Research Article

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www.itmedicalteam.pl

International Journal of Drug Development and Research
ISSN 0975-9344

2024
Vol.16 No.2:011

# Design, Evaluation and Comparison of Various Mucoadhesive Buccal Tablets of Fluoxetine

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**Received date:** Jan 08, 2024, Manuscript No. IJDDR-24-14354; **Editor assigned date:** Jan 11, 2024, PreQC No. IJDDR-24-14354 (PQ); **Reviewed date:** Jan 25, 2024, QC No. IJDDR-24-14354; **Revised date:** Feb 28, 2024, Manuscript No. IJDDR-24-14354 (R); **Published date:** Mar 06, 2024, Invoice No. IJDDR-24-14354

**Citation:** Amita S, Sunil B (2024) Design, Evaluation and Comparison of Various Mucoadhesive Buccal Tablets of Fluoxetine. Int J Drug Dev Res Vol: 16 No:2

## Abstract

The aim of the study was to develop a drug delivery system that could improve the therapeutic efficacy and onset of action of Fluoxetine, a commonly used antidepressant. FXT acts by blocking serotonin transport receptors.

Two different methods were used to create mucoadhesive buccal tablets containing fluoxetine hydrochloride. The first method used was the direct compression method, in this method the tablets were prepared by compressing the crystalline ingredients together. The optimized batch (B2) was selected based on the results obtained from various studies conducted. Also, a lyophilized tablet batch (L1) was prepared using the same polymer as in the B2 batch. This was achieved through the solvent casting freeze drying method. Several studies were performed on the developed tablets, including *in-vitro* and *ex-vivo* release studies, pre-formulation studies, and characterization of the tablets. Compatibility studies using techniques like DSC (Differential Scanning Calorimetry) and FTIR (Fourier Transform Infrared Spectroscopy) were conducted to ensure that the drug was compatible with the tablet components. Cytotoxicity studies were also carried out using L929 (fibroblast) cells as a cell line model. The results of these various studies were then compared, and it was observed that the lyophilized batch (L1) demonstrated better performance than the directly compressed batch (B2).

Overall, the current work highlights the development and comparison of fluoxetine hydrochloride-containing mucoadhesive buccal tablets using different techniques. The aim was to enhance the effectiveness and convenience of fluoxetine administration, potentially leading to improved patient compliance and faster onset of therapeutic effects in the treatment of depression.

**Keywords:** Mucoadhesive; Depression; Lyophilization; Fluoxetine; Cytotoxicity

## Introduction

Depressive illness is one of the largest problem from which a large proportion of world's population is suffering. Treatment of the depression is a time consuming process and sometimes patient struggles to adhere the regime. Major Depressive Disorder (MDD) primarily identified by depressive mood episodes which lasts for more than two weeks [1].

### Symptoms of depression

- Capricious feeling, depressed behavior and anxious.
- Feeling guilty, helpless, worthless etc.
- Restlessness, exhaustion, irritation and loss of energy.
- Loss of interest, suicidal, fatal, or attempted thoughts [2].

Mental disorders account for about 13% of global population is living with mental disorder. The World Health Organization has estimated that 970 million people worldwide, or 1 of the 8 population, experience depression in 2019, so it is becoming one of the major problem with the population [3].

Therefore, there is a need for the development of a buccal tablet that has a speedier onset of action, avoids first pass metabolism, and improves patient compliance, bioavailability, and simplicity of administration (Figure 1).


**Figure 1:** Site of oral cavity for sublingual and buccal drug delivery.

Fluoxetine, sertaline, fluoxamine, paroxetine and escitalopram belong to the group of SSRI's which is the first line treatment for

the MDD. Reason behind this is the relative selectivity, low toxicity and ease of dosing than other groups of the medications. SSRI have very fewer side effects in comparison with other anti-depressive medications, that's why patient compliance is there. According to Taurine et al., fluoxetine is the first antidepressant that should be used to treat depression in kids and teenagers [4]. The 2011 APA guideline supports the use of fluoxetine as the first-line medication for late onset depression (Figure 2).

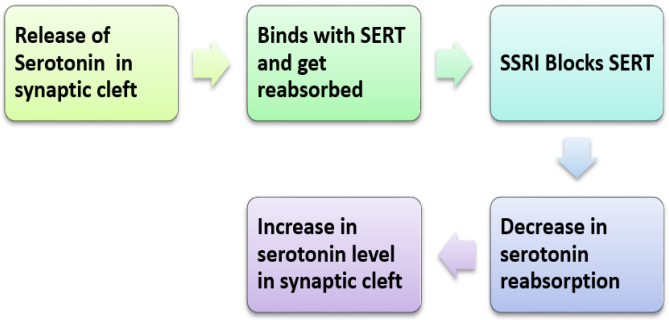


**Figure 2:** Flowchart of MOA of SSRI drugs.

In 1947, gum tragacanth and dental adhesive powder were used to attach penicillin to the oral mucosa, introducing the first bio-adhesive drug delivery formulations [5].

Buccal route of administration provides higher bioavailability and faster action when compared to the oral route administration as medicines do not pass through the gastrointestinal system. Which helps to avoid first pass metabolism [6]. The medication is held in the cheek, diffuses *via* the oral mouth mucosal tissues, and reaches the bloodstream *via* this route.

The oral mucosa is made up of a layer of stratified squamous epithelium on the outside. Below this is a basement membrane, followed by a lamina propria and, finally, the innermost layer sub-mucosa [7].

Due to the possibility of avoiding the gastrointestinal disturbance and hepatic first-pass metabolism mucoadhesion is becoming the subject of the great interest nowadays.

Bio-adhesion can be explained as the attachment of synthetic or natural macromolecules to the mucus and/or epithelial surface for extended period of time. The bond between two materials is governed by interfacial forces [8].

## Theories of bio-adhesion

- Wetting theory.
- Diffusion theory.
- Electronic theory.
- Adsorption theory.
- Fracture theory.

## Wetting theory

Appropriate wetting as a prerequisite of mucoadhesion is suggested by the wetting theory. This theory says that the mucoadhesive force is the capacity of the subject to spread over the mucosa this theory is mainly applicable to the liquids and semisolids.

Adhesion can be expressed in the term of the interfacial tension, when the interface is formed there is a release of energy per $cm^2$ that can be defined as work of adhesion. Contact angle and thermodynamic work adhesion are the two aspects with whom wetting theory deals [9].

## Diffusion theory

According to diffusion theory polymer chains get linked to the mucus and combine to form a semi-permanent sticky bond [10]. Factors like diffusion coefficient, duration of contact and other experimental factors affects the precise depth to which polymer chain must pierce to obtain significant bio-adhesion. As the cross linked density increases the diffusion coefficient get decrease rapidly and which is totally depends upon molecular weight [11]. The exact penetration depth needed for good bio-adhesive bonds are not clearly established, but it is estimated to be in the range of 0.2 µm–0.5 µm.

## Electronic theory

Theory states that due to the presence of the variations in the electrical properties electronic transfer occurs between polymer and mucus glycoprotein layer, which results in the formation of an electric double layer at the interface, and while crossing this double layer adhesive force is generated that provides adhesion [12].

## Adsorption theory

Surface force operating between the atoms in the two surfaces makes them to attach. This theory suggests that when two surfaces make first contact, the muco-adhesive substance will get absorbed on the biological surface due to the force present. At the surfaces weak forces like van der Waals force plays an essential role.

## Fracture theory

This is perhaps the most used theory in studies on the mechanical measurement of mucoadhesion. It analyzes the force required to separate two surfaces after adhesion. Concerned only with the force required to separate the parts, it does not take into account the interpenetration or diffusion of polymer chains. Consequently, it is appropriate for use in the calculations for rigid or semi-rigid bio-adhesive materials, in which the polymer chains do not penetrate into the mucus layer [13].

## Factors affecting muco-adhesion

**Polymer related factors**

Molecular weight [14,15].

Hydrophilicity [16].

Concentration of active polymer.

Flexibility of polymer chains [17].

Swelling [18].

pH [19].

**Environment related factors**

Contact time [20].

Spatial Conformation [21].

## Lyophilization technique

For the development of the solid dosage forms having APIs of low solubility and dissolution rate lyophilization have great advantages. Lyophilization helps to improve drug wetting, product stability and solubility as well as it is helpful for the API's that sensitive to the high impact force applied in the direct compression [22].

Lyophilisation process has some crucial stages like removal of the water molecules from liquid phase components and converts them into solid form. Ideal experimentation conditions for the whole process specially include the deep freezing and vacuum which involves sublimation followed by desorption [23].

**Advantages of lyophilization**

- Helps to improve the stability of hydrolysable products.
- Water molecule degradations get reduced.
- Increase the stability of the final drug components.
- Reduce shear and shipping related stresses.
- Reduce molecular motions.

**Limitations of lyophilization**

- Expensive approximately three times higher.
- More energy consumption.
- Time consuming (usually more than 24 hours cycle).

Therefore, the aim of work in this study is to formulate and evaluate FXT buccoadhesive lyophilized tablets using different tablet-forming mucoadhesive polymers for the intention of delivering the drug through the buccal route aiming to avoid the first pass metabolism and to improve the bioavailability of the drug and also patient compliance [24].

# Materials and Methods

Fluoxetine hydrochloride was sponsored by Shodhana laboratories limited, Hyderabad, India. HPMC (K4M) and Carbopol 934 was purchased from Colorcon Asia pvt. ltd., Goa, India and Central Drug House (P) Ltd., New Delhi, India respectively. Loba Chemie pvt. ltd., Mumbai, India provided MCC200, Mg stearate, talc and aerosil as a gift. Di-sodium hydrogen phosphate anhydrous, potassium dihydrogen orthophosphate (anhydrous), and methanol was purchased from Merck life sciences, Mumbai, India.

Fresh buccal mucosa of Goat was obtained from the local slaughter house and used within 2 hr of slaughter.

## Drug characterization

**Infrared Spectroscopy (FTIR):** FTIR spectroscopy helps to identify the functional groups present in the structure so it was used for the drug characterization. FTIR shows a peak at a specific wavelength for the every functional group as it works as a fingerprint. Then peaks which were at specific wavelengths are compared with the standard references and functional groups were identified.

**Differential Scanning Calorimeter (DSC):** In DSC (Thermo-analytical method) the purity of the compound was assessed by DSC is a thermo-analytical method for assessing the purity of a chemical by comparing the amounts of heat needed to raise the temperature of the test compound in response to a change in temperature. The DSC aluminum pan was filled with a dose of around 2 mg of the drug sample, and it was sealed. Then, temperature was increased from 0°C to 250°C, heated in the constant flow of nitrogen at a scanning rate of 10°C/minute. The endothermic peaks were noted using the DSC curves. The reference object used was an empty aluminum pans.

## Standard plot of FXT in methanol and PBS pH 6.8

Stock solution of 100 µg/ml, was prepared in volumetric flasks a primary stock then 10 ml of the primary stock solution was diluted to 100 ml with methanol to obtain a stock solution of 10 µg/ml as a secondary stock solution.

From the secondary stock solution aliquots ranging from 0.5 ml, 1 ml, 1.5 ml ….and 4.5 ml were pipetted out and diluted to get the concentration of 0.5 µg/ml, 1 µg/m, 1.5 µg/ml……and 4.5 µg/ml, respectively. Filtered through Whatmann no. 1 filter paper and filtrate analyzed at 227 nm by using UV-visible spectrophotometer (Model-1900i, Shimadzu, Japan) against methanol as a blank. Absorbance was recorded and standard curve was plotted absorbance on y axis and concentration on X axis for a linear relationship.

## Drug-excipient compatibility studies

The goal of the drug-excipient compatibility studies is to detect, quantify, and forecast potential interactions (physical or chemical), as well as the effects of these interactions on the ability to be manufactured, the quality of the final formulation, and its performance [25].

**Fourier-Transform Infrared Spectroscopy (FTIR):** Infrared spectroscopy is the most widely used non-thermal technique for determining drug-excipient compatibility. Based on their physical and chemical properties, these approaches provide the API and excipients a distinct fingerprint. Because of the highly sensitive nature of these approaches, any small changes in the physicochemical properties of the API as a result of interactions with the excipients are easily recognized. The physical mixture of medication (FXT) with several excipients (HPMC, HPMC K4M, Carbopol 934, NaCMC, microcrystalline cellulose, magnesium stearate, silica, PVA) was made in a 1:1 ratio, and their spectra was recorded in the 4000-400 $cm^{-1}$ region. To confirm, the

obtained results were compared with those of FXT, HPMC, HPMCK4M, NaCMC, Mg stearate, MCC, Carbopol 934, and others. The IR peaks of the drug are observed for any new peak as a manifestation of possible interaction.

## Pre-formulation studies for powder blend

**Bulk density and tap density:** The weighed amount of mixed powder was poured into the tap density apparatus's 100 mL measurement cylinder, and the initial volume mark was recorded. The instrument was programmed to produce 10, 500, and 1250 taps, and the ultimate tapped volume was measured. Lab-India Tap Density tester (TD 1025).

**Carr's index and Hausner ratio:** From the values of bulk density and tapped density, Carr's index and Hausner ratio were calculated using the following formulae and the flow type of the powder being examined was evaluated.

Hausner ratio=Tapped density/Bulk density

Carr's index=((Tapped density−Bulk density)/Tapped density) × 100

**Angle of repose:** It is the greatest angle that can be made between a powder pile's surface and the horizontal plane. With the aid of a funnel and cotton plug, it was measured. The funnel's cotton plug-blocked tip was used to receive the powder. The cotton stopper was carefully removed to release the powder onto the brown paper, which was stored underneath the funnel. The base covered by the powder pile was traced out, and the average diameter of the base was calculated. The height of the heap was measured.

Angle of repose ($\theta$)=$\tan^{-1}$ (Height of the pile/Radius of the base)

## Prepration of FXT mucoadhesive tablets

**Direct compression method:** Mucoadhesive tablets were developed using a previously developed procedure with minor modifications [26]. Tablets were punched by direct compression technique, using various proportion of the polymers. After weighing precisely, FXT was mixed with Carbopol. In a separate pouch, the remaining polymers were mixed with talc. After passing through a 40 mesh filter, these two mixtures were mixed for 5 min in a separate pouch, MCC 200 and aerosil were mixed together for 2 minutes and then it was mixed with the prior mixture for approx 5 min. Finally, Magnesium stearate was added, and the resulting mixtures were mixed before being compressed into tablet press. Table 3 shows compositions for different batches prepared.

**Lyophilization technique:** Using casting/freeze drying technique lyophilized tablets were prepared using each HPMC and Carbopol alone or in a mixture with PVA in the desired concentration. Table 3 shows the composition. To make HPMC and carbopol buccoadhesive tablets, 1% and 2% w/v polymer solutions were prepared by progressively adding the necessary amounts of the HPMC or carbopol to the mixture while stirring continuously [27]. The FXT was then added and dissolved in the polymer solution in the amounts required. Finally, the prescribed amounts of PVA were added and constantly mixed until a homogeneous solution was achieved. The polymer solutions were made so that each 1.5 ml contained 20 mg fluoxetine. To obtain clear, bubble free solutions, the medicated polymer solutions were left at room temperature overnight. An amount of 1.5 ml of each medicated polymer solution was poured into each of the pocket of an oblong PVC blister pack resulting in a dose of 20 mg fluoxetin per tablet. The plastic molds were frozen at −20°C in a refrigerator for 24 h and then freeze-dried at −45°C under a vacuum of $7 \times 10^{-2}$ mBar (Freeze Dryer, NovalypheNL500, Savant, Holbrook, USA). 30 tablets from each formulation were prepared and the tablets were stored in air tight glass containers in desiccators at room temperature till time of the evaluation tests (Figure 3).

**Figure 3:** Lyophillized tablets and directly compressed tablets.

## *In-vitro* evaluation of tablet properties

**Physicochemical parameters**

**Weight variation:** Twenty tablets were chosen and weighed one at a time to study the weight fluctuation investigation. The weight of each of the 20 tablets was calculated on average. The average weight was used to calculate the % variance in weight of each tablet. According to the Indian pharmacopoeia's stated parameters, the batch was deemed to be either pass or fail.

**Hardness:** Using the Pfizer hardness tester, the tablets hardness was assessed. Hardness was measured in kilograms per square centimeter. The amount of force needed to break the tablet was interpreted as the tablet's hardness. The conclusion was reached in triplicate.

**Thickness:** The dimensions of the tablets were evaluated with the help of Vernier caliper. The determination was made in triplicate.

**Friability:** Twenty tablets (n=20) of each batch were weighed and put into the friabilator drum (Roche friabilator). After 100 revolutions of friabilator for 4 minutes (25 revolutions per minute), tablets were recovered. The tablets were then freed from dust and weighed. Friability was calculated from the following equation.

% Friability=(Initial wt.-Final wt.)/(Initial wt.) × 100

**pH:** With the use of a pH metre (the CyberScan pH 510 from Eutech instruments), the pH of the tablet dispersion was measured. After calibrating the pH metre with several buffers (pH 4.2, 7, and 9.2), the pH was then measured by dipping the pH electrode into the tablet solution that was created after the tablet was dissolved in 10 mL.

**Matrix erosion:** Tablets initial weight was noted down (W1). Swollen tablets were dried at 60°C for 24 h in an oven and kept in dessecator for 48 h and reweighed (W3). % matrix erosion were calculated using following formula [28].

% Matrix erosion=(W1-W3)/W3 × 100

**Swelling index:** Three tablets were chosen at random from each batch, Weighed (W1), and then placed in separate petri plates with 10 mL of buffer. They were removed from the petri dish at the predetermined intervals and the extra water was drained using filter paper. The swollen tablets were reweighed (W2) and the following equation was used to determine each tablet's percentage of hydration [29].

% Swelling index=(W2-W1)/W1 × 10

**Drug content:** Ten tablets from each batch were individually crushed to a fine powder with a mortar and pestle. A quantity equal to 20 mg of FXT was weighed, diluted in 20 mL of methanol, and passed through a 0.44 µm membrane filter. 1 ml of the filtrate was collected and exposed to UV. The absorbance was measured at 227 nm, and the drug content was estimated using the standard plot.

***In-vitro* release study:** The *in-vitro* dissolution profile of FXT was observed using a United States Pharmacopoeia dissolution apparatus II (Lab India DS 8000) with a paddle rotation speed of 50 rpm. The releasing medium (USP buffer for FXT) was added to 900 mL of the dissolving vessel. 37°C, ± 0.5°C, was kept as the temperature. The dissolving studies were done three times. At each interval, 5 mL of the media was withdrawn and replaced with new media kept at 37°C. At 227 nm, the absorption was seen.

**Cytotoxicity screening:** Cell lines are an efficient way to investigate the effect of any questioned molecule on a given cell type. It also gives a more complex view on the *in-vitro* toxicity profile of the formulation. This study also helps in deciding the dose that can be administered in animal studies.

Cell cytotoxicity potential of the prepared tablets was determined, employing the established procedure of MTT assay. L929 fibroblast cells ($1 \times 10^5$ cells/well each) were harvested in individual 96-well plates, each containing the culture medium (100 µL), and kept overnight for adherence to plate surface. Subsequently, the cells were washed and exposed to varied serial concentrations, *i.e.,* 10, 15, 20 and 25 µL of API and formulation each in triplicate, and incubated for 48 hr. The excess medium containing drug and formulation was removed, and the cells were subsequently washed using phosphate buffer saline (pH 7.4). After washing, the cells were treated as per the documented MTT assay protocol [30].

## *Ex-vivo* evaluation of tablet properties

***Ex-vivo* mucoadhesion time:** After applying the buccal tablet to newly sliced goat buccal mucosa, the time of *ex-vivo* mucoadhesion was evaluated. Fresh goat buccal mucosa was connected to a glass slide, and each tablet's mucoadhesive core side was moistened with 1 drop of phosphate buffer pH 6.8 and pasted to the goat buccal mucosa for 30 seconds with light pressure. After that, the glass slide was placed in the beaker, which had been preheated to 37 ± 1°C and contained 200 mL of phosphate buffer with a pH of 6.8. After two minutes, a slow stirring rate was used to mimic the environment in the buccal canal, and tablet adhesion was tracked for 12 hours. The time for the tablet to detach from the goat buccal mucosa was recorded as the mucoadhesion time (Figure 4) [26,31].

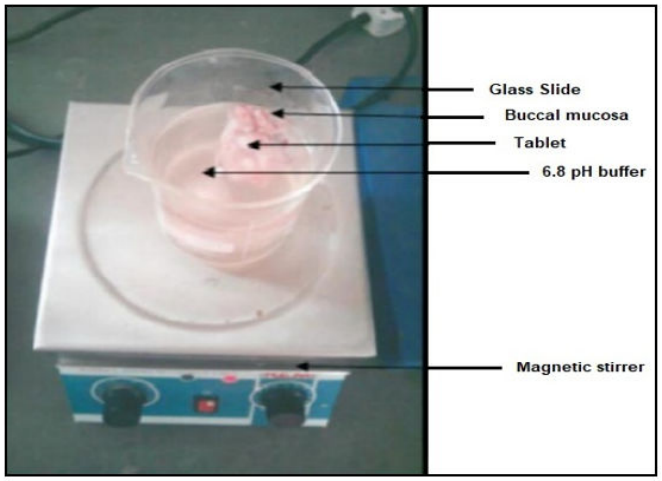


**Figure 4:** Setup for mucoadhesion time study.

***Ex-vivo* permeation studies:** *Ex-vivo* tissue permeation studies were carried out using Franz diffusion cell on goat buccal mucosa. The hairs were expelled from the extracted tissue, and subcutaneous fat was removed with a surgical tool. The tissue was cleaned with ACN and further washed with distilled water. The tissue was mounted on the Franz diffusion cell. The receptor chamber with cross-sectional region of 7.065 $cm^2$ was loaded up with 30 mL of diffusion medium (FDA approved buffer for FXT). Just before placing tablet 1 ml of simulated saliva was poured in the centre and was allowed to spread over the entire film. After that one tablet was placed gently on buccal mucosa. The receptor chamber was stirred at 100 rpm at temperature of 37°C ± 0.5°C. The samples (1 mL) were withdrawn from the receptor compartment at predetermined time intervals (10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h) and immediately replaced with fresh diffusion medium maintained at the same temperature. The sampling was carried out in triplicate. The samples were analyzed using UV-VIS spectrophotometer at 227 nm [32,33].

# Results

## Drug characterization

**DSC (Differential Scanning Calorimetry):** DSC thermogram showed sharp peak at 158.51°C illustrating in Figure 5 this sharp peak suggest that drug is pure without any degradation product and impurities which is corresponding to the melting point of product as denoted in the reference standard.

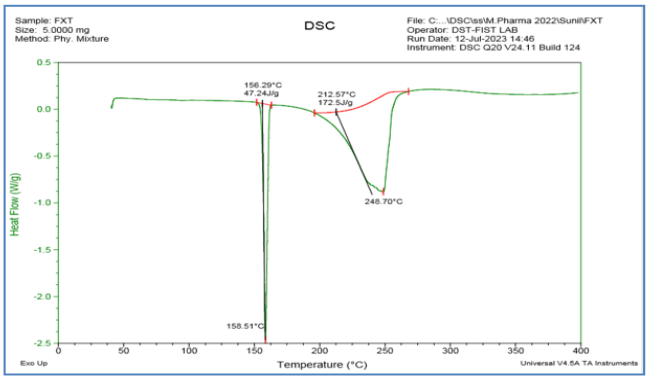


**Figure 5:** DSC Thermogram of FXT.

**FTIR (Fourier Transform Infra-Red spectroscopy)**

Infrared spectroscopy of the FXT was studied for identification purpose. Peaks were found according to functional groups present in the compounds as reported in the literature (Figure 6).

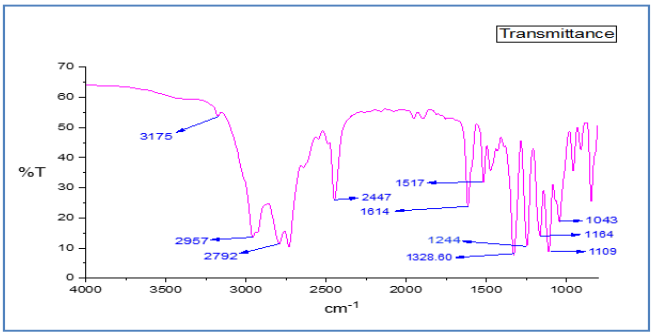


**Figure 6:** IR Spectrum of fluoxetine.

A broad and strong peak at 3175 $cm^{-1}$ indicates the stretching vibration of the N-H bond, usually found in primary amines. A sharp peak at 1614 $cm^{-1}$ represents the stretching vibrations of the aliphatic C-H bonds. Peak at 1244.78 $cm^{-1}$ indicate the stretching vibrations of carbonyl groups. Peak at 1517 $cm^{-1}$ associated with stretching vibrations of aromatic C=C bonds. Peak at 1328.60 $cm^{-1}$ correspond to Halide stretching vibrationC-F in the molecule (Table 1).

**Table 1:** IR peaks of Fluoxetine Hydrochloride (FXT).

| | |
|---|---|
| 3175 $cm^{-1}$ | Amine stretching vibration (N-H) |
| 2957 $cm^{-1}$ | (N-C) stretching |
| 1614 $cm^{-1}$ | Aromatic (C-H stretching) |
| 1517 $cm^{-1}$ | Aromatic (C=C stretching) |
| 1328.60 $cm^{-1}$ | Halide stretching vibration (C-F) |
| 1244.78 $cm^{-1}$ | Phenoxy stretching vibration (C-O aromatic group) |
| 1069.96 $cm^{-1}$ | Alkane (C-H stretching) |

The actual FTIR spectrum of fluoxetine showing additional peaks and variations are depending on the specific experimental conditions and sample preparation.

**Standard plot of Fluoxetine (FXT) in methanol and PBS**: Standard plot of FXT was made in methanol, Figure 7 showing the standard plot where the $r^2$ value was 0.995.

Standard plot of FXT was made in PBS pH 6.8, Figure 7 showing the standard plot where the $r^2$ value was 0.997.

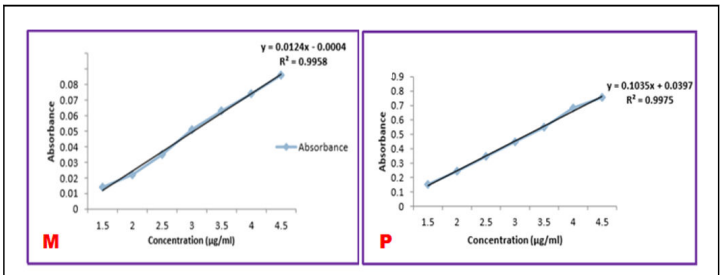


**Figure 7:** Caliberation curve of FXT in methanol (M) and PBS (P).

## Drug-excipient compatibility studies

The physicochemical compatibility of the drug and excipients was established through FTIR. Fluoxetine gave peaks at respective wave numbers *i.e.*, 3175 $cm^{-1}$ stretching vibration (N-H), 2957 $cm^{-1}$ (N-C) stretching, 1517 $cm^{-1}$ aromatic (C=C stretching), 1328.60 $cm^{-1}$ halide stretching vibration (C-F), 1069.96 $cm^{-1}$ alkane (C-H stretching) (Figure 8).

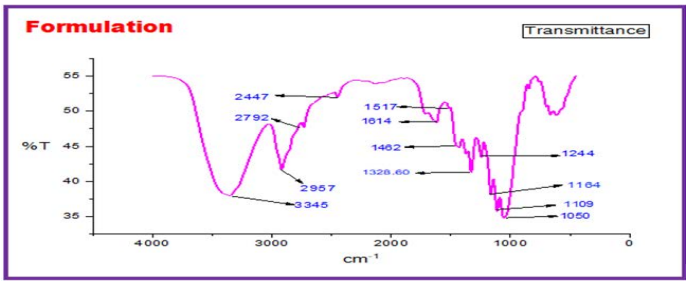


**Figure 8:** FTIR spectra of formulation (HPMC, NaCMC, CP934, MCC, PVA, FXT).

However, additional peaks were observed in the formulation mixture which could be due to the presence of various excipients and indicated that there were no chemical interactions between FXT and other excipients which were shown in Figure 8, as there was no major shifting in the peaks.

## Pre-formulation studies for powder blend

The angle of repose of all the batches was found to be less than 40° which indicated fair flow property (Table 2).

**Table 2:** Precompression study data for powder blend bulk density, tap density, Carr's index, Hausnerr's ratio and angle of repose.

| Batch | Bulk density | Tap density | Carr's index | Hausners ratio | Angle of repose |
|---|---|---|---|---|---|
| B1 | 0.64 | 0.78 | 17.9 | 1.21 | 32.4 |
| B2 | 0.63 | 0.79 | 20.2 | 1.25 | 34.73 |
| B3 | 0.62 | 0.77 | 19.4 | 1.24 | 33.64 |
| B4 | 0.64 | 0.78 | 17.9 | 1.21 | 33.58 |

The values of Carr's index and Hausner ratio were found to be less than 20% and lesser than 1.25, which was indicative of fair flow properties of powder blend according to the Indian pharmacopeia limits.

## Formulation chart

Compositions for formulation shown in Table 3.

**Table 3:** Compositions for formulation.

| Excipients (% w/w) | B1 | B2 | B3 | B4 | L1 |
|---|---|---|---|---|---|
| Fluoxetine | 11.76 | 11.76 | 11.76 | 11.76 | 13.33 |
| Carbopol 934 | 14.7 | 14.7 | 14.7 | 14.7 | 16.66 |
| HPMC K4M | 17.64 | 26.47 | - | 8.82 | 30 |
| MCC 200 | 47.05 | 44.11 | 47.05 | 47.05 | - |
| NaCMC | - | - | 17.64 | 8.82 | - |
| Mg. Stearate | 2.94 | 2.94 | 2.94 | 2.94 | - |
| PVA | - | - | - | - | 40 |
| Silica | 2.94 | 2.94 | 2.94 | 2.94 | - |
| Talc | 2.94 | 2.94 | 2.94 | 2.94 | - |

## *In-vitro* evaluation of tablets

**Physicochemical properties:** The friability lay in the range of 0.11% to 0.9%. As friability was not more than 1% for any formulation so all the batches passed this test as per pharmacopoeia (Table 4).

**Table 4:** *In-vitro* evaluation data of buccal tablets, wetting time, hardness, friability, dimension, weight variation and pH.

| Batch | Wetting time (min) | Hardness (Kg/cm$^2$) | % Friability | Thickness (mm) | Weight variation (mg) | pH |
|---|---|---|---|---|---|---|
| B1 | 25.55 | 4.47 | 0.51 | 1.76 | 171.32 | 6.5 |
| B2 | 20.3 | 4.55 | 0.63 | 1.73 | 170.22 | 6.81 |
| B3 | 23 | 5.8 | 0.52 | 1.81 | 173.34 | 6.73 |
| B4 | 22.17 | 5.55 | 0.93 | 1.79 | 168.93 | 6.98 |
| L1 | 8 | - | 0.2 | 1.62 | 151.28 | 6.78 |

The tablets prepared with the combination of HPMC K4M, NaCMC and Carbopol 934 reported hardness between 4.3-5.8 kg/cm$^2$ (B1 to L1).

The thickness was found to be in the range of 0.314 mm to 0.317 mm.

The weight variation allowed as per IP limit is 7.5%. Values of weight variation for all the individual batches were found to be within the permissible limits of conventional oral tablets stated in the I.P.

The solution pH of all the tablets was within the range of 6.58 to 7.01 which is close to neutral pH. Buccal cavity is also having almost same pH *i.e.,* 6.7 to 7.3. There was negligible or no change in the solution pH of the tablets. Hence, no irritation to the buccal cavity was assumed.

## Drug content

As shown in Table 5, the drug content varied between 95.13 to 104.34 which reflects good uniformity in drug content among different batches.

**Table 5:** Percent drug content of all batches.

| Batch | Amount of drug (mg) | % Drug present |
|---|---|---|
| B1 | 19.65373 | 98.26865 |
| B2 | 19.66708 | 98.33542 |
| B3 | 20.8689 | 104.3445 |
| B4 | 19.02611 | 95.13057 |
| L1 | 19.68044 | 98.40218 |

## Swelling index

In Figure 9 the graph illustrate that, the swelling index after 4 hr was in the range of 200% to 350% for formulation (B1 to B4). B2 is showing highest swelling index in 4 hrs and the lyophilized batch was showing the highest swelling index in first hour and later it is decreasing as the matter getting eroded and dispersing in the media [34,35].

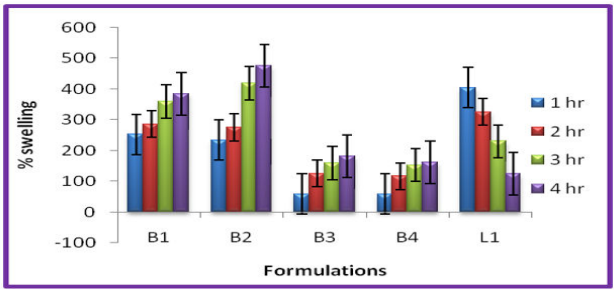


**Figure 9:** Comparative study of swelling index for different batches of fluoxetine mucoadhesive tablets at different time intervals.

## Matrix erosion

The percent amount of the matter getting eroded from the surface of the tablet and getting dissolved or disperse in the media was calculated and shown in Table 6.

**Table 6:** Numerical data of % swelling index of different batches of luoxetine mucoadhesive tablets at different time intervals.

| Swelling index | | | | | Matrix erosion |
|---|---|---|---|---|---|
| **Time** | **1 hr** | **2 hr** | **4 hr** | **8 hr** | |
| B1 | 252.86 | 286.277 | 359.423 | 384.55 | 56.92 |
| B2 | 234.063 | 275.266 | 419.103 | 474.531 | 59.85 |
| B3 | 58.52 | 125.321 | 158.667 | 182.208 | 33.7 |
| B4 | 58.834 | 116.095 | 152.86 | 160.774 | 37.88 |
| L1 | 404.73 | 325.97 | 229.96 | 125.57 | 81.1 |

## *In-vitro* release

Among all directly compressed batches batch B2 shows the best sustained drug release profile so selected as optimized batch. The batch prepared with same composition used in batch B2, but by lyophilized technique shows better results than batch B2 *in-vitro* drug release test as shown in Figure 10. So, batch B2 and L1 was selected as the optimized batch and finalized for further investigation (Table 7).

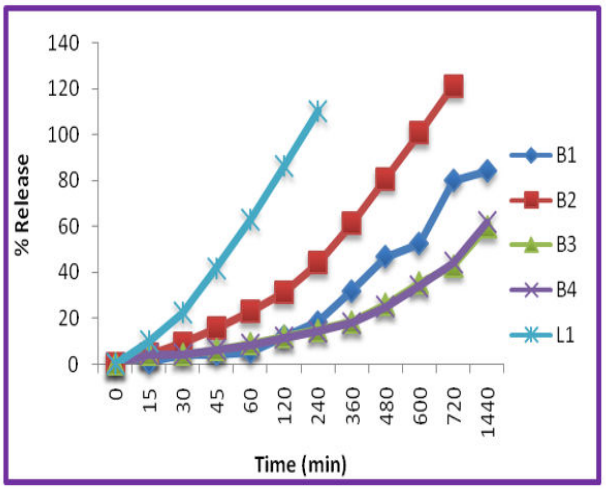


**Figure 10:** *In-vitro* drug release of all batches.

**Table 7:** *In-vitro* drug release data.

| Time | ***In-vitro* cumulative % release data** | | | | |
|---|---|---|---|---|---|
| | B1 | B2 | B3 | B4 | L1 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 15 min | 0.816 | 4.225 | 4.28 | 4.28 | 9.95 |

| | | | | | |
|---|---|---|---|---|---|
| 30 min | 4.333 | 9.11 | 4.33 | 4.33 | 22.54 |
| 45 min | 4.34 | 15.6 | 6.36 | 6.33 | 41.78 |
| 1 hr | 5.174 | 22.97 | 8.8 | 8.57 | 63.16 |
| 2 hr | 12.056 | 31.19 | 11.91 | 11.42 | 86.32 |
| 4 hr | 18.523 | 44.08 | 14.99 | 14.31 | 110.38 |
| 6 hr | 32.097 | 61.29 | 18.5 | 18.06 | - |
| 8 hr | 46.932 | 80.85 | 26.15 | 25.18 | - |
| 10 hr | 52.541 | 100.69 | 35.42 | 34.2 | - |
| 12 hr | 80.156 | 121.3 | 43 | 44.31 | - |
| 24 hr | 84.115 | - | 59.71 | 61.87 | - |

## Cytotoxicity studies

Figure 11, depicts the concentration versus percent cell viability data for the fibroblast cells incubated with pure FXT and formulation containing drug. The pure drug showed a concentration dependent reduction in percent cell viability but the formulation showed increased percent cell viability as compared to pure drug. This signified that pure drug alone showed toxicity but when the drug was given in the form of formulation *i.e.,* tablets the cell toxicity of drug decreases. Which ensure that,

- **Solubility:** The change in the solubility and bioavailability of the fluoxetine. As the formulation has enhanced the solubility of fluoxetine, leading to better cellular uptake and metabolism, resulting in higher cell viability.
- **Drug release profile:** A prolonged and optimal distribution of fluoxetine to cells was ensured by the formulation's regulated drug release profile. Compared to an abrupt exposure to fluoxetine in its pure form, this controlled release has a beneficial effect on cell viability.
- **Excipients:** Excipients in the formulation enhanced Fluoxetine's stability and cell compatibility. These excipients helped to reduce the potential harmful effect of the fluoxetine and increase the cell viability.
- **Cellular uptake mechanism:** The cellular uptake mechanism of the fluoxetine has been facilitated due to the specific mechanism of drug delivery from the formulation which was not efficient with the pure fluoxetine. This leads to increase in the cell viability

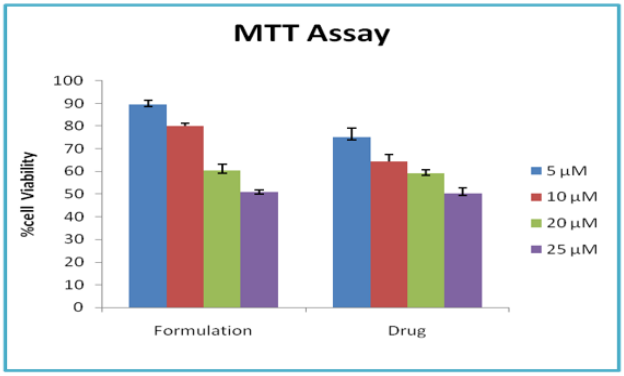


**Figure 11:** Percent cell viability of cells after treatment with pure FXT and formulation containing drug.

## *Ex-vivo* studies

**Mucoadhesion time:** The results of mucoadhesive time of prepared formulations construe that the detachment time was almost same for all the batches which varies from 6 hrs to 9 hrs as displayed in Figure 12.

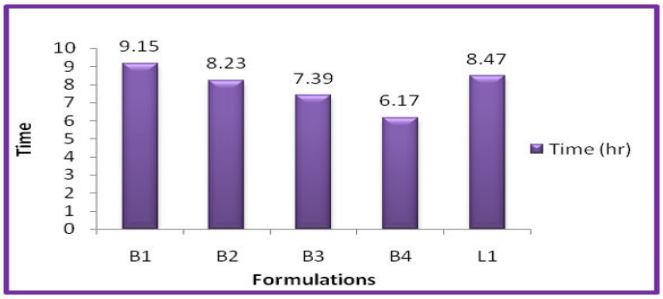


**Figure 12:** Data of mucoadhesion time test performed for all batches.

***Ex-vivo* permeation test:** Batch L1 and B2 were taken for *ex-vivo* permeation test, batch L1 was showing higher percentage of permeation than batch B2 as in 2 hrs batch L2 shows nearly 80% drug permeated and in comparison with that, batch B2 was showing approx 25% of drug permeation. That's why it was concluded that batch L1 was showing faster and better drug permeation than batch B2 (Figure 13).

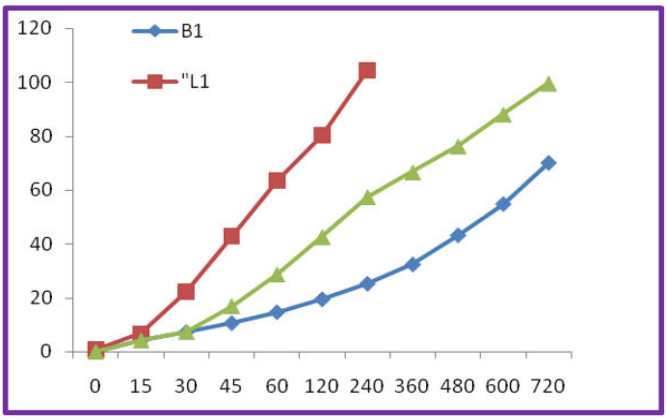


**Figure 13:** Comparison between amount of drug permeated from pure drug dispersion, batch B2 and Batch L1.

## Discussion

The tablet batches were evaluated for various parameters such as diameter and thickness using a Vernier caliper, hardness using Monsanto apparatus, wetting time, disintegration time, drug content, swelling index, *in-vitro* drug release, *ex-vivo* drug release, *ex-vivo* drug permeation, buccal mucoadhesion time and cytotoxicity studies.

The desired batches were finalised on the basis of powder flow properties from precompression studies and *in-vitro* evaluation and were further tested for cytotoxicity studies and *ex-vivo* parameters.

The final formulations obtained were B2 and L1 on the basis of various studies performed.

### *In-vitro* and *ex-vivo* evaluation results of final batches

FXT buccal tablets were prepared by direct compression and Freeze drying method using Mucoadhesive polymers (HPMC K4M and Carbopol 934).

The thickness of the all batches was found to be in the range of 1.61 mm to 1.81 mm.

The hardness of the final tablet batches was found to be in the range of 4.55 ± 0.31 kg/cm$^2$ (B2).

The prepared tablet batches passed the weight variation test as per limits given in IP *i.e.,* out of 20 tablets, NMT 2 tablets deviated from 7.5% limit.

The drug content of the final batches was found to be 98.33 ± 0.73% (B2) and 98.40 ± 0.18% (L1).

The final batch B2 showed % swelling in the range of 234.44% to 474.54% from 1 hr to 8 hr and batch L1 depicted the same in the range of 404.73% to 125.57%.

*In-vitro* drug release of the final batches was found to be 61.29 ± 0.05% at 6 hr and 121.3 ± 0.09% at 12 hr (B2) and 41.78 ± 0.11% at 45 min and 110.38 ± 0.06% at 2 h.

*Ex-vivo* drug release of final batches was observed to be 44.57 ± 0.02% at 6 hr and 95.376 ± 0.14 % at 12 h (B2) and for batch L1, 40.97 ± 0.01% at 1 hr and 80.20 ± 0.15% at 2 hr.

The cytotoxicity studies justified the selection of appropriate type and concentration of excipients for formulating fluoxetine buccal tablets.

## Conclusion

From the batches prepared by direct compression method (B1 to B4) Batch B2 selected as an optimized batch from the evaluation studies performed *i.e., in-vitro* drug release studies and swelling test study as it was showing the optimized results in desired range. Then the batch L1 was prepared by lyophilization technique by using the same composition and all the tests are performed similar to the direct compressed tablets.

The above batches composite had shown satisfactory results in the parameters such as thickness, hardness, drug content, swelling index, matrix erosion, mucoadhesive time, *in-vitro* dissolution and *ex-vivo* permeation. The satisfactory formulation shows a zero order drug release profile depending on the regression value and shown a satisfactory dissolution profile. Slow, controlled and maximum permeation of fluoxetine over a period of 12 hr was obtained from buccal tablets B2 formulation and maximum permeation of fluoxetine over a period of 2 hrs was obtained from batch L1.

After all the tests were performed, both B2 and L1 batches were compared, from the comparative study it was seen that the batch L1 was showing better results than batch B2 comparatively. Hence it was concluding that the lyophilized batch shows a better performance than batch B2 and the lyophilization techniques for mucoadhesive tablet prepration should be preferred rather than direct compression tablet technique.

## Author Contributions

**Dr. Amita Sarwal:** Agreement to be accountable for all aspects of the work in ensuring that questions related to accuracy and integrity are properly investigated and resolved. **Sunil Bharti:** Substantial contributions conducting the experimentation work and the interpretation of the data for the work acquisition and analysis.

## Fellowship

The first co-author was getting monthly fellowship from All India Council of Technical Education (AICTE) for completing their master's degree.

## 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.

## Acknowledgement

The authors would like to acknowledge Shodhana laboratories limited, Hyderabad for providing the fluoxetine HCL sample.

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