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FORMULATION, OPTIMIZATION AND EVALUATION OF QUICK DISPERSIBLE TABLETS OF SUMATRIPTAN

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ABSTRACT
The main objective of this study was to prepare quick dispersible tablets of drug sumatriptan succinate, which can rapidly disintegrate in the saliva using three different superdisintegrants that is, sodium starch glycolate, crospovidone, and croscarmallose sodium with taste masking polymer beta-cyclodextrin and aspartame as a sweetener. The taste masking of the drug was done by mixing it with the polymer beta-cyclodextrin using solvent evaporation method and then mixing optimized quantity of aspartame to it. The quick dispersible tablets were prepared by direct compression technique using taste masked drug and other formulation excipients. The effect of various super disintegrants in three different concentrations has been studied. The prepared tablets were evaluated for wetting time, in-vitro disintegration time, strength, and in-vitro dissolution time. As per the results obtained, it was found that the formulation batch no. 4 was found to be the best formulation, as the data's obtained by it was found to be in the required range of mouth dissolving tablets.

INTRODUCTION

Tablet is the most popular conventional dosage form for oral administration, because of ease of self administration, compact in nature, easy to manufacture, and that it can be delivered in accurate dose [1]. Instead of so many advantages there are some disadvantages of solid dosage forms, the main drawback is difficulty in swallowing "dysphagia" in pediatric and geriatric patients, some other problems that occurs are, fear of choking, hand tremors, dysphasia, in young patients due to underdeveloped muscular and nervous system, etc., which leads to patient incompliance, for this reason, tablets that can be rapidly dissolve or disintegrate in the oral cavity are to be formulated. Due to this reason, the fast dissolving tablets have attracted a great deal of attention in the field of pharmacy [2]. United state food and drug administration (FDA), defined fast dissolving tablet as "a solid dosage form containing medicinal substance or active ingredient which disintegrate rapidly usually within a matter of seconds when placed upon the tongue" [3]. Orodispersible tablets are also known as mouth dissolving tablets, melt-in-mouth tablets, fast dissolving tablets,

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repimelts, porous tablets, quick dissolving tablets. Orodispersible tablets dissolve or disintegrate in the oral cavity without the need of water. As orodispersible tablets are, tend to be dissolved in mouth the drug will be partially dissolved in the taste buds [4]. Thus, the taste inside the mouth becomes critical for the patient acceptance. For the bitter or partially bitter drugs, taste masking becomes an important criterion for the formulation of orodispersible tablets. The orodispersible tablets must include taste-masking agent for masking the bitter taste of drugs as they are formulated to be dissolved in mouth. The time for disintegrating tablets is generally considered less than one minute [5].

Orodispersible dosage forms have several advantages over other dosage forms such as; allow high drug loading, ability to provide advantages of liquid medication in the form of solid preparation, cost-effective, adaptable and amenable to existing processing and packaging machinery [6]. Inspite of having so many advantages the fast dissolving drug delivery system have some limitations, such as, tablets usually have insufficient mechanical strength, more susceptible to degradation by humidity and temperature, and it is sometimes difficult in developing extremely high doses [7].

Migraine is a primary episodic headache disorder characterized by various combinations of neurological, gastrointestinal, and autonomic changes. Migraine is one of the most common causes of severe and reoccurring headache. Usual symptoms in adults include extreme pain on one or both the sides of the head, throbbing in nature, pain in the eye, jaw, face or neck, nausea, vomiting, etc. The migraine attack may start with premonitory symptoms, following aura and resolution phases. Thus, the patients suffering from such disease needs quick relief from the severe headache, for this it is required to formulate such a dosage form that can provide quick relief from such severe condition. Fast dissolving drug delivery system is such an approach that may provide the required benefits for the treatment of the disease [6].

Sumatriptan is an antimigraine drug, it is a $5\text{-HT}_{1B/1D}$ serotonin receptor agonist, it has a short half life of approximately 2 hours, its oral dose is 50- 100 mg. It is absorbed from the completely GI tract. It has low oral bioavailability of about 14%, the reason behind its low bioavailability is first pass metabolism. The bioavailability of the drug may increase by formulating the drug as fast dissolving tablet. Because the drug is taken without water, thus some of its residue may remain in the oral cavity from which some part of drug may absorbed

from oral cavity, pharynx and esophagus leads to reduce the first pass metabolism [8]. Sumatriptan is available as subcutaneous injection but the problem is that this is painful and dosage is not economic and easily available to all patients. It is also available as conventional tablet, which has less advantage than fast dissolving drug delivery system [8]. The main Aim of the research work is to formulate, evaluate and optimize the taste masked Quick Dispersible tablets of Sumatriptan. The main objective behind formulating such dosage form is to provide quick relief from the severe conditions of the disease by decreasing the disintegration time also increasing the bioavailability of the drug by avoiding first pass metabolism. The other conventional dosage form of the drug has many disadvantages i.e. patient incompliance which includes dysphagia (difficulty in swallowing), for children and elderly people, bitter taste of the drug, etc. These drawbacks of conventional tablets can be overcome by the fast dissolving drug delivery system.

MATERIALS & METHODS

All materials used in the present research were of analyzed grades. Drug Sumatriptan Succinate was obtained from Nosch Labs Private Ltd. Hyderabad, polymers Beta-cyclodextrin, Sodium Starch Glycolate, Microcrystalline Cellulose, Magnesium Stearate, Talc, Sodium Starch Glycolate, and di-potassium hydrogen phosphate were obtained from SD Fine Chem Ltd. Mumbai, Vanillin and Mannitol were obtained from Loba Chemicals, Mumbai, Crospovidone was obtained from Signet, Mumbai.

Preformulation studies of pure drug
Identification of drug sample using FTIR
This was carried out for the identification of the drug sample. For this, 10mg of drug sample and 400 mg of KBr were taken in mortar and triturated. A small amount of the triturated sample was taken and kept onto the sample holder and scanned from 4000cm^{-1} to 400cm^{-1} in F.T.I.R. Spectrophotometer. The spectra obtained were obtained and interpreted for the functional group peaks.

Determination of melting point
As per pharmacopoeia range of melting point of Sumatriptan Succinate is given between 218°- 255°C. The melting point of Sumatriptan Succinate was determined by using melting point apparatus. For this, take a small amount of drug sample in capillary tube which was one sided closed and placed in a

melting point apparatus and the temperature at which drug melts was noted.

Solubility analysis

A semi quantitative determination of the solubility was made by adding solvent in small amount to a test tube containing fixed quantity of solute or vice-versa. After each addition, the system is vigorously shaken and examined visually for any undissolved solute particles.

Preparation of calibration curve in pH 6.8 phosphate buffer

An accurately weighted amount of Sumatriptan Succinate equivalent to 100mg was dissolved in small amount of pH 6.8 Phosphate Buffer in 100ml volumetric flask and volume made up to 100ml with the same pH 6.8 Phosphate Buffer. From this stock's solution, 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml and 10ml were withdrawn and diluted up to 10ml with the pH 6.8 Phosphate Buffer in 10ml volumetric flask to get concentration of 1µg, 2µg, 3µg, 4µg, 5µg, 6µg, 7µg, 8µg, 9µg and 10µg respectively. The absorbance of each solution was measured by UV-Visible Spectrophotometer at 226 nm using pH 6.8 Phosphate Buffer as blank.

Drug Excipients Interaction Study by FTIR

To check the compatibility drug and polymer in preparation of mouth dissolving tablet of Sumatriptan Succinate, the drug and excipients mixture of quantity 10 mg and 400 mg of KBr were taken in a mortar and were triturated. A small amount of the triturated sample was taken and kept onto the sample holder & scanned from $4000 – 400 cm^{-1}$ in F.T.I.R. Spectrophotometer. The spectra obtained were matched with that of the peaks obtained from the FTIR study of the pure drug sample and interpreted for the interaction of drug and excipients if any.

Preparation of drug and beta-cyclodextrin complex for Taste Masking

Drug cyclodextrin complex was prepared by solvent evaporation method, weighed amount of drug was taken with different concentrations of beta-cyclodextrin i.e. 1:1, 1:2, 1:3, 1:4 and 1:5 (Drug: Beta-cyclodextrin, respectively) in separate beakers and was dissolved in common solvent i.e. purified water. Both the solution mixtures were then mixed together and were allowed to evaporate. After evaporation of the solvent a powdered mass of drug cyclodextrin complex was found, which was then collected as a drug cyclodextrin complex. Also three different concentrations of Aspartame were taken i.e. 2%, 4% and 6%

Preparation of Sumatriptan Succinate tablets by direct compression method

Tablets of Sumatriptan Succinate were prepared by direct compression method. All the formulation ingredients mentioned in formulation table were weighed accordingly and mixed in a mortar and pestle. This powder blend was then allowed to dry for few moments and then again mixed well and passed through sieve no 60. Then blend were used for further processing

Evaluation of Pre-Compression Characteristics of Powder Blend

Powder blend prepared were evaluated for various rheological properties like bulk density, tapped density, Hausner's ratio, angle of repose by using standard procedures. All these properties were carried out in triplicate (n=3) and average values were reported.

Bulk density

Bulk density is determined by placing the powders blend in a measuring cylinder and the total volume is noted. The weight of powder bed is determined by using digital weighing balance. Bulk density is calculated using the following formula

Bulk Density = Weight of the powder / Volume of the powder

Tapped density

Tapped density is determined by taking the dried powders in a measuring cylinder and measures the volume of powders after 100 tapping's and take weight of the total powders.

Tapped Density = Weight of the powder / Tapped Volume of the powder

Angle of repose

Angle of repose was determined by measuring the height and radius of the heap of the powder bed. A cylindrical two side open tube of 6 cm length is place on graph paper. Powders are placed in the tube and slowly removed the tube vertically. With the help of scale the height and radius of the heap were measure and note.

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

Where, h = height of heap of granular bed, r = radius of heap of granular bed.

Hausner's Ratio

It is expressed in percentage and is expressed by

$H = D_t / D_b$

Where D_t is the tapped density of the powder
D_b is the bulk density of the powder.

Compression of powders into Tablets

After adding Lubricant (talc) and glidant (magnesium stearate) to the prepared powders, they were compressed into tablets on a tablet rotatary compression machine using 10mm diameter, flat faced punches

Table 1: Formulation Chart for the preparation of Tablets

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug-βCD complex	150	150	150	150	150	150	150	150	150
Sodium starch glycolate	6.25	12.5	18.75	-	-	-	-	-	-
Crospovidone	-	-	-	6.25	12.5	18.75	-	-	-
Croscarmallose sodium	-	-	-	-	-	-	6.25	12.5	18.75
Aspartame	15	15	15	15	15	15	15	15	15
Talc	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Magnesium Stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vanillin	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mannitol (filler)	23.75	21.67	19.6	23.75	21.67	19.6	23.75	21.67	19.6
Microcrystalline cellulose (filler)	47.5	43.33	39.15	47.5	43.33	39.15	47.5	43.33	39.15

Evaluation of compression characteristics of tablets [9,11]

The prepared tablets were evaluated for their thickness, friability, hardness, weight variation and dissolution test by using standard procedures.

Weight variation test

20 tablets are taken and their weight is determined individually and collectively on a digital weighting balance. The average weight of one tablet is determined from the collective weight. Note more than 2 of the individual weights may deviate from the average weight by more than the percentage deviation given in the monographs and none should deviate by more than twice that percentage given in the monographs.

Thickness test

The tablets were evaluated for their thickness using a venirer caliper measured in terms of micrometer. Averages of three readings were taken and the results were tabulated (n = 3)

Hardness test

Prepared tablets were evaluated for their hardness by using Monsanto hardness tester. The hardness was measured in terms of kg/cm^2. Triplicate readings were taken and average was determined.

Friability test

Roche friabilator was used for testing the friability of the tablets. For this test, 20 tablets were weighted accurately and placed in the friabilator chamber and rotated at 25rpm for a period of 4 min. Tablets were again weighted and the percentage weight loss was determining by using formula given below.

$$\% \text{ Friability} = [(W_1-W_2)100]/W_1$$

Where,

W_1 = Weight of tablet before test

W_2 = Weight of tablet after test.

Wetting Time

A piece of tissue paper folded twice was placed in a small Petri dish containing 6 ml of purified water, then a tablet was placed on the paper and the time required for complete wetting was measured.

Wetting time corresponds to the time taken for the tablet to disintegrate when placed gently on the tissue paper in a Petridish. Less wetting time indicates more porous tablets.

In-Vitro Disintegration Time

In this Method one tablet was placed in the disintegration apparatus containing 500 ml of distilled water at 37ºC ± 0.5ºC and the time required for complete dispersion was determined the reading was noted in triplicate and average was calculated.

In-vitro drug release study

Drug release study was carried out using USP dissolution rate test apparatus (Apparatus II, 50 rpm, 37˚C) for half an hour in phosphate buffer pH 6.8. For the first 5 minutes 5ml sample was withdrawn in a time interval of 1 minute, and for the next 25 minutes, the sample was withdrawn in a time interval of 5 minutes. 5 ml of the sample was withdrawn and diluted with the same media up to 10ml, and 5ml of the fresh media was poured to the dissolution medium for maintaining the sink condition. The samples were analyzed at 226nm using UV–Spectrophotometer.

Kinetic model of drug release

All the formulation of prepared tablets were subjected to invitro release studies these studies were carried out using dissolution apparatus, pH 7.2 phosphate buffer the results obtaining in vitro release studies were plotted in different model of data treatment as follows:

- Cumulative percent drug released vs. time (zero order rate kinetics)
- Log cumulative percent drug retained vs. time (First Order rate Kinetics)
- Log cumulative percent drug released vs. square root of time (Higuchi's Classical Diffusion Equation)
- Log of cumulative % release Vs log time (Peppas Exponential Equation)

Zero Order Kinetics

A zero-order release would be predicted by the following equation.

$$At = A_0 - K_0t$$

Where:

At = Drug release at time 't'

A_0 = Initial drug concentration

K_0 = Zero-order rate constant (hr^{-1}).

When the data was plotted as cumulative percent drug release versus time, if the plot was linear then the data obeys zero-order release kinetics, with a slope equal to K_0.

First Order Kinetics

A first-order release would be predicted by the following equation.

$$Log\ C = LogCo - \frac{Kt}{2.303}$$

Where:

C = Amount of drug remained at time 't'

C_0 = Initial amount of drug

K = First-order rate constant (hr^{-1}).

When the data was plotted as log cumulative percent drug remaining versus time, yields a straight line indicating that the release follows First-order kinetics. The constant 'K' can be obtained by multiplying 2.303 with slope values.

Higuchi's Model

Drug released from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation.

$$Q = \left[\frac{D\varepsilon}{\tau(2A - \varepsilon Cs)Cst}\right]^{1/2}$$

Where:

Q = Amount of drug released at time 't'

D = Diffusion coefficient of the drug in the matrix

A = Total amount of drug in unit volume of matrix

C_S = The solubility of the drug in the diffusion medium

ε = Porosity of the matrix

τ = Tortuosity

t = Time (hrs) at which 'Q' amount of drug is released.

Equation may be simplified if one assumes that D, C_S and A are constant. Then equation becomes

$$Q = Kt$$

When the data was plotted according to new equation i.e., cumulative drug released versus square root of time, yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K'.

Korsmeyer and Peppas Model

The release rates from controlled release polymeric matrices can be described by the equation proposed by Korsmeyer et al.

$$Q = K1t^n$$

Q is the percentage of drug released at time 't', K is a kinetic constant incorporating structural and geometric characteristics of the tablets and 'n' is the diffusion exponent indicative of the release mechanism. For Fickian release, less than n=0.45 while for anomalous (Non-Fickian) transport, n ranges between 0.45 and 0.89 and for zero order release, more than n = 0.89.

RESULT AND DISCUSSION
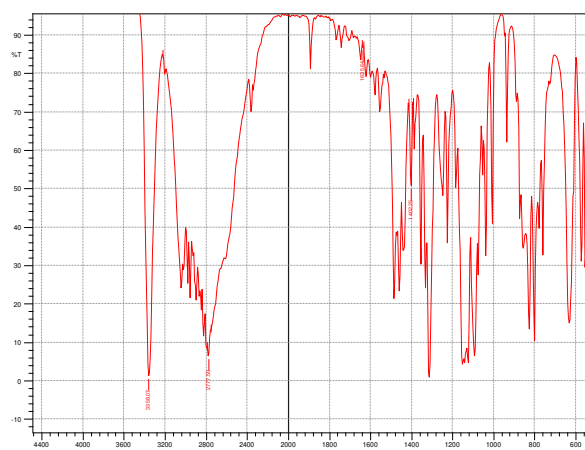### Identification of Sumatriptan Succinate by FTIR

Fig 1: IR Spectra of pure drug Sumatriptan Succinate

In the table we found that the peaks obtained by performing FTIR of pure drug were found to be in between the range of main principle peaks recorded previously as theoretical range,

hence this indicates that the drug is pure. These observations were found to be in concurrence with the structure of the drug molecule.

Table 2: IR Peak Table with principle peaks of sumatriptan succinate

Functional group of Sumatriptan Succinate	Range of Principle Peaks	Peaks Obtained
N-H of Indole	3369	3358.07
O=S=O	2964-2775	2954.95, 2777.50
conjugated C=C double bond	1640	1635.64
O-H bending vibration	1400	1402.25

Drug Polymer Compatibility Studies:

To check the compatibility drug and polymer in preparation of matrix tablet of Sumatriptan Succinate. The spectra obtained from the FT-IR studied at wavelength from $4000 cm^{-1}$ to $400 cm^{-1}$ are shown fig 2 and the characteristics peaks obtained are shown in table 3 [10,12]
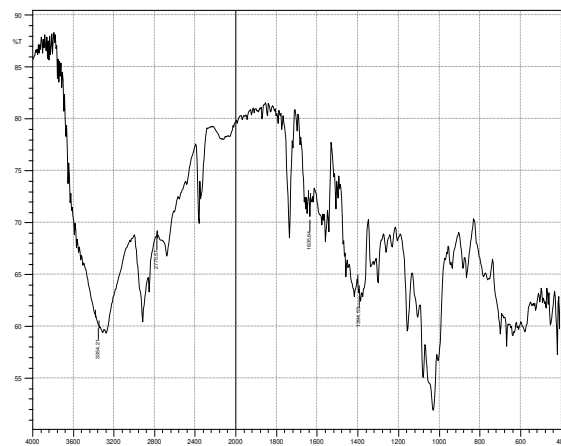

Fig 2: IR spectra of prepared Mouth Dissolving Tablets of Sumatriptan Succinate mouth dissolving Tablet

Table 3: IR peak table of prepared mouth dissolving tablets of sumatriptan succinate

Functional groups	Principle peaks	Peaks of pure drug	Peaks of formulation
N-H of Indole	3369	3358.07	3354.21
O=S=O	2964-2775	2954.95, 2777.50	2916.37, 2775.57
conjugated C=C double bond	1640	1635.64	1635.64
O-H bending vibration	1400	1402.25	1394.53

After performing FTIR of the best formulation of Sumatriptan Succinate mouth dissolving tablet it was found that the peaks obtained were in between the range of main principle peaks and were found to be very near to previously performed FTIR of pure drug Sumatriptan Succinate. No major deviation in peaks were obtained in IR spectra, hence this indicates that there is no interaction between drug and other tablet ingredients.

Determination of melting point

Melting point of pure drug was found to be 168°C that was in range of 165°- 169°C, actual melting point of Sumatriptan Succinate as per pharmacopoeia.

Solubility analysis

The pure drug sample of Sumatriptan Succinate was found to be freely soluble in water, sparingly soluble in methanol, soluble in pH 6.8 pH phosphate buffer, freely soluble in 0.1 N HCL.

Preparation of standard graphs for Sumatriptan Succinate

Standard graphs for the drug Sumatriptan Succinate was performed using pH 6.8 Phosphate buffer. Table shows the concentration of Sumatriptan Succinate in pH 6.8 Phosphate buffer and its respective absorbance. The figure shows the calibration curves of Sumatriptan Succinate performed.

Table 4: Data for standard curve of Sumatriptan Succinate

Concentration (µg/ml)	Absorbance (226nm)
1	0.13298
2	0.25839
3	0.37398
4	0.52615
5	0.62971
6	0.75891
7	0.89197

Fig 3: Standard calibration curve of sumatriptan succinate

$y = 0.1262x + 0.0055$
$R^2 = 0.999$
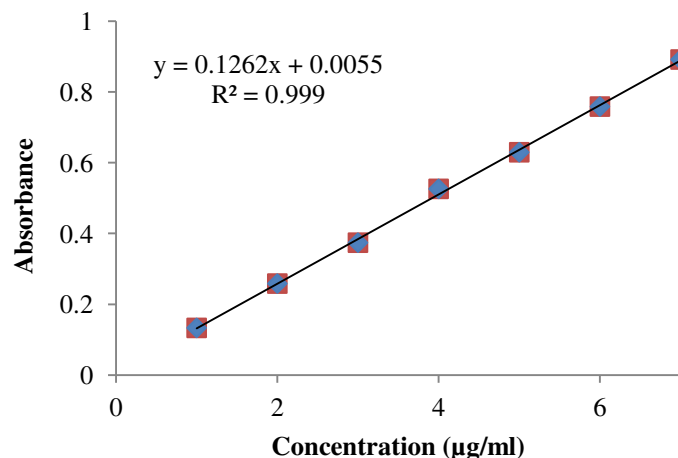
Characterization of Sumatriptan Succinate tablets

Evaluation of pre-compression characteristics of powder blend

The Pre-compression evaluations of prepared powders were shown below. The powders were evaluated for bulk density, tapped density, Hausner's ratio and angle of repose and consistency in data obtained as indicated by their standard deviation values shown in table.

Table 5: Data of pre-compression characteristics of powder blend

Batch	Bulk Density	Tapped Density	Hausner's ratio	Angle of repose (θ)
F1	0.416±0.003	0.563±0.002	1.35±0.017	21.73±0.20
F2	0.461±0.002	0.581±0.003	1.25±0.013	34.13±0.20
F3	0.452±0.002	0.571±0.004	1.26±0.008	35.03±0.20
F4	0.414±0.003	0.6±0.002	1.44±0.005	32.39±0.08
F5	0.451±0.002	0.583±0.002	1.29±0.012	29.29±0.07
F6	0.423±0.003	0.545±0.003	1.28±0.014	30.58±0.02
F7	0.490±0.001	0.585±0.002	1.19±0.007	30.87±0.35
F8	0.477±0.003	0.559±0.002	1.17±0.012	31.36±0.14
F9	0.472±0.002	0.574±0.001	1.21±0.008	33.27±0.31

Pre-compression characteristics were investigated for all 9 formulations and the study showed following results. Bulk density and tapped density of different formulations were calculated. The result of bulk density range from 0.414 to 0.477 and tapped density from 0.545 to 0.6. Hausner's ratio was found to be in between 1.17 to 1.35. Angle of repose showed good to excellent flow properties of the powdered blend. F1, F5, F6 and F7 were found to be having excellent flow properties.

Evaluation of mouth dissolving tablet of Sumatriptan Succinate

The evaluations of prepared Mouth Dissolving tablet were shown below. The tablets were evaluated for thickness, hardness, Weight variation, friability, wetting time, in-vitro disintegration time, dissolution study and consistency in data obtained as indicated by their standard deviation values, shown in table No. 4. All prepared batches were found to have small variations in between thickness of all formulation but in a particular formulation there was no such variation. The thickness of all formulation was found in between 3.1±0 to 3.5±0 mm. The hardness of the compressed tablets was determined by using hardness tester (Monsanto), it was found that the tablets prepared were of adequate strength. Hardness of tablet of all formulations was found in between the range of

3.7±0.05 kg/cm^2 and 4.2±0.49 kg/cm^2. The hardness of all formulation showed variation because of formulation combination and powder properties. The friability of all formulation was in the range of 0.32 % to .85%. All formulations were found to be under standard limit i.e. less than 1%, hence passed the test for friability. The weight variation of all formulation was in the range of 250.3±1.38 to 250.85±1.26. The weight variation test was performed according to the procedure in the pharmacopoeia. In the weight variation test, pharmacopoeial limit is 7.5%. The individual deviation of all the tablets formulation was found to be within the limit and hence passed the test for uniformity of weight. The wetting time and disintegration of the formulation F4 was found to be least as compared to other formulations.

Table 6: Data of compressed mouth dissolving tablets of sumatriptan succinate

Formulation No.	Thickness (mm)	Hardness (Kg/cm^2)	Friability (%)
F1	3.13±0.05	4.1±0.17	0.74±0.01
F2	3.2±0	3.9±0.05	0.32±0.005
F3	3.1±0	3.9±0.11	0.65±0.02
F4	3.5±0	3.8±0.11	0.43±0.01
F5	3.3±0	4.2±0.49	0.77±0.005
F6	3.2±0	3.7±0.05	0.72±0.02
F7	3.1±0	4±0	0.85±0.03
F8	3.1±0	3.9±0.01	0.76±0.02
F9	3.13±0.05	3.9±0.011	0.74±0.03

Table 7: Data of compressed mouth dissolving tablets of sumatriptan succinate

Batch	Weight variation (%)	Wetting time (seconds)	Disintegration time (seconds)
F1	Passed	42.63±.18	50.66±2.08
F2	Passed	46.16±1.6	65.66±3.51
F3	Passed	51±2	65.33±2.88
F4	Passed	38.16±1.25	41±3
F5	Passed	52.33±3.21	35.66±3.51
F6	Passed	78.66±2.08	53.23±3.34
F7	Passed	75.33±3.05	68.33±3.21
F8	Passed	54±5.29	100.33±1.5
F9	Passed	92±2.64	62.33±2.51

The values of pre-compression and post compression parameters evaluated were within prescribed limits as well as indicated a good free flowing property and post compression

parameters like hardness, friability, in-vitro disintegration time, thickness, wetting time, were also found to be within the limits.
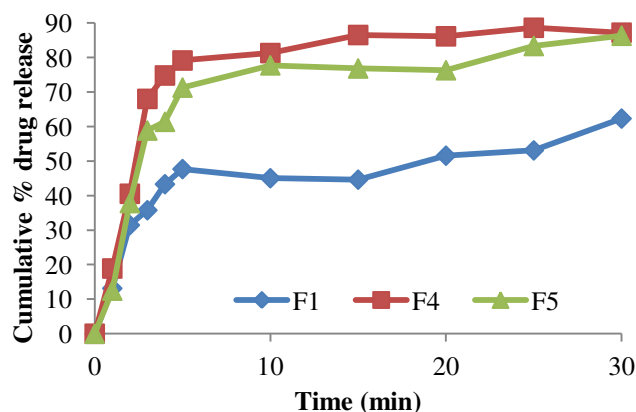

Fig 4: *In vitro* **drug release Graph**

To know the mechanism of the drug release from these formulations, the data were fixed in kinetic model i.e. zero order, first order, higuchi model, Korsmeyer Peppas model. The release rate kinetic data for all the other equation are shown in table. When the data was plotted according to the kinetic model, the formulation showed a fair linearity. The model that best fits the release data is selected based on the correlation coefficient (r^2) value in various models. The model that gives higher value of 'r' is considered to be the best fit of the release data. The value of n (diffusion constant) for all the formulations were found to be less than 0.45, this indicates that the formulations followed Fickian model for drug release.

CONCLUSION

It was concluded that mouth dissolving tablets of Sumatriptan Succinate can be successfully prepared by direct compression techniques using various superdisintegrants for the better patient compliance and effective therapy. Also the bitter drug can be easily formulated as mouth dissolving tablets by masking their taste using beta-cyclodextrin as taste masking polymer and aspartame as a sweetener without affecting any parameters of the tablet. It was also found that the superdisintegrants are effective at an optimum concentration, on increasing their concentration above their optimum concentration or mixing with other superdisintegrants it starts decreasing its effect.

FINANCIAL ASSISTANCE
Nil

CONFLICT OF INTEREST
The authors declare no conflict of interest

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