo* disintegration of the film in the oral cavity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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