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Research Article

FORMULATION AND IN VITRO EVALUATION OF CLOBAZAM ORAL THIN FILMS

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ABSTRACT
Fast dissolving drug delivery system offers a solution for those patients having difficulty in swallowing tablets/capsules. The present research work is to develop oral thin films of Clobazam by using solvent casting method. Oral thin films were developed by using various super disintegrants like Lycoat and crospovidone in different concentrations with Gelatin, Xanthan Gum as a film forming agents. The prepared formulations of films were evaluated for film thickness measurement, folding endurance study, in-vitro disintegration time, in-vitro drug release pattern (in pH 6.8 phosphate buffer). Drug content, and drug-polymers interaction study (IR spectroscopy). Among all formulations, the formulation (F12) prepared by 135 mg of Crospovidone show good drug release (99.25±1.45%). The Optimized formulation F12 follows first order.

Keywords: Clobazam, Crospovidone, oral thin films, FT-IR.

INTRODUCTION
The 40–50 cell layer known as mucus, which is composed of proteins and carbohydrates, is the oral mucosal epithelium. The mucosal thickness at the mouth base, tongue, and gums ranges from 100 to 200 µm. A tiny quantity of mucus, a gel-like substance made up of 90%–99% water, 1%–5% water-insoluble glycoprotein, and other substances such proteins, enzymes, electrolytes, and nucleic acids, is released from the submucosal layer. Saliva and parotid are secreted from the salivary duct next to the sublingual canals and submandibular teeth by the salivary glands, which are made up of lobules. The mucosa of the lips and cheeks is where small salivary glands are most frequently located. About 1-2 milliliters of saliva are released in a single minute. Mucus, water, the enzymes amylase and lysozyme, mineral salts, immunoglobulins, and blood clotting factors make up saliva. Additionally, saliva and mucin act as a barrier for the oral mucosa.[1,2] There are two distinct regions in the mucosal epithelial structure: the lipophilic, intercellular membrane of the stratified epithelium and a more hydrophilic region.[3] The permeability of the oral mucosa to chemicals is comparable to that of the intestinal mucosa and the epidermis.The buccal mucosa's permeability is thought to be 4–4000 times greater than the skin's[2]. The mucosal epithelium provides two main channels for drug absorption: the paracellular (intercellular) pathway and the transcellular (intercellular) pathway. The polarity of the intercellular space allows more hydrophilic molecules to penetrate, whereas the lipophilic structure of the cell membranes allows molecules with a high partition coefficient to flow through the cells. The medication molecule's absorption is determined by whether it is hydrophobic, hydrophilic, or amphiphilic.[3]
Numerous pharmacological preparations are used in liquid, powder, granule, and tablet form. Patients are often given tablets in a form that allows them to chew or swallow a specific dosage of medicine. However, chewing or swallowing solid dose forms is challenging for patients, particularly those who are elderly or young.[4]
In these circumstances, oral thin film (OTF) drug delivery devices are a better option. Many medications have inadequate oral bioavailability because of the stomach's pH, typical first-pass metabolism, and enzymes. When given parenterally, several traditional medications have demonstrated poor patient compliance. These kinds of

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circumstances have made it possible for the pharmaceutical industry to create thin, dispersible, and dissolveable films in the mouth as alternative drug delivery vehicles.[5,6]

One 1,5-benzodiazepine with antiepileptic qualities is clobazam (CLB). By binding to postsynaptic GABAA receptors, it causes the neuron to become hyperpolarized, raising the action potential threshold and lowering the frequency of action potentials and, subsequently, the risk of seizures.[7]

MATERIALS

Clobazam was procured from Kekule Pharma Limited, Xanthan Gum, Propylene Glycol, Citric acid, Crospovidone, Aspartame from S.d.fine chemicals Mumbai, Gelatin was procured from INR chem. Mumbai, Lycoat from Signet Chemical Corp., Mumbai, Trusil mixed flavor R.S.V from International flavours of fragnance India Ltd

METHODOLOGY

Pre formulation studies are of great importance for the development of high-quality dosage formulations in the short duration. In this, physical & chemical characteristics and derived properties of API are determined and evaluated effectively. This is the first learning phase before formulation development. By this study, we can minimize errors, reduce the costing, and reduce no. of trials. Solubility studies of Clobazam in different buffers.

Solubility studies:

Solubility of Clobazam was carried out in different buffers. Saturated solutions were prepared by adding an excess drug to the vehicles and shaking on the shaker for 24 hrs at 25°C under constant vibration. Filtered samples (1ml) were diluted appropriately with suitable buffer and solubility of Clobazam was determined spectrophotometrically at suitable nm.

Flow properties of the pure drug:

Angle of repose

The angle of repose is determined by using funnel method. The accurately weighed blend is taken in a funnel. The height of the funnel is adjusted in such a way that the tip of the funnel just touches the apex of the heap of the blend. The drug-excipient blend is allowed to flow through the funnel freely onto the surface. The diameter of the powder cone is measured and angle of repose is calculated using the following equation. The Angle of Repose less than 30° shows the free flowing of the material.

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

Bulk density

Apparent bulk density is determined by pouring a weighed quantity of blend into graduated cylinder and measuring the volume and weight.

$$Db=M/Vo$$

Tapped density:

It is determined by placing a graduated cylinder, containing a known mass of drug-excipients blend. The cylinder is allowed to fall under its own weight onto a hard surface from the height of 10 cm at 2-second intervals. The tapping is continued until no further change in volume is noted.

$$DT=M/Vt$$

Compressibility index :

The simplest way for measurement of free flow of powder is compressibility, an indication of the ease with which a material can be induced to flow is given by compressibility index(I) which is calculated as follows,

$$\text{Carr's Index (I)} = (\text{Tapped Density} - \text{Bulk Density})/(\text{Tapped Density}) \times 100$$

Hausner's Ratio :

Hausner's ratio is an indirect index of ease of powder flow. It is calculated by the following formula

$$\text{Hausner's Ratio} = \frac{\text{Bulk Density}}{\text{Tapped Density}}$$

Preparation of Standard Stock Solution:

10mg of Clobazam was accurately weighed into 10 ml volumetric flask and dissolved in a small quantity of 6.8 pH Buffer solution. The volume was made up to 10 ml with the 6.8pH Buffer solution to get a concentration of (1000 µg/ml). From this, 1 ml was withdrawn and diluted to 10 ml with 6.8pH Buffer solution to get a concentration of (100 µg/ml) SS-II.

Determination of UV spectrum: -

From stock solution (SS-II), 1 ml was withdrawn and the volume was made up to 10 ml with 6.8 pH Buffer solution to get a concentration of 10 µg/ml. UV scan range was taken between the wavelengths 200-400 nm. It gave a peak at 233 nm and the same was selected as λmax for Clobazam.

Calibration Curve in 6.8 pH Buffer of the solution:

From the standard stock solution (SS-II), 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml were withdrawn and volume was made up to 10 ml with 6.8pH Buffer solution to give a concentration of 2, 4, 6, 8, 10 and 12 µg/ml. The

absorbance of these solutions was measured against a blank of 6.8pH Buffer solution at 233 nm for Clobazam and the absorbance values are summarized in Table 6.1. The calibration curve was plotted, drug concentrations versus absorbance were given in the Fig..

Drug-excipient compatibility study

a) Physical mixtures of drug and excipients were prepared by grinding specific ratios of drug and excipients in a mortar. A sample of 3-4 grams was loaded in a glass vial, covered with the rubber stopper, sealed with an aluminum cap and labeled properly. Samples were observed and the color was recorded for initial evaluation and loaded into stability chambered at 400 c temperature and 75% relative humidity for 30 days to study the Compatibility study. Samples were removed after 15 days and 30 days and observed for any change in the color.

FTIR spectroscopy

The physical compatibility between the pure drug and polymers used in the research was tested by Infra Red (IR) spectroscopy. FTIR absorption spectra of pure drug and physical mixture were recorded in the range of 400-4000cm-1 by KBr disc method using FTIR spectrophotometer.

Preparation Method:[9]

Formulation of Oral Thin Films of Clobazam:

The oral thin films of Clobazam was prepared by solvent casting technique. The Oral Thin Films were prepared using polymers like Gelatin, PVA. Propylene glycol is used as a plasticizer and super disintegrants like Ludiflash and Crospovidone. The calculated amount of polymers i.e film forming agents and disintegrants were dispersed in the three-fourth volume of a beaker with continuous stirring using magnetic stirrer and the final volume was adjusted with distilled water. The calculated amount of Clobazam was incorporated in the polymeric solutions after levitation with required volume of Propylene Glycol, citric acid, Aspartame and Vanilla Flavor. The solution was cast onto Glass Plate then kept in hot air oven at 40^0c. The films were punched into size of 4cm^2 containing 10mg of Clobazam . By carrying out the trial and error method different concentrations for a film forming polymers were used like Gelatin, PVA. It has been found that 200mg of gelatin, 200 mg of PVA shows better films. Which these concentrations of films were prepared by dissolving different quantities of film forming polymers in required amount of water.

Table.1 Formulation details of Clobazam Oral thin films

Formulation Code / Ingredients(mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Clobazam	45	45	45	45	45	45	45	45	45	45	45	45
Xanthan Gum	100	100	100	100	100	100	-	-	-	-	-	-
Gelatin	-	-	-	-	-	-	100	100	100	100	100	100
Lycoat	22.5	45	67.5	90	112.5	135	-	-	-	-	-	-
Crospovidone	-	-	-	-	-	-	22.5	45	67.5	90	112.5	135
Aspertame	5	5	5	5	5	5	5	5	5	5	5	5
Valina Flavor(mg)	5	5	5	5	5	5	5	5	5	5	5	5
Propylene Glycol(ml)	20	20	20	20	20	20	20	20	20	20	20	20
Distilled water	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Calculation of dose for Clobazam:

The dose of Clobazam is 45 mg. Therefore, amount of Clobazam required in 4 cm2 film is 5 mg.

- Length of glass plate =6 cm.
- Width of glass plate =6 cm.
- Area of the plate =36 cm^2.
- No. of 4 cm^2 films present whole plate =36/4 =9 films.
- Therefore, Each films contains 5 mg of drug
- 9 films contain 45 mg drug (9*5).

So, the Labelled claim of drug = 5 mg

Evaluation of Oral Thin Films:

a) Physical appearance and surface texture of film:

This parameter was checked simply with visual inspection of films and evaluation of texture by feel or touch.

b) Weight uniformity of films[10]

Three films of the size 4cm square were weighed individually using digital balance and the average weights were calculated.

c) The thickness of films[11]

The thickness of the films was measured using screw gauge with a least count of 0.01mm at different spots of the films. The thickness was measured at three different spots of the films and average was taken.

d) Folding endurance of films[12]

The flexibility of films can be measured quantitatively in terms of folding endurance. Folding endurance of the films was determined by repeatedly folding a small strip of the films (approximately 4 cm^2) at the same place till it broke. The number of times films could be folded at the same place, without breaking gives the value of folding endurance.

e) Surface pH of films[13]

Surface pH was determined by the films were allowed in contact with 1ml of distilled water. The surface pH was noted by bringing a combined glass electrode or pH paper near the surface of films and allowing equilibrate for 1 min.

f) In vitro disintegration time of films[14]

Disintegration test was performed in the USP disintegration time testing apparatus. 6.8 pH Buffer solution used as a medium. The films were placed in the tubes of the container and disintegration time was recorded.

g) Drug content uniformity study of films[15]

The films were tested for drug content uniformity by a UV-Spectrophotometric method. Films of 2 cm diameter were cut from three different places from the casted films. Each film was placed in 100 ml volumetric flask and dissolved in 6.8 pH Buffer solution and 0.2 ml is taken and diluted with Buffer up to 10 ml. The absorbance of the solution was measured at 233 nm using UV/visible spectrophotometer (Single beam spectrophotometer (YIS-294)). The percentage drug content was determined using the standard graph and the same procedure was repeated for three films.

h) In-vitro Dissolution Study[16]

In vitro dissolution of Clobazam Oral thin films was studied in modified USP type 5 apparatus dissolution test apparatus 900ml 6.8 pH Buffer solution was used as dissolution medium. The stirrer was adjusted to rotate at 50rpm.The temperature of dissolution medium was maintained at 37±0.5ºC throughout the experiment. One film was used in each test. Samples of dissolution medium (5ml) were withdrawn by means of a syringe fitted with pre-filter at known intervals of time and analyzed for drug release by measuring the absorbance at 233 nm. The volume withdrawn at each time interval was replaced with the fresh quantity of dissolution medium. Cumulative percent Clobazam released was calculated and plotted against time.

i) Drug Release Kinetics

To analyze the mechanism of the drug release rate kinetics of the dosage form, the data obtained were plotted as:

1) Cumulative percentage drug released Vs time (In-Vitro drug release plots)
2) Log cumulative percentage drug remaining Vs Time (First order plots)

Zero order release rate kinetics:

To study the zero–order release kinetics the release rate data are fitted to the following equation.

$$F = K.t$$

First Order Kinetics:

A first order release would be predicted by the following equation

$$Log\ C = log\ Co \qquad Kt\ 2.303$$

RESULTS AND DISCUSSION

Solubility

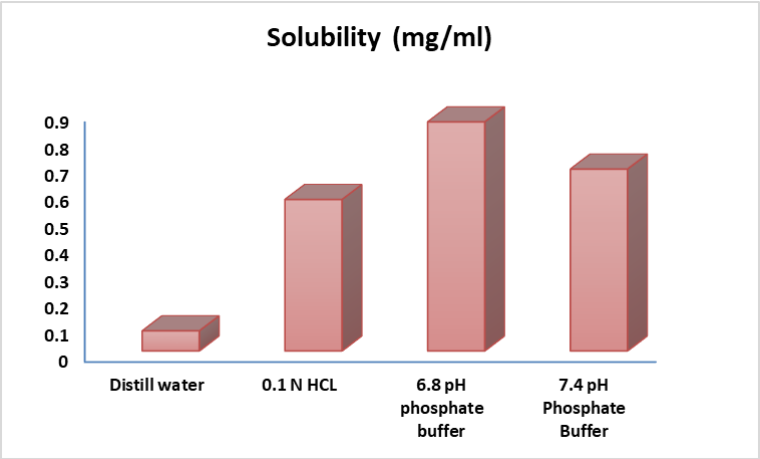

Figure.1 Solubility graph

Discussion:
From the conducted solubility studies in various solutions, we can say that 6.8 pH phosphate Buffer solutions have more solubility when compared to other buffer solutions.

Flow properties of the pure drug:

Table.2 Flow properties of the pure drug

Angle of repose	27.02±1.21
Bulk density	0.348±0.004
Tapped density	0.457±0.008
Carr's index	14.26±1.27
Hausner's ratio	1.16±0.04

Discussion: From the above flow properties of the pure drug, it was concluded that the all the parameters are within the limits indicating the free flow of drug.

UV spectrum of Clobazam:

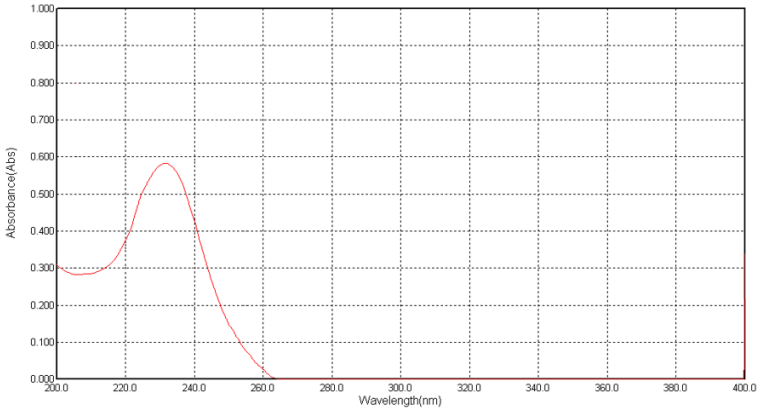

Figure.2 Absorption maxima of Clobazam in 6.8 pH phosphate buffer

Discussion: A solution of Clobazam containing the conc. 8 µg/ ml was prepared in 6.8 pH Buffer buffer and UV spectrum was taken using Single Beam Spectrophotometer(YIS-294). The solution was scanned in the range of 200 – 400 nm. The maximum absorbance was found to be at 233 nm.

Standard Calibration Curve of Clobazam In 6.8 pH Phosphate Buffer:

Standard calibration curve of Clobazam was drawn by plotting absorbance vs concentration. The λmax of Clobazam in 6.8 pH phosphate buffer was determined to be 233 nm as shown in Fig. The absorbance values are tabulated in Table. Standard calibration curve of Clobazam in the Beer's range between 0-30 µg/ml is shown in Fig.

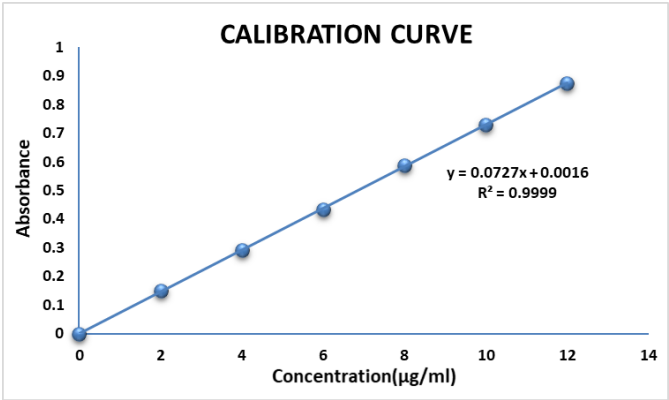

Figure.3 Standard calibration curve for Clobazam in 6.8 pH phosphate buffer at λmax 233 nm.

Discussion:
The linearity was found to be in the range of 2-12 g/ml in 6.8 pH phosphate buffer. The regression value was closer to 1 indicating the method obeyed Beer-lambert's law.

Compatibility Study:

Pure Drug:

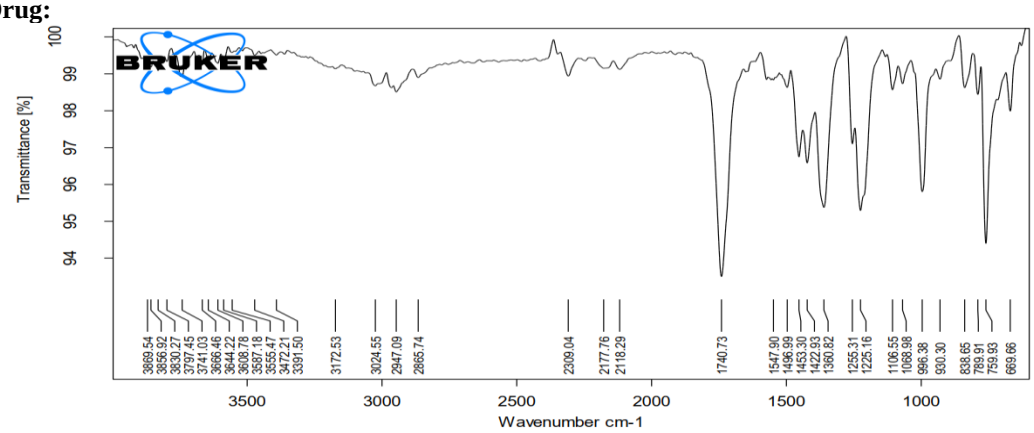

Figure.4 I.R. Spectra of pure drug

Optimized formulation:

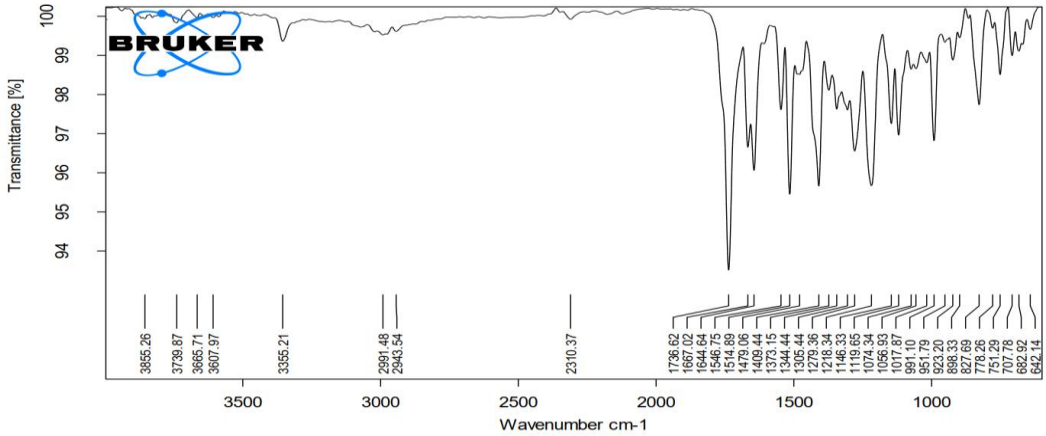

Figure.5 I.R. Spectra of optimized formulation

Discussion: From the drug excipient compatibility studies we observed that there are no interactions between the pure drug (Clobazam) and optimized formulation (Clobazam + excipients) which indicates there are no physical changes.

Evaluation of Oral Thin Films Formulations:

Table.3 Physical appearance and surface texture of films

Formulation Code	Physical appearance	Surface of Film
F1	Semi transparent	Smooth surface
F2	Semi transparent	Smooth surface
F3	Semi transparent	Smooth surface
F4	Semi transparent	Smooth surface
F5	Semi transparent	Smooth surface
F6	Semi transparent	Smooth surface
F7	Transparent	Smooth surface
F8	Transparent	Smooth surface
F9	Transparent	Smooth surface
F10	Transparent	Smooth surface
F11	Transparent	Smooth surface
F12	Transparent	Smooth surface

Discussion: These parameters were checked simply with visual inspection of films and by feel or touch. The observation suggests that the films are having the smooth surface and they are semitransparent and transparent enough to see.

Evaluation parameters of oral thin films

Table.4 Evaluation of Oral Thin Films of Clobazam

Formulation Code	Avg. Weight (mg)	Avg. Thickness(mm)	Avg. Folding Endurance
F1	22.05±1.45	0.15±0.01	134±1
F2	25.19±1.06	0.17±0.02	136±2
F3	27.89±1.13	0.19±0.01	137±2
F4	30.15±1.37	0.20±0.01	139±1
F5	32.35±1.45	0.21±0.02	141±2
F6	34.20±1.85	0.22±0.02	143±1
F7	21.51±1.10	0.14±0.01	133±2
F8	24.19±1.26	0.15±0.01	135±2
F9	26.54±1.46	0.13±0.01	137±1
F10	28.71±1.75	0.16±0.02	139±1
F11	31.16±1.51	0.17±0.01	143±2
F12	33.49±1.29	0.18±0.01	146±1

Discussion:

The average weight the films was found in between 21.51±1.10mg- 34.20±1.85mg. The average thickness of the films was found in between the range of 0.13±0.01-0.22±0.02mm. The average folding endurance of the films was been found in between the ranges of 134±1-146±1.

Table.5 Evaluation of Oral thin films of Clobazam

Formulation Code	Avg. Drug Content Uniformity (%)	Avg. In Vitro Disintegration(sec)	Avg. Surface pH
F1	91.12±1.16	26±1.0	6.6±0.02
F2	93.67±1.37	24±230	6.7±0.01
F3	94.45±1.45	23±1.0	6.5±0.02
F4	95.37±1.20	20±1.0	6.8±0.01
F5	94.02±1.14	17±2.0	6.6±0.02
F6	97.16±1.36	15±2.0	6.7±0.01
F7	92.42±1.66	24±1.0	6.7±0.02
F8	94.97±1.12	22±1.0	6.8±0.01
F9	96.45±1.37	21±2.0	6.6±0.02
F10	97.27±1.45	18±2.0	6.5±0.01
F11	98.45±1.20	16±1.0	6.7±0.02
F12	99.13±1.46	14±1.0	6.8±0.01

Discussion:

The average content uniformity of the formulations from F1 to F12 was found in between 91.12±1.16% - 99.13±1.46%. The Disintegration time of the films from F1 to F12 was in between the range of 14±1.0- 26±1.0. The average surface pH of the films was in the range of pH 6.5±0.02-6.8±0.01.

In-Vitro Dissolution Study:

The in-vitro drug release study of oral thin films from each batch (F1 to F12) was carried out in 6.8 pH phosphate buffer solution for 30 mins and the values are shown in Table.

Table.6 In vitro dissolution studies

Time(min)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
5	38.27±1.16	45.17±1.47	51.42±1.10	53.57±1.20	61.48±1.37	65.45±1.18
10	46.34±1.51	61.65±1.45	65.45±1.20	69.48±1.16	70.46±1.25	71.78±1.75
15	54.76±1.20	69.38±1.47	67.17±1.15	77.37±1.25	78.45±1.32	86.25±1.68
20	68.67±1.45	78.45±1.28	79.67±1.35	84.45±1.37	85.18±1.45	92.59±1.17
25	75.65±1.37	86.34±1.45	88.45±1.12	94.45±1.12	91.35±1.25	98.81±1.47
30	86.47±1.12	91.67±1.12	98.67±1.18	98.86±1.37	99.25±1.16	
35	98.32±1.48	99.85±1.75				

Table.7 In vitro dissolution studies

Time(min)	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
5	31.75±1.67	45.14±1.52	47.78±1.46	49.48±1.14	55.75±1.34	68.14±1.20
10	47.20±1.14	53.32±1.63	56.20±1.12	60.56±1.37	63.10±1.45	77.46±1.27
15	60.78±1.25	67.32±1.84	69.24±1.45	73.10±1.52	76.20±1.54	89.14±1.51
20	74.46±1.15	76.35±1.28	78.67±1.20	81.45±1.39	87.67±1.58	94.35±1.45
25	88.56±1.14	89.76±1.15	91.14±1.75	89.18±1.54	96.14±1.20	99.25±1.45
30	93.75±1.25	95.48±1.36	97.45±1.36	98.54±1.20	99.43±1.54	
35	98.27±1.12	98.51±1.75	98.85±1.24			

Discussion:

This shows that effectiveness of super disintegrants is in the order of Crospovidone> Lycoat. The concentration of super disintegrant's in the formulations also increased the dissolution rates. In all the formulations 100mg concentration of Gelatin and 135 mg of Crospovidone, there was linearly increase in dissolution rate. At higher concentration, all the formulations showed increase in dissolution rate.

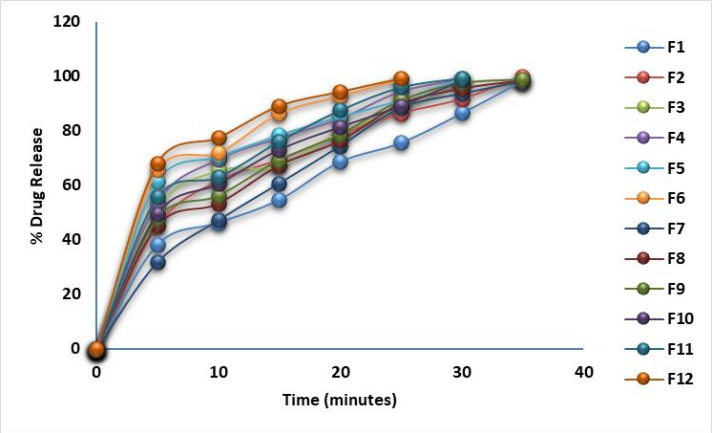

Figure.6 In-vitro drug release of formulations (F1-F12)

Drug Release Kinetics of Clobazam

Zero Order Release Kinetics

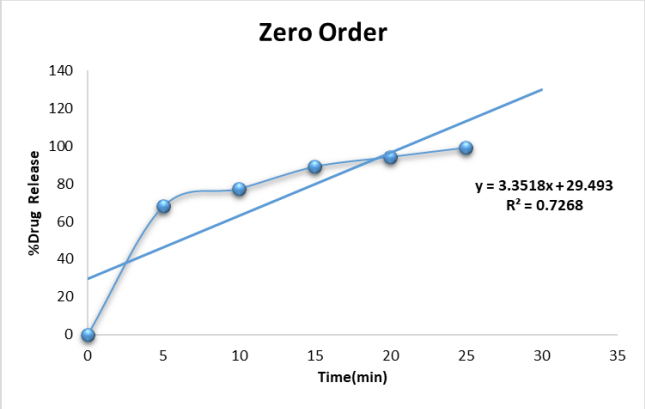

Figure.7 Zero order release profile of Clobazam Best formulation (F12)

First Order Release Kinetics Data

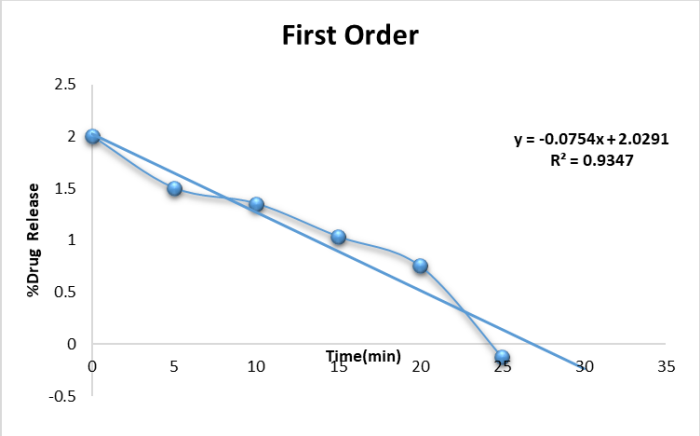

Figure.8 First order release profile of Clobazam Best formulation (F12)

Discussion: The in vitro dissolution data for best formulation F12 were fitted in different kinetic models i.e, zero order, first order. Optimized formulation F12 follows first order.

SUMMARY AND CONCULSION

In the present study Oral drug delivery system of Clobazam were successfully developed in the form of oral thin films which offers a suitable and practical approach in serving the desired objective of faster disintegration and dissolution characteristics with increase bioavailability. Oral thin films of Clobazam were prepared by using Crospovidone and Lycoat as super disintegrants. Under the pre-formulation studies, API characterization and drug-excipient compatibility studies were carried out. The API characterization showed compliance with the drug characteristics. The disintegrants and other excipients were selected based on the satisfying results produced during drug- excipient compatibility studies to develop the final formulation. The final suitable formulation (F12) was achieved fruitfully by the solvent casting method using Gelatin as film forming agent and Crospovidone as disintegrant which exhibited a rapid disintegration time (14±1.0 sec) and in vitro drug release (99.25±1.45%) ate the end of 25minutes. Considering the results of batches containing Crospovidone and Lycoat as disintegrant it can be concluded that the formulation F12 was meeting the higher in-vitro correlation limits and in less instance of time when subjected to the comparison with other formulation with Crospovidone as the disintegrating agent. It was also observed that solvent casting method was the best suitable method used for immediate drug release. Finally, in vitro dissolution data for best formulation F12 were fitted in different kinetic models i.e, zero order, first order. Optimized formulation F12 follows first order.

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