Saudi Journal of Biological Sciences 29 (2022) 1283–1297

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Formulation and *in-vitro* characterization of fast-disintegrating herbal extract sublingual immunotherapy tablet for peanut-induced allergic asthma

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ARTICLE INFO

Article history:
Received 23 November 2021
Revised 6 December 2021
Accepted 12 December 2021
Available online 22 December 2021

Keywords:
Sublingual immunotherapy
Crude peanut extract
Super disintegrants
Disintegration time
Dissolution
Kinetic study

ABSTRACT

Background: Allergen immunotherapy (AIT) involves the regimen of gradually incrementing doses of the allergen, thereby inducing desensitization and tolerance. Sublingual Immunotherapy tablets (SLIT-tablets) have been formulated for several allergies and had manifested efficacy for allergic rhinitis and allergic asthma. SLIT promises an alternative method to other routes of AIT enabling patients to self-administer AIT.
Objective: The study aimed to formulate fast disintegrating SLIT containing crude peanut extract for peanut-induced allergic asthma.
Methods: The crude peanut extract was prepared by a simple extraction method and was subjected to quantitative and qualitative analysis. The extract was also characterised for its physical properties. The preformulation study for the extract and excipients of the tablet was performed using FT-IR spectroscopy and Differential scanning calorimetry. The tablet powder blends were characterised for pre-compression properties. The SLIT tablets were developed by direct compression and the post-compression evaluation was performed.
Results: The results of the quantitative and qualitative analysis of extract confirmed the presence of peanut proteins in the extract. The preformulation studies using FT-IR spectroscopy and Differential Scanning Calorimetry revealed that there is no significant interaction between the CPE and excipients. The pre-compression characterisation showed that the powder blends had good flowproperties. Three doses of SLIT tablets were formulated with each dose containing four batches and the tablet of each dose was optimized by studying the effect of varying concentrations of super disintegrants on disintegration time and dissolution rate. The post compression characterization of the tablets was performed and the

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.12.031

optimized batch of the three doses with the concentration of 5% crospovidone and 2% croscarmellose sodium showed less wetting time and high-water absorption ratio, shorter disintegration time of 14secs and maximum drug release of >90% within 2–3 min.

Conclusion: The results indicated the suitability of formulated SLIT tablets for peanut induced allergic asthma.

1. Introduction

Peanut allergy is a common type of food allergy which induces severe fatal and anaphylactic reactions. The clinical symptoms of peanut allergy vary from cutaneous manifestations to life-threatening anaphylactic reactions. The manifestations usually develop within minutes after the ingestion of very trace amount of peanut which affect the gastrointestinal, cardiovascular, and respiratory systems (Hafsa and Vidya, 2020). Also, the upper and lower respiratory symptoms may worsen in nearly fatal cases (Julie, 2019). Asthma is a common chronic disease affecting both children and adults which is ranked 28th among the primary causes of the burden of diseases. Allergic asthma is a common type of asthma which is triggered by the allergens like pollen, dust mites or food substances. The food allergens are categorised as one of the major allergens causing allergic asthma. The major food allergens are eggs, fish, peanut or groundnut, milk, wheat etc. The seeds of peanut plant *Arachis hypogaea L* is composed of an array of allergens that induces the immunoglobulin E (IgE) mediated immune reaction. Presently, 16 peanut proteins (Ara h proteins) are considered as peanut allergens. The peanut allergy is generally regarded as an IgE- mediated hypersensitivity reaction (Palladino and Breiteneder, 2018). The production of IgE specific antibody is stimulated by the initial sensitization to allergens. The ingested peanut allergens pass through the intestinal epithelium by various transport mechanisms and encounter the mast cells (Hendrik and Jennifer, 2018). When IgE antibodies that are bound to the mast cells by high affinity FcεRI receptors recognises and bind to the allergens, the crosslinking occurs. This results in the release of preformed hypersensitivity mediators and production of leukotrienes, prostaglandins, and platelet-activating factor which promotes the vasodilation and enhance vascular permeability contributing to the bowel wall swelling, mucus production, smooth muscle contraction resulting in vomiting and diarrhoea. The allergen and IgE-sensitized basophils and mast cells react when distributed systemically, resulting in anaphylactic reactions. The primary treatment of anaphylactic reaction is the intramuscular administration of epinephrine. The long-term management involves strict allergen avoidance, pharmacotherapy and the most novel approach termed allergen immunotherapy (AIT) (Fischer et al., 2018).

AIT involves the administration of slowly incrementing doses of specific allergen to the patients which alters the natural course of allergic disorders. The AIT induces two immune states: desensitization and tolerance. The daily allergen exposure increases the threshold of sensitivity to the food resulting in desensitization. Thereby, the patients develop tolerance to the food proteins during food challenge while on the treatment. The ultimate goal of AIT is to provide tolerance, the ability to consume food without allergic symptoms even after the discontinuation of therapy (Onyinyel et al., 2018).

The allergen dose exposure induces allergen-specific $CD4^+$ Treg cells which in turn produce the regulatory cytokines, interleukin-10, TGF-β, and other surface molecules that lead to the immune suppression of allergic responses. Th2 cells, basophils and eosinophils are suppressed by Treg cells which also induce allergen-specific Breg cells. These control the IgE production and favours the production of IgG from B cells. The other regulatory cells of the immune system contribute to the allergen-specific tolerance.

Sublingual immunotherapy (SLIT) is a form of AIT which involves the administration of allergen extract as drops or tablets under the tongue. Sublingual route induces allergen-specific tolerance and specifically targets oral dendritic cells to promote allergen specific immune mechanisms. The SLIT induces a local immune response in the oral mucosa and results in the shift of immune response from Th2 to Th1 response. When the allergens are delivered in the form of SLIT, the allergens are directly taken up by the Langerhans cells present in the oral mucosa. This leads to the IL-10 release promoting the T cell production of tolerogenic cytokines like TGF-β. This induces the Treg cells and downregulates the Th2 cytokines. There is a shift of immune response from Th2 to Th1 response. This inhibits the IgE response while promoting the allergen-specific production of IgG antibodies which acts as blocking antibodies. These immunological changes also lead to changes in clinical responses indicating the immune response to the specific allergens.

A decreased nonspecific basophil and mast cell reactivity is associated with SLIT. "Initially, the serum peanut-IgE levels increases and then gradually decreases whereas peanut-IgG level increases" (Cantrell et al., 2017). Currently, SLIT tablets are available in the form of freeze dried or compressed formulations of allergenic extracts. Oralair® and Grastek® are sublingual grass pollen immunotherapy tablets; in Japan, House Dust Mite (HDM) SLIT tablets (Miticure and Actair) have been available since 2015, Ragwitek was approved for the treatment of ragweed pollen allergy, Odactra was approved to treat dust mite allergy. But currently, there is no SLIT approved for peanut allergy (Saritha et al., 2020; Nurmatov et al., 2017; Moingeon et al., 2017). The FDA has not approved allergen drops of peanut for the treatment of peanut allergy. The only FDA approved allergen immunotherapy is Palforzia which is in a powder form which often presents some GI side effects and systemic reactions. Currently, tablets are the only approved form of SLIT for the treatment of allergies caused by dust mites and grass pollens. Also, the tablets will increase the tolerance and help reduce the symptoms. Hence, the tablet form of SLIT was selected for the study. This is the rationale of this study and due to the pressing need from physicians, we have undertaken this study to formulate fast disintegrating SLIT tablets to treat peanut induced allergic asthma.

A fast-disintegrating sublingual tablet (FDSTs) is a solid dosage form which disintegrates rapidly in saliva when placed beneath the tongue. Several strategies have been developed in the formulation of FDSTs to ensure the fast drug release, dissolution and drug delivery by reducing the disintegration time. In this study, lyophilization of the crude peanut extract and direct compression have been used to manufacture FDSTs containing peanut extract. Direct compression technique involves the incorporation of super-disintegrants in the formulation and utilization of water-soluble excipients to attain faster disintegration of tablet (Moote and Kim, 2011). The study aimed to formulate fast-disintegrating SLIT tablets for the treatment of peanut-induced allergic asthma (Kito et al., 2019; Ohashi-Doi et al., 2020).

2. Materials and methods

2.1. Materials

The raw peanut (local market, Kochi); Hydroxypropyl methylcellulose (HPMC) and Mannitol (Yarrow chemicals, India); Crospovidone, Croscarmellose Sodium and sodium hydroxide, Bovine serum albumin (Himedia Laboratories Pvt. Ltd, Mumbai); Magnesium stearate (Nice chemicals Ltd, Kochi); Talc (Merck, Mumbai); Ether, acetone and ethylenediamine tetraacetate (EDTA) (Research lab fine chem industries, Mumbai); Potassium dihydrogen orthophosphate (New India chemical Enterprises, Kochi); Folin's reagent, Sodium carbonate (Sigma-Aldrich Chemicals Pvt. Ltd, Bangalore); Copper sulphate (Thermo Fisher Scientific India Pvt.Ltd).

2.2. Preparation of allergen extract (Crude peanut extract) (Singh and Chandni, 2018)

The crude peanuts procured from the local market were washed, cleaned and subjected to the following processes (Fig. 1).

Grinding: The dried crude peanuts were dried and grounded to fine powder to obtain the peanut powder.

Defatting: 50 g of the peanut powder, along with acetone was taken in a conical flask in a ratio of 1:3 (g: ml). The conical flask was shaken simultaneously at 4 °C, and the upper oily layer was decanted after few minutes. This process was continued until no colour is visible. It was then filtered using Whatman filter paper No. 1, freeze-dried, and stored in a plastic container at −20 °C.

Extraction: A specific amount of the defatted peanut powder was extracted with an equal amount of alkaline buffer in a pH ranging from 6.4 to 8.6 in an Erlenmeyer flask to evaluate the effect of pH on the yield of the product obtained. Protease inhibitors such as Ethylene diamine tetraacetate (EDTA) was also added to the buffer mixture to aid the extraction process. It was then subjected to continuous agitation at 4 °C for 8 to 20 h (Koppelman, 2018).

Centrifugation: The extracted sample was centrifuged at 10,000 rpm for about 30 min at a temperature of 4 °C to separate the soluble ingredients.

Purification: The supernatant obtained from centrifugation was filtered using Whatman filter paper to obtain any product residue.

Lyophilization: The obtained crude peanut extract was lyophilized under vacuum of 50–100 microbar (freeze dry system) (FreeZone 2.5 Liter, Labconco) and stored at −60 °C to −70 °C.

2.3. Standardization of the extract

2.3.1. Qualitative analysis

The qualitative tests such as Biuret test, Xanthoproteic test, Ninhydrin test and Millon's test were performed to standardize the crude peanut extract. The UV absorption was evaluated using a UV-double beam spectrophotometer, UV-1700, Shimadzu (Kyoto, Japan). The samples were uniformly mixed at 0.1 mg/mL in the corresponding extraction buffer and then scanned from 240 nm to 600 nm at 25 °C. The FT-IR analysis of the obtained crude peanut extract and all the excipients was done by Shimadzu (Kyoto, Japan), to identify the specific functional groups of the drug. LC (Agilent 1290 series, Agilent Technologies, Palo Alto, CA) was performed by injecting 5.0 μL of the obtained peanut extract onto a 0.32 × 150 mm Symmetry300 C18 5 μm particle size column (Waters, Bedford, MA) at a flow rate of 0.3 ml/min. A 0–50% acetonitrile with 0.5% acetic acid gradient was used for the separation. Mass peaks of the allergens were detected using Mass spectrometer (Agilent 6460 QQQ) in positive ionization mode at mass range scanned from 200 to 2000amu. The run time is about 40 min.

2.3.2. Quantitative analysis

The protein quantification was performed using UV–Vis spectrophotometric technique using the standard Lowry's method with

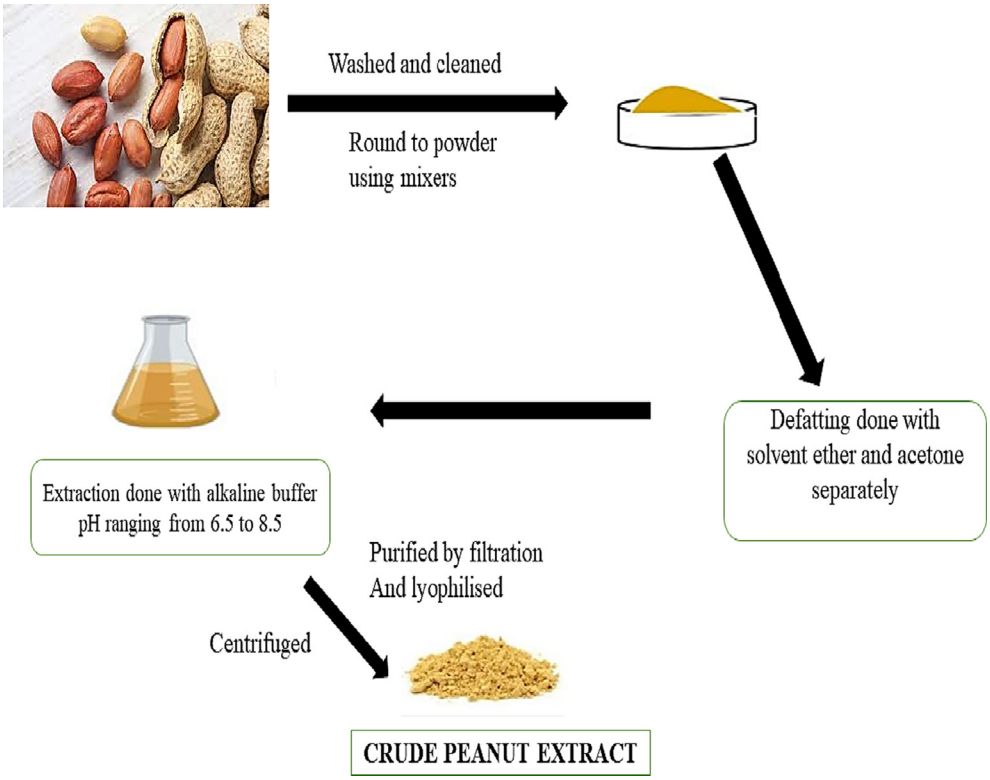

Fig. 1. Method of preparation of crude peanut extract.

Bovine Serum Albumin (BSA) as standard. Distilled water was used as blank. To the test tubes containing different standard dilutions and sample extract, 4.5 ml of Reagent A (50 ml of 2% sodium carbonate in 0.1 N sodium hydroxide + 1 ml of 0.5% copper sulphate solution) was incubated for 15 min. Then, 0.5 ml of Reagent B (Folin's reagent + distilled water in the ratio 1:2) was added and left for 30 min for incubation. The absorbance was measured at 660 nm and the protein concentration was estimated from the standard graph (Sudipta et al., 2020).

2.4. Characterization of the extract

2.4.1. Physical characterisation

(a) Wettability

A weighed amount of powder (1 g) was kept on a glass slide, which was placed above distilled water in a small container at 25 °C. The powder was allowed to fall on the water surface by quickly removing the glass slide. The time required for particles to wet was noted. The wettability was calculated as:

$$w = m/t$$

w is wettability in g s^{-1}; m is the mass of sample in g; t: time in s.

(b) Hygroscopicity

The accurately weighed powder sample (±1 g) was transferred in to a container with sodium chloride saturated solution at 25 °C for a week, followed by further reweighing of the powder. Hygroscopicity was calculated as:

$$H = A/(B * a) \times 100$$

H is hygroscopicity in %; A is absorbed water mass in g; B is water content of the powder in g g-1; a is the mass of the sample in g.

(c) Apparent density

About 10 g of the peanut extract was transferred into a 10 ml measuring cylinder without tapping. The apparent density was determined as,

$$\rho a = M/Vt$$

ρa is apparent density in g mL^{-1}; M is the mass of the solid in g; Vt is total volume in mL.

(d) Compact density

A 10 ml graduated cylinder was filled with a weighed mass of peanut extract. The graduated cylinder was tapped 50 times and the compact density was calculated as,

$$\rho c = m/Vc$$

ρc is compact density in g mL^{-1}; m is the mass of the solid in g; Vc is volume of the solid after compaction in mL.

(e) Carr's index

Carr's index (CI) is calculated with the equation:

$$CI = (\rho c - \rho a)/\rho c \times 100$$

(f) Hausner ratio

The Hausner ratio (HR) was determined using the equation:

$$HF = \rho c/\rho a.$$

2.4.2. The identification of peanut allergen extract was done by performing organoleptic evaluation, determination of melting point and partition coefficient, solubility studies and stability studies.

(a) Organoleptic valuation

Organoleptic characters like color, odor, and state of the drug were evaluated by visual assessment and recorded using descriptive terminology.

(b) Melting point determination

The melting point of the compound designates the pureness of the sample. The presence of a relatively small amount of impurity can lower the melting point. The melting point of peanut extract was determined using Dynamic light scattering (DLS) method.

(c) Partition coefficient

10 mg of drug was taken into a standard flask with 30 ml of n-octanol & pH 5.5 buffer. Then the funnel was equilibrated for 1hr with continuous shaking. The aqueous layer was separated, 2 ml of this solution was pipetted and volume was made up to 10 ml with pH 5.5 buffer solution. The concentration of solute in aqueous solution was assessed by UV spectroscopy at 294 nm, and log p was calculated using the equation.

$$\text{Partition coefficient} = \frac{\text{concentration of drug in organic solvent}}{\text{concentration of drug in aqueous solvent}}$$

(d) Solubility studies

The solubility studies of crude peanut extract were carried out in different solvents like methanol, ethanol, acetonitrile, DMSO, and water. A saturated solution was made by adding an increased amount of pure CPE in 1 ml of ethanol, methanol, acetonitrile, DMSO, and water as solvents. The drug was solubilized into the solvent by shaking. The addition was continued until the part of the solid was left undissolved attaining saturation. The clarity and presence of undissolved particles were analyzed.

2.5. Preformulation studies

2.5.1. Drug-polymer interaction studies

(a) Fourier transform infrared (FT-IR) spectrophotometric study

The infrared (IR) spectrum of the drug (CPE) and the drug-excipient mixture was recorded on FT-IR Spectrophotometer (Shimadzu, Kyoto, Japan) by using 1–2% of the finely grounded powder sample to study any drug-excipient interaction.

(b) Differential scanning calorimetry (DSC)

The DSC studies of pure drug (CPE) and the physical mixture of drug-excipients were carried out using NETZSCH DSC 204F1 Phoenix instrument. The samples were heated at temperature ranging from 20 °C to 300 °C at a heating rate of 10 °C/min in an inert nitrogen atmosphere.

2.6. Formulation and optimisation of SLIT tablets

The sublingual immunotherapy tablets containing peanut extract were developed by direct compression according to the

Table 2. SLIT mainly comprises of an escalation dose and maintenance dose. In this study four batches of immunotherapy tablets were prepared in three varying doses. Mannitol is used as the diluent/filler in the formulation. Mannitol is non-hygroscopic in nature and hence it is of great importance as the peanut protein is hygroscopic in nature. Also, mannitol is widely used in rapidly disintegrating dosage forms because it reduces the disintegration time due to high solubility than other excipients. As there are three doses of the CPE or active ingredient, the amount of mannitol used in the formulation is higher and it changes accordingly.

The crospovidone and croscarmellose sodium were selected as super disintegrants in the formulation. The concentration of super disintegrants was varied for each batch of three doses of sublingual immunotherapy tablets. The CPE and the excipients were precisely weighed, gently blended and were passed through 60# mesh size. Finally, the weighed amount of lubricant and glidant were added and mixed thoroughly and compressed using multi station rotary tablet compression machine (Cadmach, D8, Ahmedabad) to obtain SLIT tablets.

The fast-disintegrating sublingual tablet was optimized by a custom design approach, which allows to build smart designs more quickly and to balance the need to maximize the information that could be gathered from the experiment whilst minimizing resources and time. Custom design with 2 factors, 2-levels and 8 runs were selected for the optimization study using JMP version 16 Pro. The concentration of Crospovidone and Croscarmellose were chosen as independent variables and they were set at low (−1) and high (+1) based on the preliminary trials. which resulted in repetition of runs. The concentration of croscarmellose sodium used in the tablet formulation ranges from 0.5% to 5.5% w/w. In the direct compression, croscarmellose sodium is used at a concentration of 2% w/w. Crospovidone is commonly used in tablet formulations at a concentration range of 2% − 5%. Hence, the lowest concentration of 2% and highest concentration of 5% were used for the study (Table 1). The formulation chart of fast disintegrating SLIT tablets as per custom designed is described in Table 3. In the current design, it was aimed to minimize the disintegration time and maximize the dissolution time in 2 min (see Table 4).

2.7. Pre-compression evaluation (Sem et al., 2018)

(a) Angle of Repose

About 10 g of powder was passed through a funnel, the height of which was adjusted so that the tip of the funnel was placed 2 cm above the hard surface. The angle of repose was calculated by,

$$\theta = \tan^{-1} h/r$$

Where h is height of the heap; r is radius of the base of the heap, θ is angle of repose.

(b) Bulk and tapped density

10 g of each powder formulation was placed into 100 ml measuring cylinder. The initial volume was noted. The cylinder was then tapped 100 times on a hard surface at an interval of one second. The tapped volume was noted. The bulk and tapped density were calculated from the obtained bulk and tapped volume respectively by dividing the mass to the corresponding volumes.

Table 1
Variables and their levels in Custom design of Fast disintegrating SLIT tablets.

Independent variables	Levels	
	−1	1
Concentration of Croscarmellose (%w/v)	0	2
Concentration of Crospovidone (HMW, % w/v)	2	5
Dependent Variable		
Disintegration time(sec)	Minimum	
Dissolution (% drug release) in 2 min	Maximum	

(c) Hausner's ratio and Carr's index

Hausner's ratio was calculated by dividing the tapped density by bulk density and Carr's index (compressibility index) was calculated from the equation,

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

2.8. Post compression evaluation

The developed SLIT tablets were evaluated for its characteristics (Hamadet al., 2021).

2.8.1. Hardness and thickness

The hardness of minimum 3 tablets from each batch were measured using Monsanto hardness tester (Sentwin India) and the thickness of tablets was evaluated using a Vernier caliper.

2.8.2. Friability

About twenty tablets were weighed collectively and placed in Roche friabilator apparatus (Veego instruments corporation Mumbai, VPT-1D) and the tablets were subjected to 100 revolutions at 25 rpm. Then the tablets were reweighed. The friability was determined as,

$$\text{Percentageloss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

2.8.3. Weight variation

Twenty tablets from each batch were randomly selected and weighed individually to ensure the weight uniformity. The average weight and percentage variation of weight were calculated.

2.8.4. Wetting time

A tissue paper was double folded and placed in a petri dish containing about 6 ml of distilled water and three tablets from each batch were placed individually at the center of paper. The time taken for complete wetting of the tablet was determined as wetting time (Bhabani et al., 2017).

2.8.5. Water absorption ratio

A tissue paper which was double folded and wet was placed in a petri dish and a tablet from each batch was placed on the center of the paper. The completely wet tablet was removed and reweighed (Yadav et al., 2020).

2.8.6. In-vitro disintegration time

The *in vitro* disintegration time of 6 tablets was determined in the tablet disintegration tester (Electrolab). The disintegration test was performed using simulated saliva (pH 6.8) as the disintegration medium at 37 ± 0.5 °C and the disintegration time was represented as an average of six determinations (Ali et al., 2019).

2.8.7. Drug content uniformity

6 tablets from each batch were individually crushed in a mortar and transferred into 100 ml volumetric flask and the volume was made up with phosphate buffer (pH 6.8) and sonicated for 10 min. Then 1 ml from the above solution was pipetted out and

Table 2
Formulation of fast disintegrating SLIT tablets containing CPE.

Formula code	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)	F7 (mg)	F8 (mg)	F9 (mg)	F10 (mg)	F11 (mg)	F12 (mg)
Drug (CPE)	4.8	4.8	4.8	4.8	7.2	7.2	7.2	7.2	9.6	9.6	9.6	9.6
Mannitol	127.38	137.88	131.88	130.2	120.3	130.8	124.8	127.8	117.9	128.4	122.4	125.4
HPMC	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Croscarmellose sodium	3	–	3	–	3	–	3	–	3	–	3	–
Crospovidone	7.5	7.5	3	3	7.5	7.5	3	3	7.5	7.5	3	3
Magnesium stearate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Talc	3	3	3	3	3	3	3	3	3	3	3	3

Table 3
Formulation chart of fast disintegrating SLIT tablets as per custom design.

Run	Croscarmellose (%)	Cross povidone (%)
1	0	5
2	2	5
3	0	2
4	0	5
5	2	5
6	2	2
7	0	2
8	2	2

Table 4
Physical characteristics of peanut extract.

Sl. No.	Analysis done	Obtained values ± S.D
a)	wettability (g s^{-1})	0.006 ± 0.002
b)	hygroscopicity (%)	5.643 ± 0.03
c)	apparent density (g mL^{-1})	0.362 ± 0.02
d)	compact density (g mL^{-1})	0.437 ± 0.02
e)	Carr's index (%)	17.162 ± 0.06
f)	Hausner ratio	1.207 ± 0.03

filtered through a 0.45um filter paper. The absorbance of the solution was determined spectrophotometrically using a UV/Vis double beam spectrophotometer (Shimadzu, Tokyo, Japan) at 276.5 nm and the drug content was determined from the calibration graph of the peanut extract.

2.8.8. In-vitro dissolution studies

The *In-vitro* dissolution study of three SLIT tablets from each batch was conducted using a customized dissolution apparatus. The dissolution medium used was simulated saliva of pH 6.8 (35 ml) at 50 rpm by maintaining the temperature at 37 ± 0.5 °C. About 5 ml of samples were withdrawn at intervals of 1, 2, 3, 4, and 5 mins which was subsequently replaced with 5 ml of simulated saliva. The obtained samples were filtered and the absorbance was measured at 276.5 nm. The dissolution data was fitted to various mathematical models.

2.9. Stability study

The optimized formulations F1, F5 and F9 were stored in an air tight container at room temperature and refrigerated condition (2–8 °C) for 3 months. The samples were withdrawn to determine the organoleptic properties, drug content and in-vitro dissolution profile.

3. Results

3.1. Preparation of crude peanut extract

The crude peanut extract was prepared by a simple extraction method using buffer of pH 8.6. The yield of product obtained using buffers of different pH is depicted in Fig. 2. The percentage yield of peanut extract obtained was 84.1%.

3.2. Standardization of the extract

The reaction of peanut extract with copper (II) ions resulted in the formation of a violet-coloured complex called biuret. The peanut extract yielded a yellow-coloured substance which was due to the formation of xanthoproteic acid by the nitration of specific amino acids present in the protein, such as tyrosine and tryptophan. An intense purple-blue coloured complex was formed upon the reaction of peanut extract and the reagent. The mixture was first observed as a white coloured precipitate, which then changed to brick red on boiling. The λ_{max} of the prepared peanut extract was found to be 276.5 nm.

The FT-IR spectra of the prepared peanut extract obtained indicated the presence of amide groups. The presence of amide functional groups in the obtained peanut extract confirms the presence of proteins. The FT-IR of the extract is depicted in Fig. 3.

The prepared peanut extract was subjected to LC-MS/MS scanning in order to identify the molecular mass of the proteins present. The LC-MS/MS spectrum contributes to the confirmation that the CPE sample contains the allergen. The LC-MS/MS spectra of crude protein extract is given in the Fig. 4.

Estimation of total protein content was performed by Lowry's method. The total amount of protein present in the crude peanut extract (CPE) was found to be 208.86 µg/ml. Therefore, 100 mg of CPE contains approximately 20.8 mg protein.

3.3. Characterization of the extract

The physical characterization of the prepared peanut (with skin) extract was performed, and data obtained after three replicates ± standard deviation.

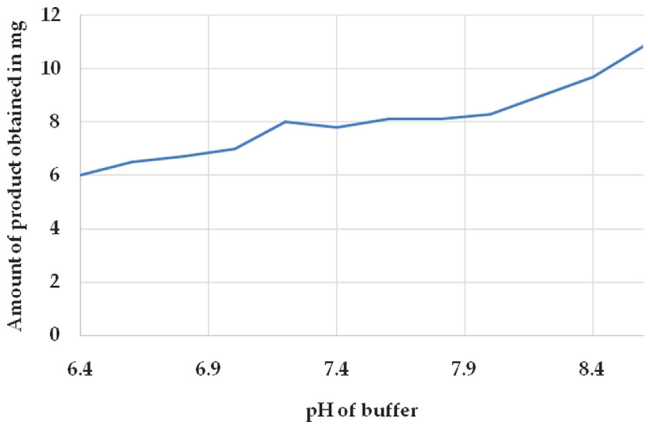

Fig. 2. Effect of pH on product obtained.

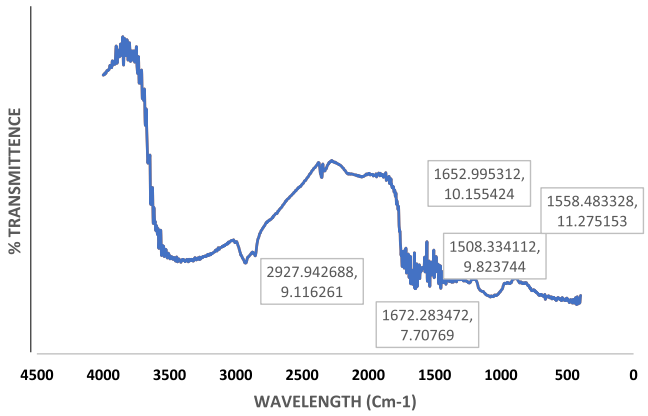

Fig. 3. FT-IR of the prepared peanut extract.

3.4. Preformulation studies

3.4.1. Identification of the peanut allergen extract

The lyophilized peanut extract was found to be off white to light brown in color, odorless, powder and solid in nature. The melting point and partition coefficient of the peanut extract was found to be 164 °C and 0.7. The peanut extract was slightly soluble in ethanol, moderately soluble in water, and insoluble in DMSO and acetonitrile.

3.4.2. Drug-polymer interaction studies

Fourier transform infrared (FT-IR) spectrophotometric study

The FT-IR data indicates that there is no significant interaction between the CPE and physical mixture (CPE-excipient) as presented in Fig. 5. The FT-IR of crude peanut extract (CPE) exhibited peaks at 1600–1700 cm^{-1}, 1500–1600 cm^{-1} and 1674.21 cm^{-1} indicating the presence of major peanut proteins in the sample.

Differential Scanning Calorimetric study (DSC)

The DSC was performed to study the compatibility of CPE and the excipients used. In this study, the CPE and the physical mixture were evaluated. The DSC thermograms of CPE and physical mixture exhibited a pointed endothermic peak at 165.8 °C and 169.4 °C respectively. The DSC thermograms are depicted in the Fig. 6 and Fig. 7.

3.5. X-Ray diffraction study (XRD)

The XRD diffractograms of the CPE and the formulation is presented in Figs. 8 and 9. The CPE showed the characteristic sharp peaks at 2θ angle, namely, 15.288, 19.940, 21.508, 22.519, 30.357 and 33.998° indicating the crystalline nature of CPE (see Fig. 10).

3.6. Precompression evaluation

The directly compressible tablet blends were evaluated to determine the flowability and compressibility. The results are depicted in Table 5. The directly compressible tablet blends were evaluated to determine the flowability and compressibility.

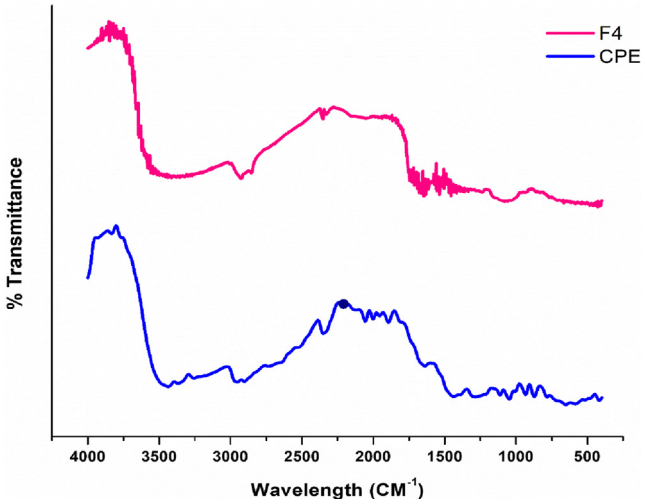

Fig. 5. FT-IR of CPE and CPE-excipients.

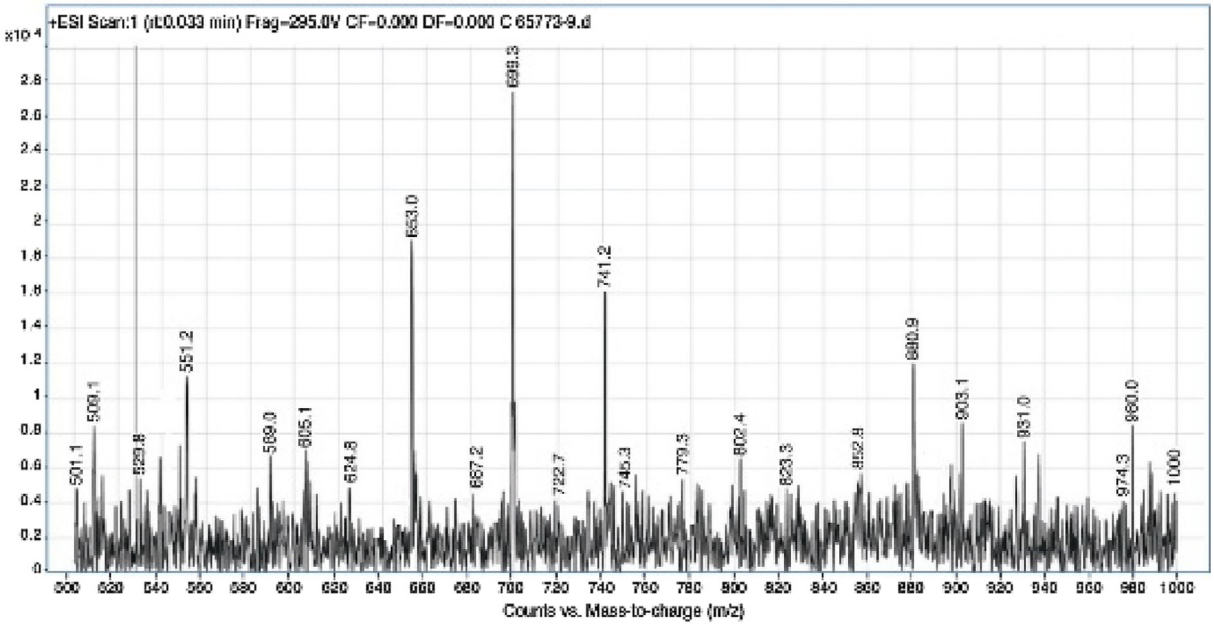

Fig. 4. LC-MS/MS spectra of CPE.

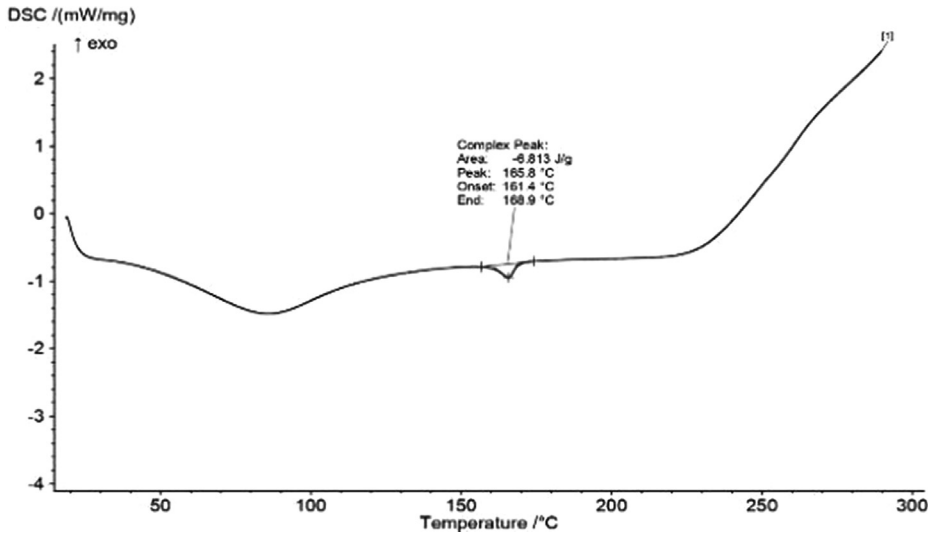

Fig. 6. DSC thermogram of CPE.

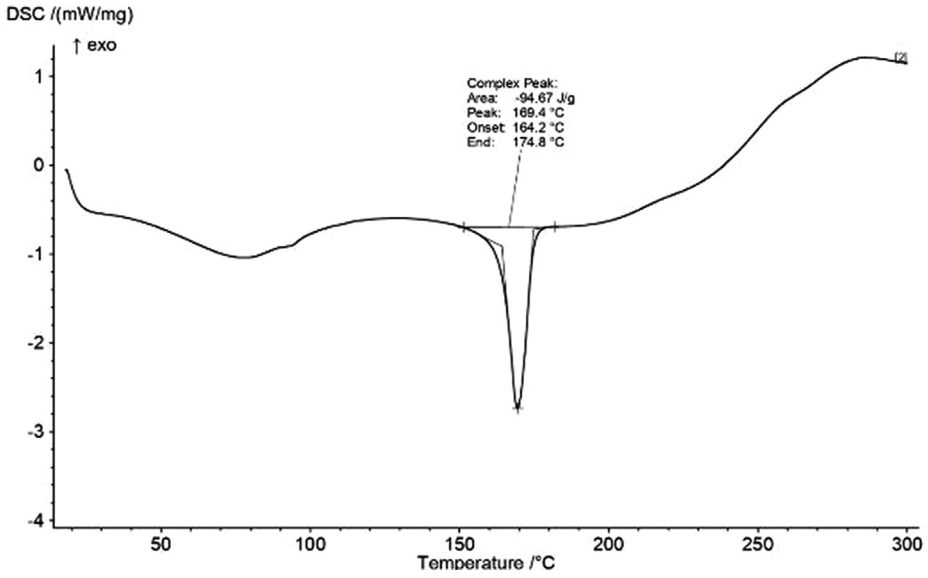

Fig. 7. DSC thermogram of physical mixture.

3.7. Post compression evaluation

The post compression evaluation was performed and the results are presented in Table 6. All the formulations gave appreciable results and the values were observed to be within the limits.

The drug content of formulation F1 to F12 ranged between 76.1 ± 0.57% to89.7 ± 0.12%. The drug content of formulations F1, F5 and F9 were found to be 89.7 ± 0.12%, 85.7 ± 1.74% and 86 ± 0.63%, respectively. The F1 of first dose, F5 of second dose and F9 of the third dose showed shorter disintegration time and higher drug release, F1, F5 and F9 were selected as the optimized batches for three doses respectively.

The *in-vitro* dissolution profile of optimized fast disintegrating sublingual tablets exhibited a rapid drug release of more than 90% within 3 min as given in the Fig. 11. The *in vitro* drug release of optimized batches of three doses of formulation named F1, F5 and F9 was found to be 94.1 ± 1.18%, 93.4 ± 0.82% and 94.9 ± 0.2 5%, respectively within 3 min.

3.8. Drug release kinetics study

The drug release kinetic study was conducted by employing various mathematical models (Radhakant Gouda et al., 2017; Constantin Mircioiu et al., 2019). The regression coefficient of the various drug release kinetic models of the optimized formulations F1, F5 and F9 are shown in Fig. 12 and the calculated parameters are depicted in Table 7 (see Table 8, Fig. 13).

3.9. Design evaluation

The responses from the study for the selected factors were incorporated into experimental design and the model was evaluated for its suitability of fit. The actual verses prediction plot for the responses of disintegration time and dissolution (% drug release) in 2 h is represented in Figs. 14 and 15 respectively.

According to the predictive model's disintegration time ($p < 0.0001$=, $R^2 = 0.99815$) and dissolution % ($p = 0.0005$ = ,

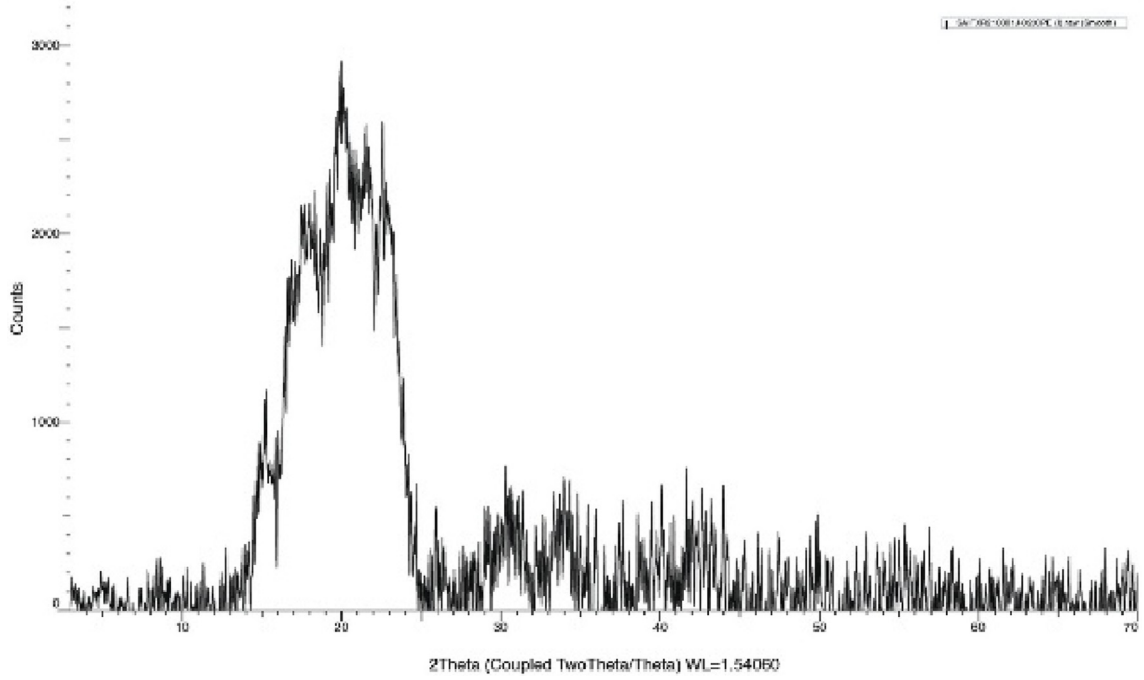

Fig. 8. XRD diffractogram of CPE.

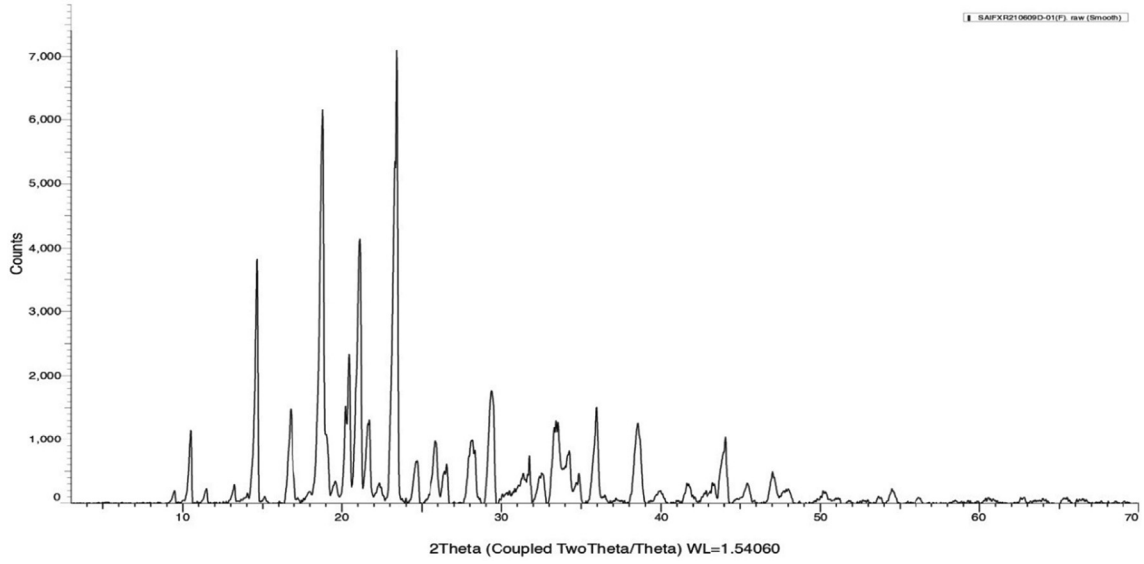

Fig. 9. XRD diffractogram of formulation.

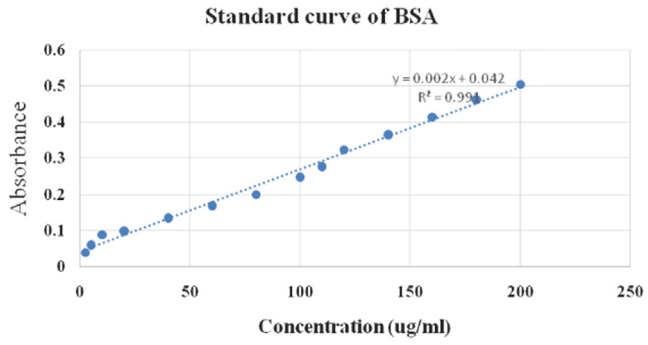

Fig. 10. Standard curve of BSA.

R^2 = 0.95), it assures the rationality of the model, where the data points were found to be almost linear for disintegration time and dissolution %. The maximum desirability for was observed at (0.6703) (Fig. 16) at a Croscarmellose of 2 %, and Crospovidone 5% the predicted disintegration time (14.075 sec) was and the dissolution (94%).

3.10. Stability studies

The selected formulations were kept at refrigerated temperature (2–8 °C) and room temperature for 3 months. After 3 months, the selected formulations were assessed for its physical appearance, tablet characteristics and the drug release. The physical appearance of the formulations that were stored at room temperature were not good, as it had moisture content which may be

Table 5
Pre-compression parameters.

Formula code	Angle of repose (θ)	Bulk density (g/ml)	Tapped density (g/ml)	Hausner's ratio	Carr's index
F1	35.7 ± 0.56	0.11 ± 0.004	0.13 ± 0.007	1.1 ± 0.003	15.3 ± 0.42
F2	39.8 ± 0.68	0.14 ± 0.08	0.18 ± 0.18	1.2 ± 0.015	22.2 ± 0.08
F3	41 ± 0.07	0.15 ± 0.005	0.20 ± 0.02	1.3 ± 0.009	25 ± 0.14
F4	43 ± 0.09	0.18 ± 0.07	0.23 ± 0.07	1.2 ± 0.08	25 ± 0.03
F5	36.4 ± 0.18	0.13 ± 0.13	0.17 ± 0.13	1.3 ± 0.012	23.5 ± 0.121
F6	36 ± 0.12	0.14 ± 0.01	0.18 ± 0.001	1.2 ± 0.007	22.2 ± 0.005
F7	38.9 ± 0.018	0.12 ± 0.015	0.16 ± 0.05	1.3 ± 0.14	25 ± 0.13
F8	40 ± 0.08	0.16 ± 0.06	0.20 ± 0.13	1.3 ± 0.06	25 ± 0.08
F9	36.5 ± 0.15	0.15 ± 0.03	0.19 ± 0.075	1.26 ± 0.194	21.05 ± 0.04
F10	39.2 ± 0.04	0.13 ± 0.008	0.17 ± 0.17	1.3 ± 0.047	23.5 ± 0.194
F11	42.1 ± 0.07	0.14 ± 0.02	0.19 ± 0.03	1.3 ± 0.017	24.3 ± 0.04
F12	42.7 ± 0.12	0.16 ± 0.08	0.18 ± 0.07	1.1 ± 0.14	25.7 ± 0.14

Table 6
Post compression parameters.

Formula code	Average weight (mg)	Thickness (mm)	Hardness Kg/cm^2	Friability (%)	Wetting time (secs)	Water absorption ratio (%)	Drug content (%)	*In-Vitro* disintegration time (secs)
F1	144.6 ± 0.24	2.56 ± 0.17	3.5 ± 0.01	0.73 ± 0.12	44 ± 1.24	50.6 ± 0.11	89.7 ± 0.12	14.5 ± 0.4
F2	143.79 ± 0.12	2.66 ± 0.08	3.78 ± 0.09	0.74 ± 0.65	43 ± 0.57	46.7 ± 2.31	77.1 ± 0.49	25.7 ± 1.1
F3	145.89 ± 0.19	2.13 ± 0.12	3.2 ± 0.07	0.7 ± 1.13	57 ± 2.14	44.8 ± 1.64	81.5 ± 0.26	29.4 ± 0.75
F4	143.17 ± 0.07	2.82 ± 0.14	3.4 ± 0.16	0.6 ± 0.12	61 ± 0.19	39 ± 0.17	82.3 ± 1.34	42.3 ± 0.08
F5	142.96 ± 0.11	2.1 ± 0.03	3.7 ± 0.11	0.7 ± 0.04	42 ± 3.1	49.5 ± 0.26	85.7 ± 1.74	15 ± 1.35
F6	144.45 ± 0.09	2.15 ± 0.13	3.5 ± 0.19	0.6 ± 0.07	45.5 ± 0.09	47.1 ± 1.57	77.5 ± 0.49	26.3 ± 2.1
F7	144 ± 0.14	1.75 ± 0.09	2.9 ± 0.14	0.3 ± 1.02	57 ± 0.57	40.8 ± 0.25	79.2 ± 1.08	37.1 ± 1.5
F8	143.01 ± 0.13	1.7 ± 0.19	2.6 ± 0.07	0.7 ± 0.01	64 ± 0.18	35 ± 0.02	78.8 ± 0.42	43 ± 0.05
F9	146.24 ± 0.17	1.53 ± 0.15	2.5 ± 0.17	0.75 ± 0.02	47 ± 2.7	49.3 ± 0.76	86.2 ± 0.63	16.2 ± 2.3
F10	144.79 ± 0.02	1.75 ± 0.17	2.75 ± 0.08	0.56 ± 0.15	52 ± 1.37	47.6 ± 1.78	81.6 ± 0.29	26.9 ± 1.7
F11	143.83 ± 0.06	1.12 ± 0.04	2.5 ± 0.1	0.73 ± 0.06	56 ± 1.55	45.5 ± 2.05	76.1 ± 0.57	39.3 ± 0.5
F12	144 ± 0.18	1.8 ± 0.09	2.64 ± 0.04	0.54 ± 0.16	63.1 ± 0.19	38 ± 0.07	81.4 ± 1.03	46.7 ± 0.27

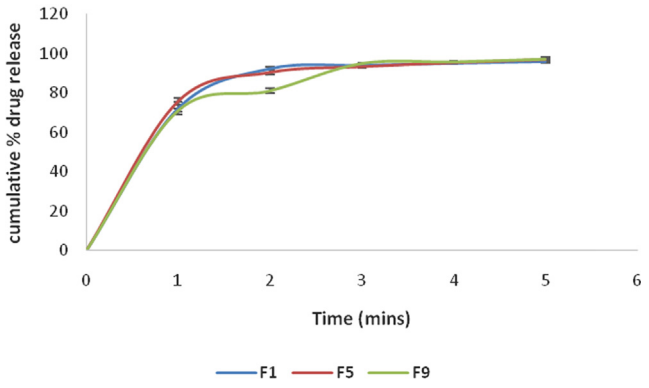

Fig. 11. Dissolution profile of SLIT tablets.

attributable to the high mannitol content. So, they were not selected for further studies. The formulations stored at refrigerated condition were appealing in its physical appearance and the *in-vitro* drug release study revealed that there is no remarkable difference in thedrug release pattern. The stability study of the optimized batches of F1, F5 and F9 showed a drug content of 87. 7 ± 0.19%, 84.2 ± 0.74% and 84.5 ± 0.68%, respectively which indicates there is no significant loss of drug during storage. The *in-Vitro* dissolution profiles of F1, F5 and F9 resulted in 92.4 ± 5.58% , 90.1 ± 1.4% and 93.5 ± 1.24%, respectively within 3 min. Hence these formulations were considered as stable formulations.

4. Discussion

4.1. Standardization of the extract

The crude peanut extract was prepared by a simple extraction method using buffer of pH 8.6. The oral mucosa is the primary site of interaction between the immune cells and allergens. Hence, it is necessary to extract the proteins in a pH that mimics the salivary pH conditions and thus the alkaline buffer was selected for the extraction to mimic the salivary pH. The peanut proteins exhibited poor solubility in the pH range of 3 to 6, while its solubility increased at very low pH of 1.5 and pH > 8. The release of peanut protein was found to be more within the pH range of 6.4 to 8.6. Hence, the alkaline buffer with a pH range of 6.4 to 8.6 was selected for extraction.

The alkaline borate buffer of pH 8.6 yielded the maximum product weighing 10.85 g after lyophilisation at a temperature of 4 ± 2 °C. Typically, 100 g of peanut contains 25.80 g of proteins. In the procedure, 50 g of peanut powder was used for extraction. Therefore, the theoretical yield obtained should be 12.9 g. The practical yield obtained was 10.85 g. Hence, the percentage yield of peanut extract obtained was 84.1%.

The UV absorption spectra of the prepared peanut extract were obtained. The spectral region between 240 nm and 600 nm was used for analysis. Lambda max (λmax) refers to the wavelength along the absorption spectrum, where a substance has its most substantial photon absorption. Therefore, λmax of the prepared peanut extract was found to be 276.5 nm.

The FT-IR spectra of the prepared peanut extract obtained indicated the presence of amide groups. The proteins in the peanut are presented as allergens that cause allergic reactions. The presence of amide functional groups in the obtained peanut extract confirms the presence of proteins.

The FT-IR spectra of the prepared peanut extract obtained indicated the presence of amide groups:

1. The amide I region (1600–1700 cm^{-1}) corresponding to the C = 0 stretch weakly coupled with C-N stretch and N-H bending.
2. The amide II region (1500–1600 cm^{-1}) representing C-N stretch strongly coupled with N - H bending.

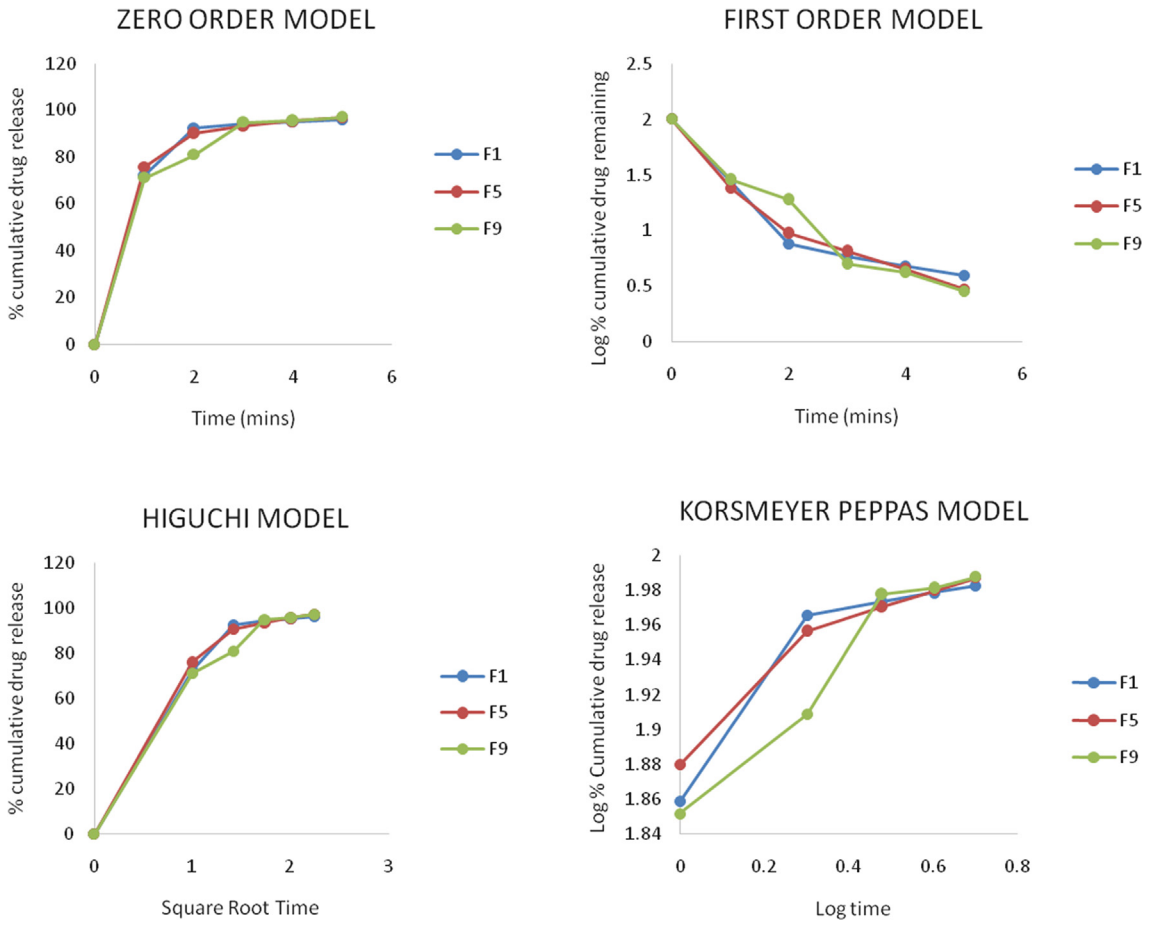

Fig. 12. Graph of kinetic studies.

Table 7
Drug release kinetic models.

Formulations	Zero order ($Q_t = Q_0 + K_0t$)	First Order ($\ln Q_t = \ln Q_0 + K_1t$)	Higuchi ($Q_t = K_Ht^{1/2}$)	Peppas ($Q_t/Q_{co} = K_K t^n$)	
	R^2	R^2	R^2	R^2	n
F1	0.6056	0.8411	0.8618	0.8116	0.1707
F5	0.6006	0.9102	0.8583	0.9106	0.1496
F9	0.6732	0.9421	0.9057	0.9429	0.2087

Table 8
Predicted and experimental optimized formulation.

Factors		Model		Experiment		PDI
Concentration of Croscarmellose (%)	Concentration of Crospovidone (%)	Disintegration time(sec)	Dissolution (% drug release) in 2 h	Disintegration time(sec)	Dissolution (% drug release) in 2 h	
2	5	14.075	93.69	14.5	94%	0.67

Fig. 13. Data indicating p value.

3. The amide III region (1200–1350 cm^{-1}) is N-H in-plane bending coupled with C-N stretching and also includes C-H and N-H deformation vibrations.

In LC-MS study, complex protein mixture was first subjected to enzymatic cleavage, and then the resultant peptide products were analysed using a mass spectrometer. The quantitative bioanalysis for proteins is complicated when compared with small molecules, because of their structural complexity. The molecular ion peak showed a molecular mass of 699.3 Da and 605.1 which are similar to those observed in the Ara h1 standard (Kevin et al., 2006). Several product ions of the Ara h proteins such as *m*/*z* 687.2, *m*/*z* 722.7, *m*/*z* 745.3, *m*/*z* 779.3, *m*/*z* 852.8, and *m*/*z* 974.3 were identified in MS/MS spectrum of CPE. The peptide ions like *m*/*z* 551, *m*/*z* 605, and *m*/*z* 678 corresponding to Ara h2 proteins were also present. Other product ions and fragment ions ranging from *m*/*z* 500 to *m*/*z* 1100 resembling the molecular ion peak reported in the literature (Shefcheck and Musser, 2004). The presence of small addi-

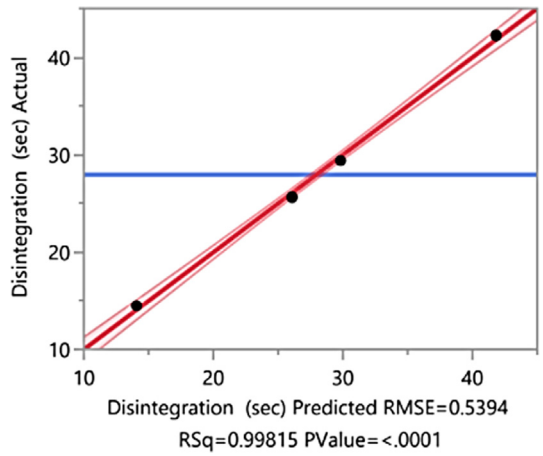

Fig. 14. Response disintegration in secs (Actual by predicted plot).

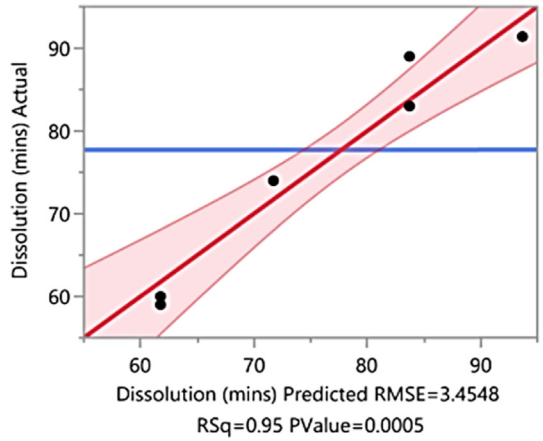

Fig. 15. Response dissolution in mins (Actual by predicted plot).

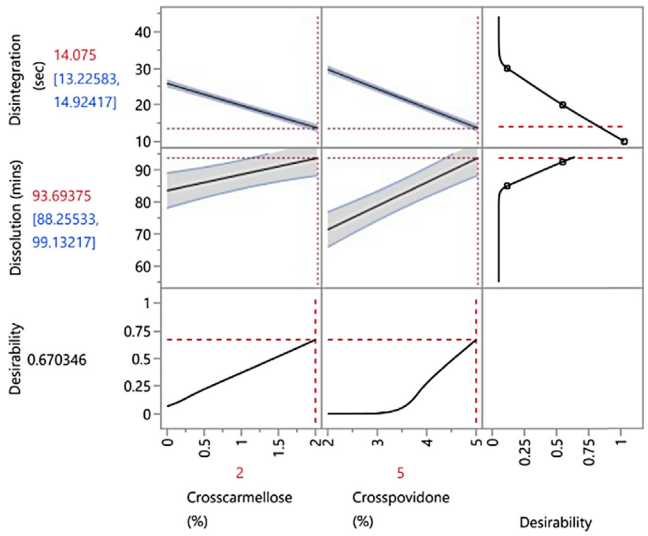

Fig. 16. Prediction profiler.

tional peaks confirmed the possibility of fragmentation and isoform formation. Thus, this LC-MS/MS spectrum gives the affirmation that the sample of CPE contains allergen. The disturbance and irregularity in the spectrum may be attributed to the analysis of the crude peanut extract rather than a single isolated protein extract.

Lowry's test

Lowry's method is the most widely accepted procedure when it comes to determine the amount of protein present in any biological sample (either already in solution or easily soluble in dilute alkali). The main perception behind the Lowry method of determining protein concentrations is based on the reaction between the peptide nitrogen and the copper [II] ions under alkaline environment, followed by reduction reaction of the FolinCiocalteau phosphomolybdic and phosphotungstic acid that turns into heteropoly molybdenum blue.

From the standard graph, r^2 value was found to 0.9956. The total amount of protein present in the crude peanut extract (CPE) was found to be 208.86 μg/ml. So, 1 ml contains 208.85 μg or 0.208 mg protein. Therefore, 100 mg of CPE contains approximately 20.8 mg protein, which is largely enough to induce allergic reactions.

4.2. Characterization of the extract

The physical characterization of the prepared peanut (with skin) extract was performed, and data obtained after three replicates ± standard deviation. The wettability was found to be good with the peanut extract with skin which may be due to the fact that the particles of instantaneous powder get wetted faster than normal powder. The peanut extract exhibit hygroscopicity values ranging from 5.643 %. The peanut powder extracts are non-hygroscopic, which may be attributable to the microstructural features of the material, which in turn aids an elevated lifetime to the product.

The apparent density and the mean value of compact density of the peanut extract was found to be 0.362 g mL^{-1} and 0.437 g mL^{-1} respectively. The CI and HR values are indication of the flow property of powders. The CI values ranging from 15 and 20% reveals good fluidity of powder indicating that the peanut extract exhibits good fluidity. The HR of the extract was found to be 1.207. The powders with Hausner ratio ranging from 1.2 and 1.4 indicate intermediate cohesiveness. Thus, the extract showed intermediate cohesiveness, which could be advanced by incorporating suitable adjuvants.

4.3. Preformulation studies

4.3.1. Identification of the peanut allergen extract

The lyophilized peanut extract was found to be off white to light brown in color, odorless, powder and solid in nature. The protein's melting point (TM) is defined as the temperature at which the protein denaturation occurs. The melting point of the peanut extract was found to be 164 °C. The partition coefficient of peanut extract was found to be Log Po/w = 0.7, indicating that the extract is hydrophilic. If the P value > 1, then the compound is said to be lipophilic and the P value < 1, then the compound is hydrophilic. From the result, it is confirmed that the compound is hydrophilic. The peanut extract was slightly soluble in ethanol, moderately soluble in water, and insoluble in DMSO and acetonitrile. The solubility highly depends on the pH and isoelectric point of the solvent used. A methanol-water system showed a higher solubility than the plain methanol solvent. The lyophilized form of peanut extract showed more stability both in room temperature as well as refrigerator temperature. Microbial contamination and denaturation were observed in the liquid form of peanut extract. Hence the lyophilized peanut extract was found to be more stable.

4.3.2. Drug-polymer interaction studies

Fourier transform infrared (FT-IR) spectrophotometric study

The FT-IR of crude peanut extract (CPE) exhibited peaks due to C = 0 stretch weakly coupled with C-N stretch and N-H bending at 1600–1700 cm^{-1}, C-N stretch strongly coupled with N - H bending at 1500–1600 cm^{-1} which corresponds to the amide I region and amide II region respectively. Also, a peak was exhibited at range of 3600–2500 cm^{-1} represents strong carboxylic acid OH stretch. The FT-IR of CPE-excipient mixture exhibited peak at range of 3600–2500 cm^{-1}, at 1674.21 cm^{-1} due to C = 0 stretch weakly coupled with C-N stretch and N-H bending, at 1510 cm^{-1} C-N stretch strongly coupled with N - H bending.

Differential Scanning Calorimetric study (DSC)

The DSC thermogram of CPE displayed an endothermic peak at 165.8 °C relating to the melting of the CPE while the physical mixture exhibited a pointed endothermic peak at 169.4 °C corresponding to the melting point of the CPE. The comparison of DSC of CPE and physical mixture shows that the melting point values of the physical mixture are higher than the CPE which may be due to the presence of excipients. This also indicates that the CPE is converted to amorphous form (Mathure et al., 2018). The results showed that there is a very light shift in the peak of CPE-excipient mixture when compared to the DSC of the drug stipulating no significant interaction among the CPE and excipients used. The area of the peak has been increased in the DSC of CPE-physical mixture which may be due to the complete incorporation of the drug in the formulation mixture. Also, the results indicated the crystalline nature of the drug which is most stable form, with high melting point, but may have low solubility (Kaoud et al., 2021).

4.4. X-Ray diffraction study (XRD)

The CPE showed the characteristic sharp peaks at 2θ angle, namely, 15.288, 19.940, 21.508, 22.519, 30.357 and 33.998° indicating the crystalline nature of CPE. The XRD diffractogram of the formulation exhibited few characteristic peaks with reduced intensity which may be due to the lesser fraction of CPE in the formulation and the complete incorporation of the CPE in the polymer chains. The results also shows that crystalline nature of drug may be reduced due to the reduced intensity of peaks indicating that the drug would be amorphized and the sharp peaks observed in the XRD of formulation could be attributable to the highly crystalline nature of excipient like mannitol which was used as the diluent. The results of XRD and DSC of CPE may be related, both indicating the crystalline nature of the extract (Samvedna et al., 2018).

4.5. Precompression evaluation

The results of the precompression studies shows that all formulations had good flow properties revealed by the values of angle of repose ranging from 35 to 45, Carr's index values of 15–25 and Hausner's ratio ranging from 1.1–1.3. The results indicate that the powder blends have good flow properties which plays a crucial role in the unit processes such as blending, compression transportation etc.

4.6. Dose fixation

The SLIT involves the administration of gradually increasing doses of the allergen to induce desensitization and tolerance. The SLIT involves the administration of few micrograms to milligrams of allergen in an increasing manner. SLIT mainly comprises of 2 stages: The escalation dose and maintenance dose. The maximum maintenance dose can be up to 2–3 mg as per clinical studies. Hence, we ought to formulate 3 doses of SLIT tablets with dose ranging from 1 to 2 mg of proteins which could render it as safe dose. The doses selected were as 4.8 mg, 7.2 mg and 9.6 mg of CPE corresponding to 1 mg, 1.5 mg and 2 mg protein (Zhang et al., 2018).

4.7. Post compression evaluation

The thickness was maintained from 1 to 2 mm and the hardness was within 2.5–3.5 kg/cm^2. All the tablets have shown lower hardness values which are preferred for sublingual tablets. The weight variation test for the tablets was performed by calculating the average weight of ten tablets from each batch. The weight of the tablets was between 142 mg and 145 mg which were within the acceptable limit (7.5%). The friability of the tablet is required for the shipment of the product. The percentage friability of all the formulations was not more than 0.7% which is within 1% limit. The results indicate that the tablets were mechanically stable. The formulation with 2% croscarmellose sodium and 5% crospovidone, that is, higher concentrations of super-disintegrants showed a lower wetting time of 44 ± 1.24 secs and water absorption ratio of 50% compared to other formulations with varying concentrations of both super-disintegrants. The lowest wetting time and highest water absorption ratio is thought to be ideal for sublingual tablets for its rapid disintegration. Formulation with 2% croscarmellose sodium and 5% crospovidone had lesser disintegration time than other formulations. This could be attributed to the water insoluble and spongy nature of crospovidone resulted in highly porous tablet. The disintegration may be due to the capillary movement and the swelling and wicking action of croscarmellose (Dilebo and Gabrie, 2021). The highest disintegration time was observed with formulation containing only 2% crospovidone. In addition, increasing super-disintegrant concentration significantly reduced the disintegration time which could be due to the rapid disintegration induced by super-disintegrants. The F1 of first dose, F5 of second dose and F9 of the third dose showed shorter disintegration time and higher drug release, F1, F5 and F9 were selected as the optimized batches for three doses respectively.

The *in-vitro* dissolution profile of optimized fast disintegrating sublingual tablets exhibited a rapid drug release of more than 90% within 3 min as given in the Fig. 11. The drug release of optimized batches of three doses of formulation named F1, F5 and F9 was found to be 94.1 ± 1.18%, 93.4 ± 0.82% and 94.9 ± 0.25%, respectively within 3 min. Similar studies were done, where the drugs were released 90% within 10 min (Sakata and Onishi, 2020). The release profile of all other batches of three doses were lesser compared to the release data of optimize batches of three doses. Hence, the *in vitro* drug release profile of the optimized batches is only depicted in Fig. 11. The optimized formulations with 2% croscarmellose and 5% crospovidone lead to rapid release of the drug, i.e., initial burst release of more than 90% is observed within 3 min which approaches a steady state. This could be attributable to the water-insoluble and spongy nature of crospovidone resulting in a highly porous tablet which disintegrates rapidly by capillary movement resulting in the weakening of intermolecular bonds contributing to the breakdown of tablet into fine particles accelerating the dissolution (Nahed et al., 2017). The *In-vitro* drug release profile of the optimised formulations of three doses namely F1, F5 and F9 resulted in similar drug release pattern indicating the influence of 2% croscarmellose and 5% crospovidone in the formulations. This suggests that different doses for SLIT tablets can be formulated with ideal concentration of super disintegrants resulting in optimum drug release.

The rapid allergen release in a small volume of medium has a crucial role in the efficient sublingual delivery of allergen by SLIT tablets. A complete allergen release from the formulation indicates that full allergen dose of SLIT tablet is made available to the sublingual immune system (Ashok et al., 2019).

4.8. Drug release kinetics study

The drug release kinetic study was conducted by employing mathematical models (Radhakant Gouda et al., 2017; Constantin Mircioiu et al., 2019). The regression coefficient of the various drug release kinetic models of the optimized formulations F1, F5 and F9 are depicted in Fig. 12 and depicted in Table 7. From the results, it is evident that the formulation F1 was best fitted with Higuchi square root model (R^2 = 0.8618), whereas, the formulations F5 and F9 was best fitted with First order kinetic model with the highest R^2 value of 0.9102 and 0.9421 respectively, which indicates the formulation depends on the concentration of the drug. Korsmeyer-Peppas plots validated the diffusion mechanism of the formulations by demonstrating acceptable linearity (R^2 values of F1, F5 and F9 are 0.8116, 0.9106, and 0.9429, respectively), with the n values less than 0.5, showing that the drug release mechanism in the optimized tablets was diffusion controlled. According to previous published studies, this finding was confirmed (Lund et al., 2019; Basak et al., 2008; Reza et al., 2002).

4.9. Design evaluation

The disintegration time for twelve formulations was within the range of 14.5–42.3 s and % drug released in 2 min were in the range 60–91.45 %. The Analysis of variance (ANOVA) contributes the information on the effect of each factor with their interactions and the significant levels of these factors on the responses. For the fast-disintegrating tablet, both Croscarmellose and Cross povidone had a significant influence on the disintegration time and Dissolution (% Drug release) in 2 min.

The data obtained from the experimental study showed that the batches F1, F5, and F9 had a shorter disintegration time of 14 secs and >90% drug release within 2–3 min. Thus, the results from the experimental study and the predicted results of disintegration time and dissolution rate were closely related indicating F1, F5 and F9 of three doses respectively are optimized batches.

According to the predictive model's disintegration time ($p < 0.0001$=, R^2 = 0.99815) and dissolution % ($p = 0.0005$=, $R^2 = 0.95$) confirms the validity of the model, data points are found to be almost linear for disintegration time and dissolution %. The maximum desirability for was observed at (0.6703) (Fig. 15) at a Croscarmellose of 2 %, and Crospovidone 5% the predicted disintegration time (14.075 sec) was and the dissolution (94%).

5. Conclusions

Peanut allergy affects about 25% of children and over 1% of the general population in western countries. The current standard of care includes proper diagnosis, strict dietary avoidance of allergen, and promoting awareness to the patient and family. Since treatment options are inadequate, there is an unmet need for new therapeutic options. Presently there is no FDA approved sublingual tablets for food allergy, specifically peanut allergy. The designed peanut allergen-containing sublingual tablets offer a new treatment option to the susceptible community, resulting in clinical desensitization. Meta-analysis revealed that SLIT-tablets showed better efficacy over anti-allergic pharmacotherapies for allergic disorders. Remarkable reductions in the use of daily inhaled corticosteroid, risk of asthma aggravations, and asthmatic symptoms were associated with the use of SLIT tablets. The most common adverse events of SLIT are mild oral reactions with low risk of systemic allergic reactions. These evidences suggest SLIT-tablets could be considered as a supplementary treatment to pharmacotherapy for asthma.

The SLIT tablet of 3 doses containing crude peanut extract were developed and the tablet was optimized by custom design approach where the effect of varying concentration of super disintegrants on disintegration time and dissolution. The compatibility studies of FT-IR and DSC were carried out to determine any drug-excipient interaction and no interactions were observed. The pXRD analysis of the CPE and the formulation was also studied which indicated the crystalline nature of the drug due to the presence of characteristic peaks. The precompression evaluation was carried out for the powder blends which showed good flowability and compressibility. The SLIT tablets were developed by direct compression method and the tablets were characterized for its post-compression parameters. The results showed that the formulation with 2% croscarmellose sodium and 5% of crospovidone resulted in desirable lesser wetting time, higher water absorption ratio, shorter *in vitro* disintegration time and maximum of 90% drug release within 2–3 min indicated the suitability of developed SLIT tablets in treatment of peanut allergy. In-vivo animal studies and other pharmacological studies needs to be conducted to gather more insight into the toxicity and the safety of the prepared formulation so that the SLIT could be a promising platform for the AIT.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank our dean research Dr. Shantikumar Nair and Dr. Sabitha M for providing the facilities for research. Syed Mohammed Basheeruddin Asdaq, would like to thank AlMaarefa University, Riyadh, Saudi Arabia for extending support (TUMA-2021-1) to do this research.

Funding

Majid Alhomrani would like to acknowledge Taif University for support No. TURSP (2020/257).

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