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Original Article

Formulation optimization and evaluation of fast dissolving film of aprepitant by using design of experiment

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ABSTRACT

The concept of fast dissolving dosage form has become popular as new delivery system. This system will provide maximum therapeutic efficacy, increased bioavailability and maximum stability by reducing the frequency of dosage. It will also avoid first pass metabolism of the drugs. This system provides more rapid drug absorption from the pre gastric area which may provide quick onset of action. The present research aimed to prepare fast dissolving films (FDF) of aprepitant used in the prevention and treatment of chemotherapy-induced nausea and vomiting. The FDF was prepared using solvent casting method and optimized employing central composite design considering two independent variables film forming polymer (pullulan) and PEG 400. Disintegration time, wetting time, drug release and folding endurance were taken as dependent variables. The prepared optimized formulation showed minimum disintegration time (20 s), highest dissolution rate (88.87%) and satisfactory physicochemical properties. It is evident from the above results that the developed formulation can be an innovative dosage form to improve the drug delivery, onset of action as well as improve patient compliance.

1. Introduction

There has been significant interest in the development of modified release oral dosage forms because oral delivery market holds approximately 52% of the market in the overall drug delivery market. But there are some commonly associated problems with oral administration of drugs like minimizing the risk of partial loss of active ingredients due to tablet or capsule crushing or imprecise liquid administration which leads to dosage inaccuracy and drug therapy overdosing or inefficiency [1–3]. In order to overcome these issues, fast dissolving drug delivery systems are gaining considerable attention. Among them oral film strips have hit the mainstream in the last few years as a new way of freshening the breath. These gel-like wafers slip into the mouth and dissolve quickly to release the flavor [4–6]. Recent technological advancements have diverted many drug companies to explore new prospective in this technology to provides fast, accurate dosing that is expected to increase compliance, particularly among children [7–9]. Nowadays, there has been significant development in transmucosal routes of drug administration because this route has a potential to fathom such problems associated with oral administration of the drugs [9]. There is no need for water or measuring and upon melting; the dose of medicine is swallowed. Absorption of drug by oral mucosa into systemic circulation is an attractive approach because it is highly vascularized and hence highly permeable. Therefore fast dissolving films have become a popular oral dosage form for various medicaments which provide rapid disintegration due to large surface area and hence improve patient compliance.

Various hydrophilic polymers which provide rapid dissolution, acceptable mechanical properties and good mouth feel quality are used as a film forming agents. Pullulan is natural occurring polysaccharide consisting of repeating maltotriose units, which are made up of three 1,4- alpha glucosidic linkages and single 1,6 -alpha -glucosidic linkages. It is available in both food and pharmaceutical garde. It is widely used in the food industry as a low-calorie food additive. Pullulan exhibits excellent moisture retention and prevents fungal growth. These properties make it a good food preservation substance in the food industry. It is an excellent film-former, producing a film which is heat sealable with good oxygen barrier properties [10,11]. It can be used as encapsulating agent, adhesive, thickening and extending agent.

Nausea refers to forceful expulsion of contents of the stomach and intestine. Aprepitant was selected due to its selective high affinity neurokinin 1 receptor antagonist activity against both acute and

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delayed postoperative vomiting (POV) and chemotherapy-induced vomiting (CIV) [12,13]. Its efficacy against nausea, however, appears to be comparable to other treatment options. Like 5-HT_3 receptor antagonists, aprepitant is nonsedative. The oral bioavailability of aprepitant is limited due to its poor dissolution and low aqueous solubility. By reducing the particle size down to submicrons range results in increased solubility and gives complete absorption of drug [14]. Predictions from dissolution tests and theoretical considerations indicated that on reducing the particle size of the drug leads to increased dissolution and increased oral bioavailability.

Hence, based on the rationale of the proposed research work, the aim of present investigation was to develop and formulate pullulan based fast dissolving films of aprepitant by solvent casting method for the direct absorption of drug via transmucosal lining to the systemic circulation. The proposed formulation has the potential to improve compliance and presents multiple competitive advantages over its marketed oral dosage forms used in chemotherapy induced nausea and vomiting patients.

2. Materials and methods

2.1. Materials

Aprepitant was obtained from Hetero Labs Ltd, Baddi, India. Pullulan was obtained as gift sample from Gangwal Chemicals Pvt Ltd, Mumbai. All other ingredients were used of analytical grade without any further modification.

2.2. Formulation development of fast dissolving films

2.2.1. Preliminary trials for screening of components

Development of successful fast dissolving film is based on selection of polymer nature and concentration; several polymers were tried for their film forming property. Pullulan was used as a film former in present investigation. Blank formulations were prepared by dissolving different polymers and plasticizer compositions in distilled water as shown in Table A.1. The resulting solution was casted and dried in the oven at 45 °C for 24 h. Obtained films were evaluated for film clarity, surface appearance, stickiness, DT and folding endurance as shown in Table A.2.

2.3. Preparation of drug loaded fast dissolving films

Polymeric solution (Solution A) was prepared by dissolving desired amount of pullulan in sufficient quantity of distilled water (70%). Specific quantity of drug along with polyethylene glycol and other excipients were dissolved in remaining water (30%) with continuous stirring (Solution B). Solution B was slowly added in polymeric solution A with continuous stirring. Final solution obtained was kept aside for 30 mins for defoaming. After defoaming, solution was poured in petri plate and dried at 45 °C in hot air oven for 24 h [15,16]. Film casted in petri plate was then carefully peeled off and cut into pieces of desired shape and size. Different optimized combinations of film containing pullulan with PEG 400 were prepared and evaluated for the disintegration time, wetting time, folding endurance and drug release as shown in Table A.3.

Table A.1
Composition of blank fast dissolving films.

Ingredients	BF1	BF2	BF3	BF4	BF5	BF6	BF7	BF8	BF9
Pullulan (mg)	50	100	150	200	250	300	350	400	450
PEG 400 (mg)	16	32	48	64	80	96	112	128	144
Citric acid (mg)	15	15	15	15	15	15	15	15	15
Tween 80 (ml)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Mannitol (mg)	15	15	15	15	15	15	15	15	15
Water (ml)	20	20	20	20	20	20	20	20	20

2.4. Preparation of pullulan based fast dissolving film of aprepitant using experimental design

To optimize the formulation variables, factors selected for study were concentration of pullulan and concentration of plasticizer. These variables were taken at five different levels viz. –alpha, low, center point, high level and + alpha level. The variables were selected on the basis of preliminary studies carried out before the experimental design was being implemented. Diverse batches of aprepitant were prepared as per the central composite design and a total of 9 runs were presented by the Design Expert® software. The response or dependent variables studied were disintegration time, wetting time, folding endurance, and drug release [17,18]. The design matrix along with the investigated response variables are shown in Table A.4. Experimental findings were analyzed using ANOVA by fitting the response Fig. A1(a–h) in the run design.

2.5. Evaluation of prepared aprepitant loaded FDF

2.5.1. Drug excipient interaction study

2.5.1.1. Fourier transform infrared spectroscopy (FTIR). The FTIR absorption spectra of the pure drug, pullulan and their mixture were recorded in the range of 4000–400 cm^{-1} by KBr disc method using FTIR spectrophotometer (Spectrum GX, Perkin-Elmer, USA).

2.5.1.2. Differential scanning calorimetry (DSC). The thermal properties of the pure drug, pullulan and mixture of both were evaluated using differential scanning calorimeter using DSC-PYRIS-1 (Perkin-Elmer, USA). The analysis was performed with a heating range of 48–50 °C and a rate of 10 °C min^{-1} in an inert nitrogen atmosphere.

Properties such as film clarity, stickiness, homogeneity, surface appearance were evaluated by visual inspection [19,20].

2.5.2. Thickness

Thickness of every oral film was determined at five different places using screw gauge. Average thickness and standard deviation of each oral film formulation was determined [21,22].

2.5.3 wt. variation

Three films of 2 × 2 cm^2 size were cut randomly from each film formulation. Films were weighed individually on electronic balance and the mean weight for each batch was calculated [15].

2.5.4. Surface pH

This test was evaluated by placing the film in a Petri dish. Then it was moistened with 0.5 ml of phosphate buffer and kept for 30 s. The pH was noted after bringing the electrode of the pH meter in contact with the surface of the formulation and allowing equilibration for 1 min [23]. The average of three determinations for each formulation was taken.

2.5.5. Folding endurance

It was determined by folding the film of uniform cross sectional area and thickness until it breaks. The number of times film was folded without breaking computed as the folding endurance value. This test ensures the tensile strength of the film [24–26].

2.5.6. Uniformity of drug content

The drug content was estimated by random sampling of all batches. The FDF (2 × 2 cm^2) was dissolved in the phosphate buffer. This solution was filtered and injected into the HPLC [27–29]. The drug content was estimated as a mean of three determinations.

Table A.2
Preliminary trail results of blank fast dissolving film.

Formulation code	Stickiness	Surface appearance	Film clarity	*In-vitro* disintegration time (sec)	Folding endurance
BF1	Sticky	Film did not form	–	–	–
BF2	Sticky	Film did not form	–	–	–
BF3	Sticky	Film did not form	–	–	–
BF4	Non - sticky	Uniform	Clear	28 ± 1.7	97 ± 1.0
BF5	Non - sticky	Uniform	Clear	35 ± 1.5	86 ± 1.0
BF6	Non - sticky	Uniform	Clear	43 ± 1.7	84 ± 2.0
BF7	Non - sticky	Non– uniform	Turbid	–	78 ± 2.0
BF8	Non - sticky	Non– uniform	Turbid	–	71 ± 2.0
BF9	Non - sticky	Non– uniform	Turbid	–	65 ± 3.0

Table A.3
Optimized concentration of polymer for drug loaded fast dissolving film.

Independent Variables	Levels				
	Alpha level (−)	Low-1	Medium0	High1	Alpha level (+)
Pullulan %w/v	42.92	45	50	55	57.07
PEG 400 %w/v	7.92	15	17.5	20	22.07

2.5.7. Percentage moisture loss

To determine the integrity and physical stability of the film, percent moisture loss test was done. A film size 2 × 2 cm^2 was cut and weighed. After that the film was placed in a desiccator containing fused anhydrous calcium chloride for three days [30]. After three days film patch was taken out and weighed again. The percentage moisture loss of the film was calculated using the following formula:

$$\text{Percentage Moisture Loss} = (\text{Initial Weight - final weight})/\text{Initial Weight} \times 100$$

2.5.8. In vitro wetting time

A circular paper was placed in the petriplate. 6 ml of 0.1%w/v amaranth dye solution was prepared and added to the petriplate. The film strip (2 × 2 cm^2) was placed on the surface of tissue paper. The time required for the dye to appear on the surface of film was noted as the wetting time.

2.5.9. Disintegration time

Disintegration time was measured by placing the film strip (2 × 2 cm^2) in a Petri dish 6 cm in diameter containing 6 ml of phosphate buffer of pH 6.8. Time required for complete disintegration of the film was noted. All the measurements were done in triplicate and average values was reported [31,32].

2.5.10. In-vitro release study

Determination of dissolution profile of films was carried out in a beaker containing 30 ml phosphate buffer (pH 6.8) with 1% w/v SLS at 37 ± 0.50 °C. Whole assembly was then placed on a shaker. Sample aliquot (1.0 ml) was withdrawn at different time intervals and replaced with same fresh media. Samples were filtered and diluted with phosphate buffer (pH 6.8) and analyzed by using HPLC. The *in vitro* release data obtained were subjected to a zero order and first order kinetics to understand the release profile and release mechanism [33,34].

2.6. In vivo study deign study

New Zealand male rabbits were selected for animal pharmacokinetic study. All rabbits were healthy during the period of study. The protocol of study for animal experiments was approved by institutional animal ethics committee.

New Zealand rabbits (weighing 2.6–3.1 kg) were fasted overnight before administration of formulations. The study was performed into two periods, two sequence cross over design. Rabbits were randomly divided into two groups. One group received the marketed formulation (Aprepitant equivalent to 1 mg/kg) whereas the other group received fast dissolving film. Film was carefully placed on the rabbit tongue with the help of body restraint device in which animals head was exposed and lifted apart the gums with a wooden tongue depressor. Film was placed in the mouth by wetting mouth with small amount of water [35,36]. Also the innovator applied a gentle tension to restrain the mouth in order to ensure the complete disintegration of the film [37]. For pharmacokinetic study the blood samples were obtained from the peripheral vein of each rabbit at the interval of 0, 1, 2, 3, 4, 5, 7, 9, 12, 24 hrs.

2.6.1. Preparation of plasma samples for HPLC analysis

0.5 ml of blood sample was withdrawn in the centrifugation tube containing disodium EDTA. The sample was centrifuged at 5000 for 10 min. With the addition of acetonitrile, the plasma proteins were precipitated [38–40]. The supernatant was transferred to a test tube and evaporated to the dryness. The residue was reconstituted with acetonitrile and dilute phosphoric acid to determine aprepitant in blood sample by HPLC method. The chromatographic conditions were depicted in Table A.5 and standard chromatogram was shown in Fig. A2.

Table A.4
Optimization parameters of drug loaded fast dissolving films.

Run	Independent variables		Dependent variables			
	Pullulan (% w/v)	Plasticizer (%w/v)	DT (Sec)	WT (Sec)	Folding endurance	Drug release (%)
FDF1	50	22.07	22 ± 0.4	16 ± 0.2	110 ± 2	87.90 ± 0.02
FDF2	45	10	32 ± 0.2	26 ± 0.2	99 ± 1	82.71 ± 0.04
FDF3	55	10	21 ± 0.3	14 ± 0.4	92 ± 3	86.89 ± 0.02
FDF4	50	7.92	18 ± 0.1	20 ± 0.2	93 ± 2	85.88 ± 0.04
FDF5	57.07	15	24 ± 0.2	13 ± 0.3	101 ± 4	82.76 ± 0.07
FDF6	55	20	19 ± 0.4	11 ± 0.5	113 ± 2	88.87 ± 0.02
FDF7	42.92	15	40 ± 0.5	33 ± 0.2	96 ± 5	79.86 ± 0.04
FDF8	50	15	20 ± 0.2	16 ± 0.2	94 ± 3	85.00 ± 0.07
FDF9	45	20	38 ± 0.2	23 ± 0.1	94 ± 2	84.87 ± 0.02

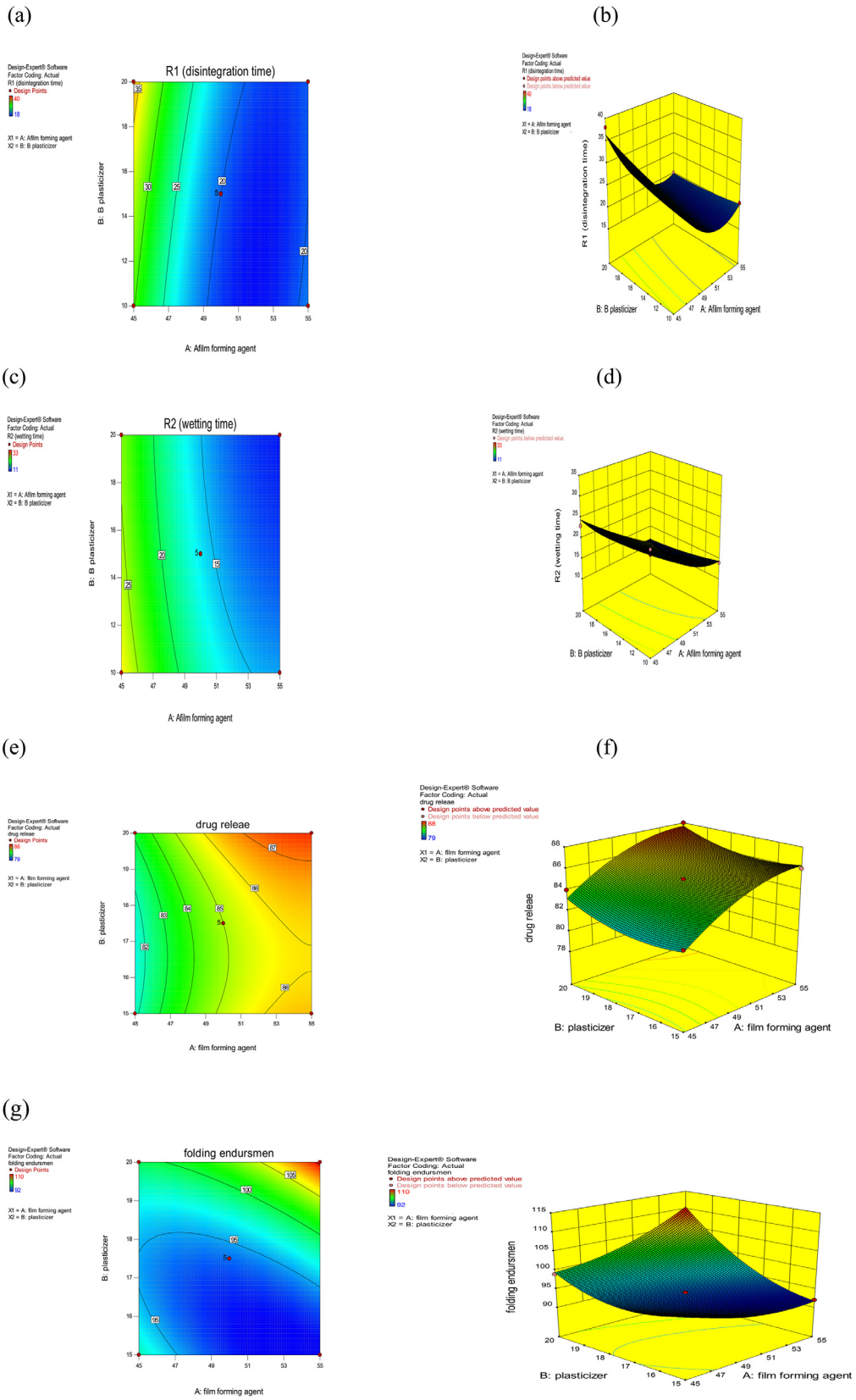

Fig. A1. Images of contour plots (a–d) and three dimensional surface response plots (e–h) showing the effect of in vitro disintegration time (Y1), wetting time (Y2), drug release (Y3) and folding endurance (Y4).

Table A.5
Chromatographic conditions.

Column	C18
Mobile phase	Acetonitrile and Dilute phosphoric acid
Flow rate	0.9 ml/min
Sample injection volume	10 µl
Temperature	35 °C

2.6.2. Pharmacokinetic studies

PK parameters analyzed in the study included maximum measured plasma concentration (Cmax), area under the plasma concentration vs. time curve (AUC), time of maximum measured plasma concentration (tmax), mean residence time (MRT) and area under the first moment curve(AUMC).

3. Results

3.1. Optimization of independent variables

Results from preliminary studies suggested that 5–45% pullulan and 20–30% PEG 400 causes stickiness in films. The films prepared with 55–95% pullulan and 5–15% PEG 400 composition was non-uniform. However, films prepared by taking a concentration of pullulan at 45–55% and PEG 400 at 15–20% were non sticky, uniform and clear as shown in Table A.2. Based on the results, polymers composition pullulan in the range of 45–55% and plasticizer in the range of 15–20% were selected for further formulation. It was found that amount of polymer above 55% showed increase in DT. Since below 15% the film did not show flexibility and above 20% film became sticky.

3.2. Optimization of dependent variables

3.2.1. Response 1 (In- vitro disintegration time Y1)

$$Y1 = +20.00 - -6.45* A + 1.33*B - -1.75*AB + 6.44*A^2 + 0.44*B^2$$

(R^2 = 0.9708, quadratic model)

ANOVA was applied to estimate the significance ($p < 0.05$) of the model and individual response parameters. The surface response plots and contour plots were analyzed to reveal the effect of independent factors on the measured responses. The quadratic model of F-value 80.73 implies the model is significant $p < 0.0001$. The contour plot and surface response plot in Fig. A1(a) and (b) showed the effect of different independent variables on disintegration time.

The *in- vitro* disintegration time of film was increases as the amount of pullulan increases and increase in concentration of plasticizer decreased disintegration time but after excessive amount of polymer increase, the film became brittle so there was slight decrease in disintegration time [41].

3.2.2. Response 2 (Wetting time Y2)

$$Y2 = + 16.00 - -6.54*A - 1.46* B + 0.000*AB + 3.00*A^2 + 0.50*B^2$$

(R^2 = 0.9761, quadratic model)

After ANOVA estimation, the quadratic model of F-value 57.72 implies the model is significant p-value 0.0001. The contour plot and surface response plot in Fig. A1(c) and (d) showed the effect of different independent variables on wetting time.

The wetting time of film was increases as the amount of Pullulan increases and decreased as the amount of plasticizer increase.

3.2.3. Response 3 (Drug release Y3)

$$Y3 = + 85.00 + 1.53* A + 0.85* B + 0.000*AB - -1.81*A^2 + 0.94*B^2$$

(R^2 = 0.8764, quadratic model)

After ANOVA estimation, the quadratic model of F-value 9.93 implies the model is significant $p < 0.0044$. The contour plot and surface response plot in Fig. A1(e) and (f) showed the effect of different independent variables on drug release.

The drug release of film was increases as the amount of plasticizer and polymer increases up to 45–55%. When concentration of polymer reached to 55% then drug release was decreases since drug remains inside the matrix of polymers.

3.2.4. Response 4 (folding endurance Y4)

$$Y4 = + 94.00 + 1.88* A + 4.51* B + 5.50*AB + 1.88*A^2 + 3.37*B^2$$

(R^2 = 0.9469, quadratic model)

After ANOVA estimation, the quadratic model of F-value 24.98 implies the model is significant $p < 0.0003$. The contour plot and surface response plot in Fig. A1(g) and (h) showed the effect of different independent variables on folding endurance. The folding endurance of film was increases as the amount of plasticizer and polymer increases.

Table A.6.1–A.6.4 shows the results of the analysis of variance (ANOVA), which was used to generate mathematical models. The high values of correlation coefficient for disintegration time, wetting time, folding endurance and drug release indicate a good fit i.e. good agreement between the dependent and independent variables.

The linear correlation plots for all the responses (Y1–Y4) were generated with the software. To graphically envision the effect of formulation/operation variables on the output variables, Contour and Response surface plot were constructed using software as shown in Fig. A1.

3.3. Characterization of aprepitant loaded fast dissolving films

The FTIR spectra obtained for aprepitant showed strong absorption at 1704 cm^{-1} which indicated C═O stretching, C-F stretching at 1132 cm^{-1}, C–H stretching over the range 1500–1600 cm^{-1}. The FTIR-ATR of pure Pullulan sample presented characteristic peak at 3423 cm^{-1} which indicated –OH stretching vibration peak. The strong peak at 2928 cm^{-1} indicated C—H bond of alkane compounds. The peaks at 1636 cm^{-1} and 1460 cm^{-1} showed a C═O bond and peaks at 928 cm^{-1}, 849 cm^{-1} & 757 cm^{-1} indicated presence of glycosidic bonds. The FTIR-ATR of the drug loaded pullulan films showed the characteristic peaks of drug and pullulan which indicates purity of drug

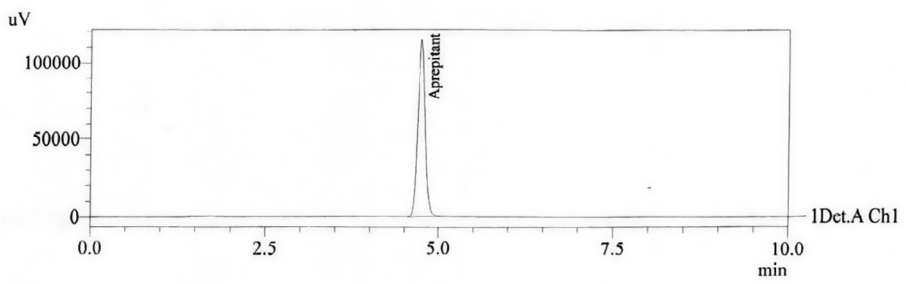

Fig. A2. Standard chromatogram of aprepitant.

Table A.6.1
ANOVA for response surface quadratic model.

Sr No	Source	Sum of Squares	Df	Mean Square	F value	p-value
1	Model	649.05	5	129.81	80.73	< 0.0001 significant
2	A-Film forming agent	333.17	1	333.17	207.21	< 0.0001
3	B- Plasticizer	14.20	1	14.20	8.83	0.0280
4	AB	12.25	1	12.25	7.62	0.0281
5	A^2	288.29	1	288.29	179.30	< 0.0001
6	B^2	1.33	1	1.33	0.83	0.3931
7	Residual	11.26	7	1.61		
	Lack of fit	11.26	3	3.75		
	Pure error	0.000	4	0.000		
8	Cor total	660.31	12			

Table A.6.2
ANOVA for response surface quadratic model.

Sr No	Source	Sum of Squares	df	Mean Square	F value	p-value
1	Model	406.12	5	84.28	57.72	< 0.0001 significant
2	A-Film forming agent	341.71	1	341.71	232.02	< 0.0001
3	B- Plasticizer	16.99	1	16.99	11.53	0.0115
4	AB	0.000	1	0.000	0.000	1.0000
5	A^2	62.61	1	62.61	42.51	0.0003
6	B^2	1.74	1	1.74	1.18	0.3132
7	Residual	10.31	7	1.47		
	Lack of fit	10.31	3	3.44		
	Pure error	0.000	4	0.000		
8	Cor total	431.69	12			

Table A.6.3
ANOVA for response surface quadratic model.

Sr No	Source	Sum of Squares	df	Mean Square	F value	p-value
1	Model	57.17	5	11.43	9.93	< 0.0044 significant
2	A-Film forming agent	18.74	1	18.74	16.27	< 0.0050
3	B- Plasticizer	5.83	1	5.83	5.06	0.0592
4	AB	0.000	1	0.000	0.000	1.0000
5	A^2	22.85	1	22.85	19.84	0.0030
6	B^2	6.11	1	6.11	5.31	0.0547
7	Residual	8.06	7	1.15		
	Lack of fit	8.06	3	2.69		
	Pure error	0.000	4	0.000		
8	Cor total	65.23	12			

Table A.6.4
ANOVA for response surface quadratic model.

Sr No	Source	Sum of Squares	df	Mean Square	F value	p-value
1	Model	405.57	5	81.11	24.98	< 0.0003 significant
2	A-Film forming agent	28.39	1	28.39	8.74	0.0212
3	B- Plasticizer	162.37	1	162.37	50.00	0.0002
4	AB	121.00	1	121.00	37.26	0.0005
5	A^2	24.46	1	24.46	7.53	0.0287
6	B^2	79.24	1	79.24	24.40	0.0017
7	Residual	22.73	7	3.25		
	Lack of fit	22.73	3	7.58		
	Pure error	0.000	4	0.000		
8	Cor total	428.31	12			

and polymer; hence there is no interaction of drug with film former Pullulan as shown in Fig. A3.

A DSC thermogram of pure phullulan showed endothermic peak at 84 °C corresponding to its melting point and pure aprepitant displayed endothermic peak at 248.2 °C. The transition temperature for the drug and pullulan was decreased by 10 °C–15 °C indicating decrease in the melting point of the mixture. So the DSC suggested that the solubilization rate of the mixture was increased which had lead to decrease in disintegration time as shown in Fig. A4.

Aprepitant fast dissolving films was evaluated according to the following parameters: thickness, weight variation, surface pH, drug content and moisture loss.

The average weight of FDF batches were found in the range of 22–42 mg (Fig. A5) indicating weight variation is uniform. The mean thickness values for all the batches were found to be 0.05–0.12 mm (Fig. A6) indicating uniform cast of respective batches. The surface pH was found to be in the range of 6.5–6.9 which was close to saliva pH (Fig. A7). All the batches contained uniform quantity of drug which indicated the uniformly and evenly distribution of the drug as shown in Fig. A8. The in vitro drug release results indicated that initial phase of release of drug was due to the amount of polymer spread the hydrophilic chain around the matrix system. F6 formulation showed rapid release (Fig. A9) but at high concentration of the polymer the release profile was decreased due to the higher percentage of polymer which will form a layer around the drug and allow the drug release at slow rate.

Thus from in-vitro drug release study, it was concluded that F6 was selected as the best formulation.

4. Pharmacokinetic data analysis

For animal pharmacokinetic study, the plasma concentration time profile obtained is shown in Fig. A10. Pharmacokinetic parameters including Cmax, Tmax, AUC, AUMC and MRT were determined from plasma profile for aprepitant by the using Winnonlin Software.

The average peak plasma concentration obtained for the marketed product and FDF, indicating increase in the extent of absorption (AUC). Similarly, there was increase in rate of absorption (Cmax) for FDF as compared to the marketed product (Table A.7), indicating that drug was more rapidly absorbed from the fast dissolving films and achieving higher plasma concentration in short interval after dosing than marketed formulation. The higher values of pharmacokinetic parameters showed the enhancement in bioavailability of the drug by formulating them into fast dissolving films. This faster absorption is an obvious advantage for the management of nausea and vomiting.

5. Discussion

In preliminary phase, attention was given to select the proper concentration of film forming agent and plasticizer to develop a successful FDF. These selections were used to impart suitable ductility, mechanical strength and flexibility to the films under different type of mechanical stress. Pullulan was used as a film forming agent due to its excellent film forming properties. PEG 400 was selected as plasticizer for Pullulan based FDF as it developed clear homogenous preparation. The FDF prepared by using combination of Pullulan and PEG 400 found to be flexible with good mechanical strength.

Preliminary trials indicated 45–55% w/v levels of pullulan and 15–20% plasticizer level in the film casting solution showed good results for mechanical properties and disintegration time. Levels of other excipients were fixed for all formulations. The selected levels of both factors were optimized using design expert software. 3^2 central composite design enabled prediction of interaction among independent variables and its effect on the dependent variables. It was helpful to investigate that how the response changes when two factors are changed simultaneously. Therefore 3^2 central composite design was

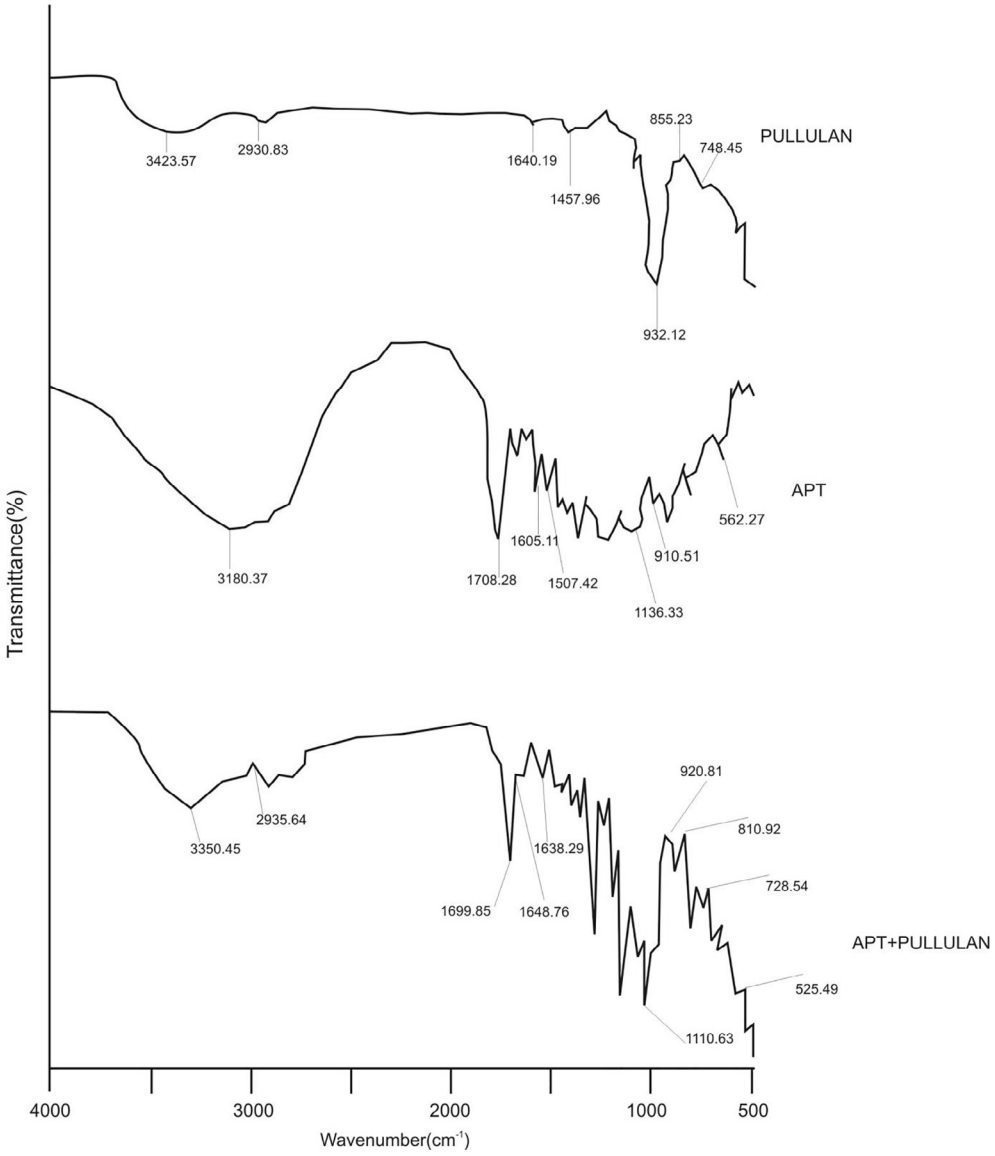

Fig. A3. Comparison of FT-IR of pullulan, aprepitant and mixture of both.

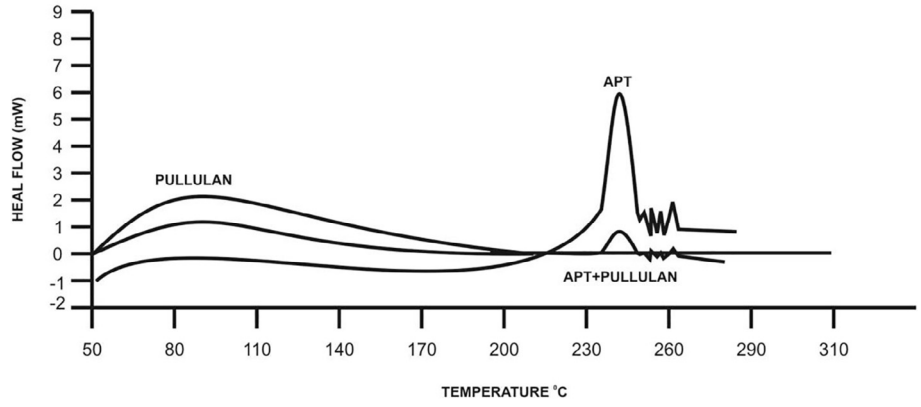

Fig. A4. Comparison of DSC of pullulan, aprepitant and mixture of both.

used to prepare aprepitant loaded FDF. The in vitro release of the drug was found to be 88% indicating that the films dissolve within minutes and the formulation excipients did not retain the drug. The results of content uniformity indicated that drug has been uniformly distributed in the film. The drug content of optimized formulation was found to be 96.45%. As no significant difference in drug content was found which indicated good content uniformity among the films. To generate optimized formulation, different equations and regression behaviors were

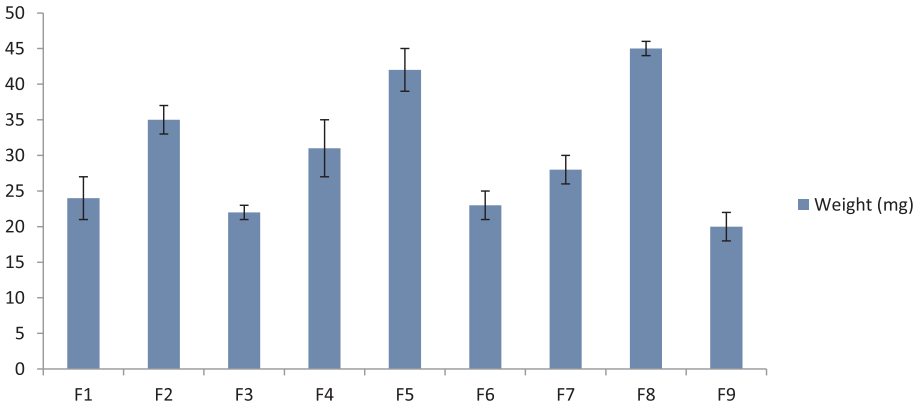

Fig. A5. Graphical presentation of weight variation of all batches (F1–F9).

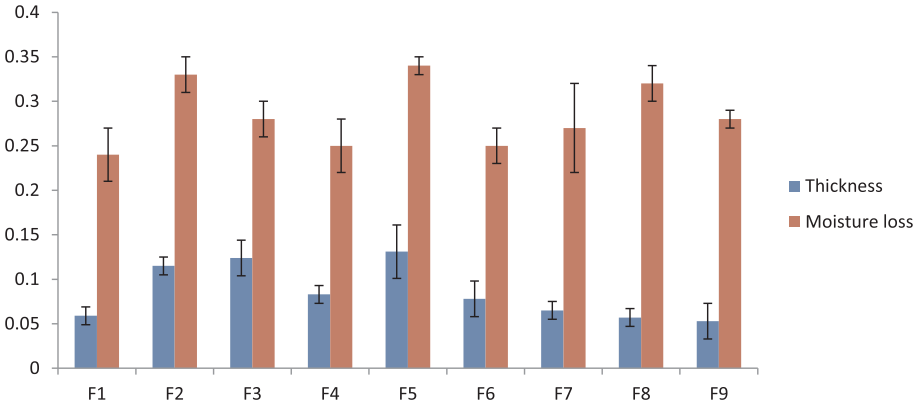

Fig. A6. Graphical presentation of thickness and moisture loss of all batches (F1–F9).

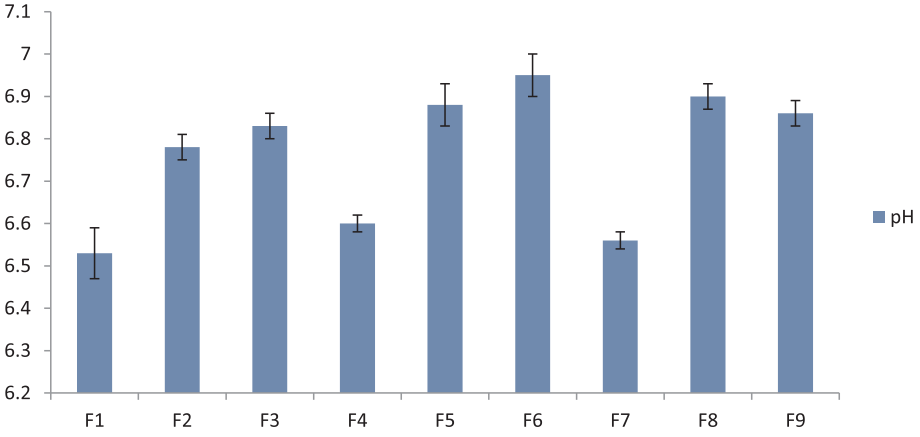

Fig. A7. Graphical presentation of pH of all batches (F1–F9).

analyzed and fitted into the software.

A statistical model polynomial term was used to evaluate the responses. The responses showed wide variation as shown in Table A.4. The data clearly indicates that the DT, WT, drug release and folding endurance values were strongly dependent on the selected independent variables. The high values of correlation coefficient indicated a good fit i.e. good agreement between the dependent and independent variables.

The surface pH results indicated that film was acceptable by the patients. Folding endurance gives an indication of brittleness of the film. As concentration of PEG 400 increased, folding endurance was also increased which affects on overall flexibility of aprepitant loaded FDF. Higher value of folding endurance for FDF indicated that the films were strong enough to withstand handling.

In vitro disintegration study represents an indication of onset of action of the drug. The results suggested that an increase in concentration of plasticizer decreased the disintegration time. Wetting time was estimated to further evaluate the disintegration behavior of the films. An increase in concentration of Pullulan leads to decrease in wetting time.

The increase in the rate of drug release could be found by the ability of hydrophilic polymer to promote the dissolution by creating pores for the drug to diffuse out of the films and enhance the release of the drug. There was direct relationship between the drug release and concentration of plasticizer, when the amount of plasticizer increases.

The optimized FDF showed a 20 sec disintegration time with sufficient wetting time, folding endurance and 88.87% drug release. Further, a significant difference in AUC and AUMC of the film formulation indicated improvement in oral bioavailability of aprepitant.

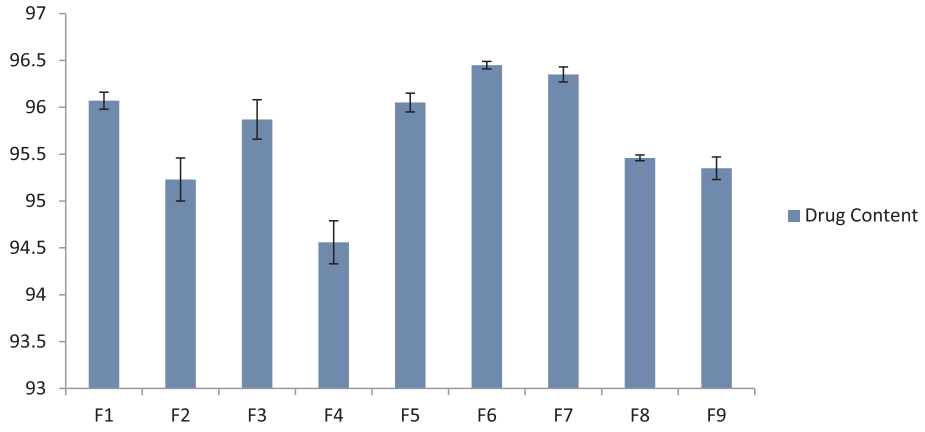

Fig. A8. Graphical presentation drug content of all batches (F1–F9).

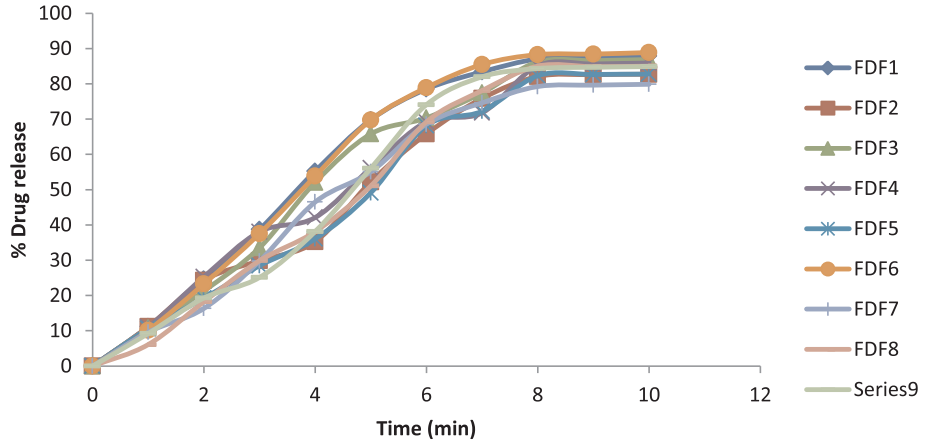

Fig. A9. Comparative study of percentage drug release of film formulations.

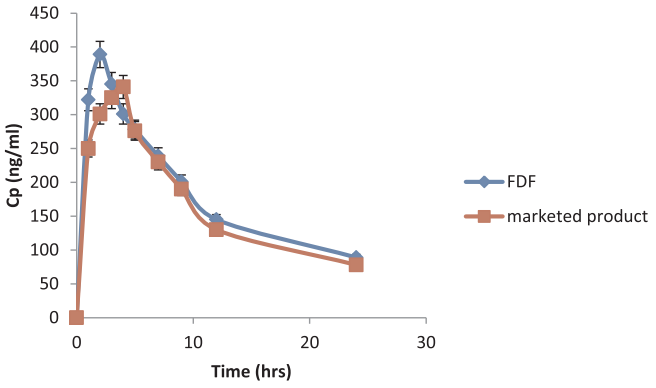

Fig. A10. The pharmacokinetic profile of optimized FDF and marketed product.

Table A.7
Pharmacokinetic parameters following after oral administration.

PK parameters	APT-FDF	Marketed product
AUC (ng.h/ml)	5120 ± 278.42	4609 ± 234.78
Tmax (h)	2.00 ± 0.45	4.00 ± 0.47
Cmax (ng/ml)	389 ± 25.45	341 ± 17.43
AUMC (ng.h^2/ml)	59534 ± 2341.88	51672 ± 1987.54
MRT (h)	11.6278 ± 0.32	11.2111 ± 0.37

6. Conclusion

The aim of present research work was to formulate FDFs by employing the 3^2 central composite design and evaluate different formulations of fast dissolving films of anti-emetic drug aprepitant to achieve faster drug release to control in nausea and vomiting. *In vivo* studies showed significant improvement in pharmacokinetic parameters (AUC, Cmax, tmax, AUMC and MRT) and in bioavailability as compared with marketed product.

The fast dissolving films of anti-emetic drugs were found to be a better option in control of nausea and vomiting by way of fast onset of action for patient convenience and compliance.

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