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Formulation and evaluation of mucoadhesive sublingual tablet of rosuvastatin calcium

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ABSTRACT

Rosuvastatin calcium is a lipid lowering agent which has been selected as a model drug for investigation, because of its very low bioavailability (20%) due to extensive first pass metabolism. In this work a new attempt was made to enhance the solubility, dissolution rate and oral bioavailability of poorly soluble rosuvastatin calcium by formulating it as direct compression method by using mucoadhesive polymer chitosan and various super disintegrant like sodium starch glycolate and croscarmelose sodium. Solubility of rosuvastatin calcium increased by using complexing agent β-cyclodextrin by kneading method. Mucoadhesive sublingual tablet were evaluated by pre and poss comprssion parameter. Precompression test like bulk density, tap density, angle of repose, cars index and postcompression test include thickness, hardness, friability, weight variation, drug content, disintegration time, wetting time, surface pH, in-vitro drug release and ex-vivo mucoadhesive time. USP II type appraratus is used for measuring mucoadhesion time with fresh got mucus membrane. The order of drug release from the dosage form has been determined. The optimized formulation followed Hixon-Crowel kinetics release. The physiochemical interaction between the drug and polymer were investigated using FTIR and DSC study.Optimized formulation (F4) showed 96.69% in-vitro release within 60 min. The present study concluded that sublingual delivery of mucoadesive rosuvastatin calcium tablets can be a good way to bypass the first pass metabolism and it will render great bio availability.

Keywords: Rosuvastatin calcium, β-cyclodextrin, chitosan, first pass metabolism, kneading method.

INTRODUCTION

The interest in novel routes of drug administration occurs from their ability to enhance the bioavailability of drugs impaired by the narrow absorption window in the gastrointestinal tract. Drug delivery via the sublinguall route using mucoadhesive dosage forms offers such a novel route of drug administration [1].The main aim of these study is enhance the bioavailability of drugs by increasing solubility, avoiding first-pass metabolism and increase absorption by increasing contact time of drug and mucus membrane.

Drug delivery via the oral mucous membrane is considered to be a promising alternative to the oral route. Sublingual route is a useful when rapid onset of action is desired with better patient compliance than orally ingested tablets. The portion of drug absorbed through the sublingual blood vessels bypasses the hepatic first-pass metabolic processes giving acceptable[2,3].

Mucoadhesion can be defined as a state in which two components, of which one is of biological origin are held together for extended periods of time by the help of interfacial forces. The important role of mucoadhesive dosage forms is to help the controlled-release of an active ingredient through increasing of the residence time of dosage forms in gastrointestinal tract. Mucoadhesive drug delivery systems its having various advantages and it has a lot of potential in formulating dosage forms for various chronic diseases [4,5]. Mucoadhesive drug delivery systems

includes the following: Buccal delivery system, Oral delivery system, Vaginal delivery system, Rectal delivery system Nasal delivery system, Ocular delivery system [6].

Rosuvastatin calcium (RC), a HMG CO-A Reductase enzyme inhibitor, is widely used in the treatment of Hyper-lipoproteinemia. Hyperlipidaemia is the condition indicating increase in lipid level. Both these conditions may cause narrowing and hardening of the arteries, i.e. atherosclerosis (coronary artery disease-CAD). Thus, hyperlipoproteinemias is one of the leading causes of ischemic heart disease, myocardial infarction and cerebral vascular accidents. Thus there is emergent need of the treatment of hyper-lipidaemia [7].

EXPERIMENTAL SECTION

Materials:
Rosuvastatin calcium was obtained as a gift sample from Watson Pharma Ltd, Mumbai, β-cyclodextrin was purchased from S. D. Fine Chemicals Ltd., Mumbai, Microcrystalline cellulose, Chitosan and Croscarmellosesodium were purchased from Ozone International, Mumbai, Mannitol, Sodium starch glycolate was obtained as a gift sample from Ranbaxy Fine Chem. Ltd., Delhi. All other reagents and chemicals used were of analytical reagent grade.

Compatibility studies:
The drug-excipient compatibility studies were carried out using Fourier Transform Infrared Spectrophotometer (FTIR) and Differential Scanning Calorimetry (DSC). IR spectra and DSC data of pure drug and physical mixture of drug and excipients were recorded.

Phase solubility study:
Phase solubility studies were carried out according to the method reported by Higuchi and Connors. An excess amount of the Rosuvastatin calcium (5mg) was added to 10ml aqueous solutions containing increasing concentrations of β-cyclodextrin (0-10mM). The β-cyclodextrin solution was prepared in Methanol. The flasks were sealed and shaken at 25^0C for 72 hrs on a rotary flask shaker. At equilibrium after 72 hrs, supernatant was filtered through a 0.45µm membrane filter. The filtered samples were diluted suitably and analyzed at 242nm using UV spectrophotometer for drug content. The apparent stability constant (Ks) of the complexes was calculated from the slope of the phase solubility diagram.

$$\mathbf{K_{1:1}=Slope / So (1 - Slope)}$$

Where $K_{1:1}$ is the stability constant for the complex and So is the solubility of the drug in the absence of β-cyclodextrin [8].

Preparation of inclusion complexes:
Inclusion complexes of Rosuvastatin calcium with β-CD were prepared by Kneading method.

1. Kneading method: RST Calcium and β-CD in 1:1mM ratio were taken in a mortar & mixed thoroughly; a small volume of ethanol (1:1) solution was added, while triturating to get slurry like consistency. The thick slurry was kneaded for 45 minutes and then dried at 45˚C for two days. The dried mass was pulverized and sieved through mesh no.100.

Formulation of mucoadhesive sublingual tablet:
Direct compression method:
Fast dissolving tablets were prepared by direct compression using RST Ca:β-CD inclusion complex prepared by kneading method. The formula included variable amounts of superdisintegrants and other excipients are shown in Table No.1. The amount of complex equivalent to 10 mg of drug per tablet were taken and then mixed with directly compressible diluents and superdisintegrant in a plastic container. Magnesium stearate were pass through sieve no. 60, mixed and blended with the initial mixture in the plastic container followed by compression of the blend. Compression was performed on 8 station Lab press tablet compression machine using 6-mm punches [9].

Evaluation test for mucoadhesive sublingual tablet:
mucoadhesive sublingual tablets were evaluated for for various tests as Thickness, Hardness, Friability, Weight variation, Content uniformity and Disintegration Time [10].

Table No. 1: Formulation composition of tablet (250mg)

Ingredients	F1	F2	F3	F4	F5
Complex (Eduivalant to 10mg of drug)	34.37	34.37	34.37	34.37	34.37
Mannitol	30	30	30	30	30
Citric acid	2	2	2	2	2
Chitosan	10	8	6	4	2
Crosscarmelose sodium	1	2	3	4	5
Sodium starch glycolate	1	2	3	4	5
Talc	2	2	2	2	2
Magnesium stearate	2	2	2	2	2
Microcrystalline cellulose	60	60	60	60	60
Lactose	107.63	107.63	107.63	107.63	107.63
Total	250	250	250	250	250

Wetting time:
The method was applied to measure tablet-wetting time. A piece of tissue paper folded twice was placed in a small Petri dish (i.d. = 6.5 cm) containing 6 ml of water, a tablet was put on the paper, and the time for complete wetting was measured. Three trials for each batch were performed and standard deviation was also determined [11].

Surface pH study:
Surface pH study of the buccal tablet was determined in order to investigate the possibility of any side effect in-vivo. As an acidic or alkaline pH may cause irritation to the mucosa, it was determined to keep the surface pH as close to neutral as possible. The Bottenberg method was used to determine the surface pH of the tablet. A combined glass electrode was used for this purpose. The tablet was allowed to swell by keeping it in contact with 1ml of distilled water (pH 6.5+ 0.05) for 2hours at room temperature. The pH was measured by bringing the electrode in contact with the surface of the tablet and allowing it to equilibrate for 1minute [12].

In vitro dissolution studies:
The release rate of Rosuvastatin calcium baccul tablets was determined using USP Dissolution Testing Apparatus II (Paddle type). The dissolution test was performed using 900 ml of Phosphate buffer pH 6.8, at 37 ± 0.5°C and speed of 50 rpm. Aliquot (5 ml) of the solution was collected from the dissolution apparatus for every 10min and were replaced with fresh dissolution medium. The aliquots were filtered through whatmann filter paper no. 41. Absorbance of these solutions was recorded at 242nm (Rosuvastatin calcium) in photometric mode for single drug and in multicomponent mode analysis for combined drugs. Aliquots were withdrawn at ten minute interval from a zone midway between the surface of dissolution medium and the top of rotating paddle not less than 1 cm apart from the vessel wall. Drug content in dissolution sample was determined by software (PCP disso v3) version [10,13].

Mucoadhesion time:
Mucoadhesion can be defined as a state in which two components, of which one is of biological origin, are held together for extended periods of time by the help of interfacial forces. The ex-vivo resident time was determined using a modified USP dissolution apparatus. The dissolution medium was composed 800ml 6.8 phosphate buffer maintain at 37± 2°C. Got buccal tissue was fixed to the surface of the glass layer, vertically attached to the apparatus. The buccal tablet was hydrated from the surface using 0.5ml of 6.8 phosphate buffer, and then hydrated surface was brought in to contact with the mucosal membrane. The time necessary for complete erosion are de-attachment of the tablet from the mucosal surface was recorded [11].

Stability studies:
The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and enables recommended storage conditions, re-test periods and shelf lives to be established [14].

RESULTS AND DISCUSSION

Phase solubility studies:
The effect of β-cyclodextrin on the solubility of Rosuvastatin calcium in phosphate buffer solution was investigated at 25°C as in Fig. No.1. and readings are given in Table No.2. It was found that the solubility of Rosuvastatin calcium was increased markedly by complexation with β-cyclodextrin. The Phase solubility study was done to determine the stoichiometric proportion of Rosuvastatin calcium with complexing agent β-CD. The phase solubility analysis indicated formation of a 1:1 molar inclusion complex of drug with β-cyclodextrin is optimum to increase the solubility of Rosuvastatin calcium.

The solubility curve was classified as type A_L which indicates the formation of 1:1-complexes at pH 6.8 at a given cyclodextrin concentration range (0 – 10 mM) used. There was 3 fold increases in the solubility of drug. Stability constant of Rosuvastatin calcium: β-CD complexes were determined to be 67.18 M^{-1} at pH 6.8. The solubility of the drug in the absence of cyclodextrin was 0.1528 mM which is in good agreement with the literature value.

Table No.2: Phase solubility study of Rosuvastatin calcium.

Sr.No.	Conc. of β-CD (Mm)	Conc. of RST (Mm)
1.	0	0.1527
2.	2	0.1814
3.	4	0.1918
4.	6	0.2013
5.	8	0.2159
6.	10	0.2251

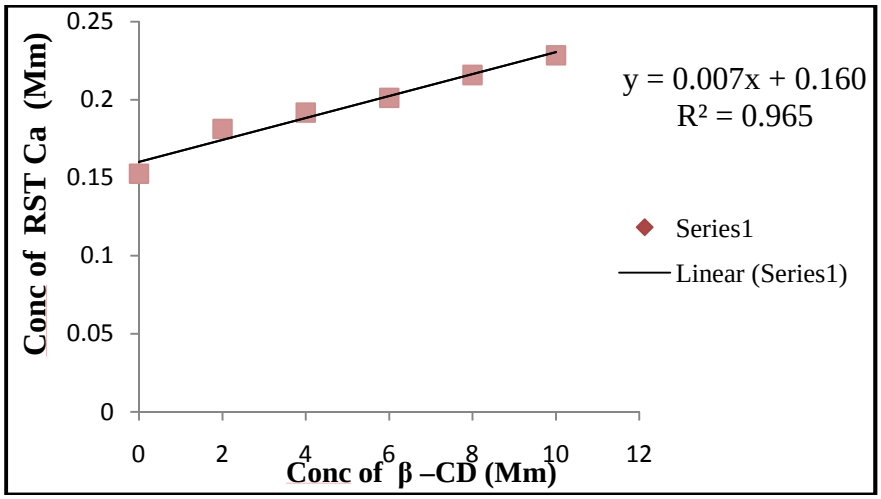

Fig. No.1: Phase solubility diagram of Rosuvastatin calcium

Characterization of rosuvastatin calcium inclusion complex:

Drug content uniformity:

Inclusion complexes of Rosuvastatin calcium with β-CD prepared by Kneading method showed consistency in drug content (almost 100%).

IR spectral analysis:

IR spectra of pure drug and inclusion complexes of Rosuvastatin calcium with β-CD are given in Fig. No.2. and Fig. No.3. It was suggested that vibrating and bending movements of guest molecule i.e. Rosuvastatin calcium were restricted due to formation of inclusion complexes. It may be the aromatic ring portion of Rosuvastatin calcium, which has been included into the cavity of β-cyclodextrin

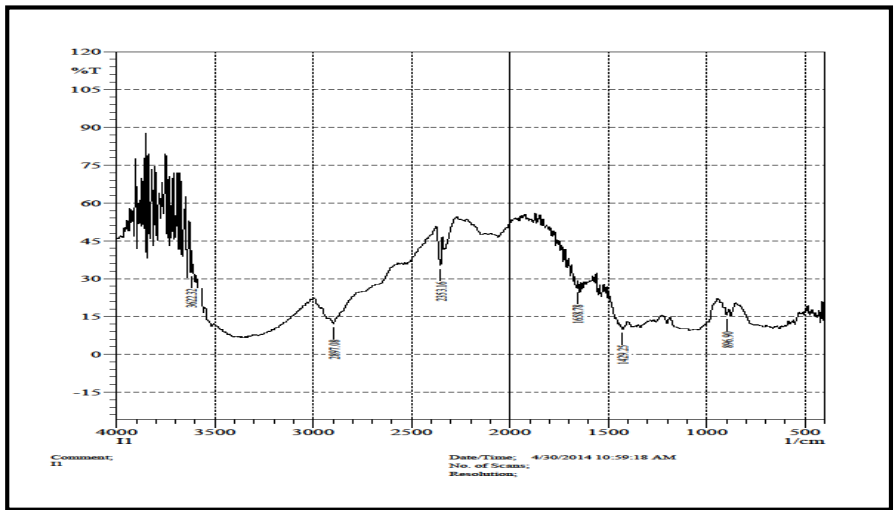

Fig. no.2: IR spectrum of Rosuvastatin calcium

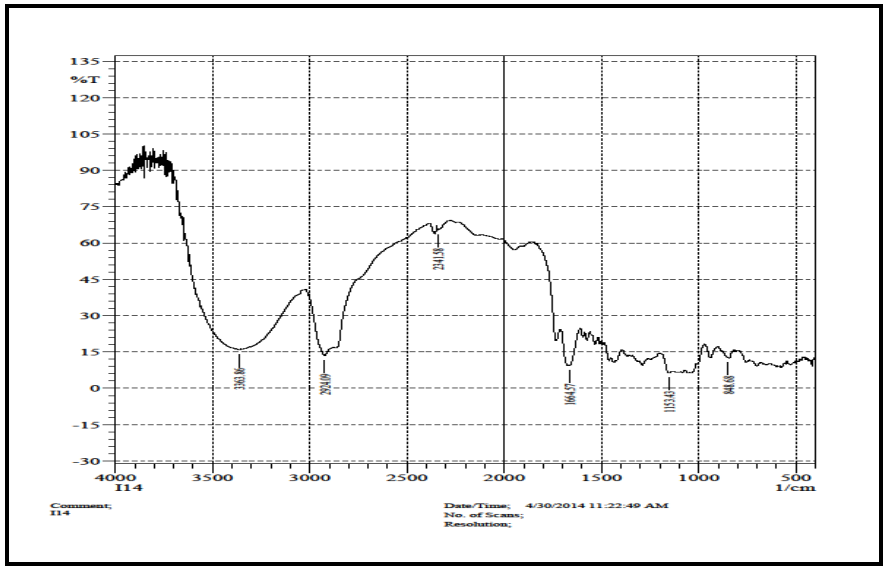

Fig. No. 3: IR spectrum of Rosuvastatin calcium and β-CD.

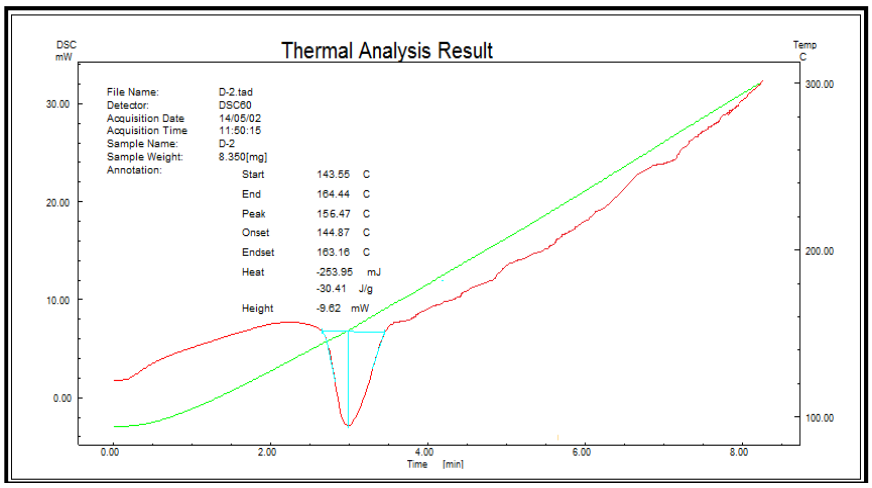

Fig. No.4 : DSC data of Pure Rosuvastatin calcium

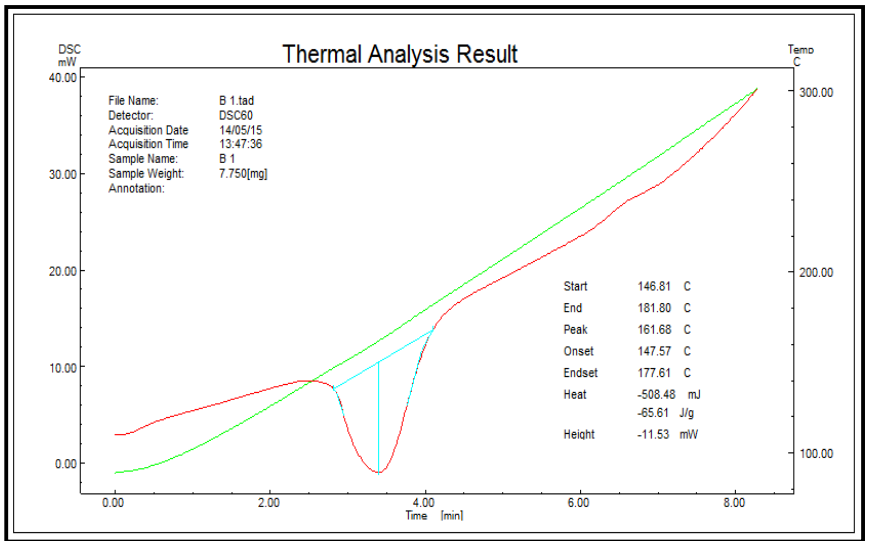

Fig. No.5: DSC data of Rosuvastatin calcium and Exicipeints

Evaluation of mucoadhesive sublingual tablet:

Differential scanning calorimetry:

The DSC thermogram shows the endothermic peak at 156.47^{0}C and 161.68^{o}C of pure Rosuvastatin calcium and formulation respectively indicated the melting point which was reported in literature. There was no sharp change in melting point of drug. Thus there was no significant interaction between the drug, and polymer. (Fig. No. 4. and Fig. No. 5.)

Mucoadhesive sublingual tablet of Rosuvastatin calcium were prepared by using direct compression method. Before compression, the powder blends were subjected to Precompression evaluation to determine the flow properties and the compressibility. The results of the Precompression evaluation are as given in Table No.3.

Table No. 3: Evaluation of pre-compression parameter of tablet (n=3)

Formulation code	Angle of repose (θ)	Bulk density (gm/ml)	Tap density (gm/ml)	% Compressibility
F1	21.17±1.1507	0.30±0.041	0.28±0.068	7.14±0.881
F2	21.11±1.017	0.34±0.034	0.33±0.073	4.03±1.831
F3	22.14±1.8615	0.32±0.037	0.30±0.040	6.66±0.479
F4	21.25±1.1663	0.34±0.110	0.32±0.073	6.25±0.881
F5	22.18±1.766	0.30±0.072	0.29±0.095	4.44±1.766

Post-compression parameters:

All the tablet formulations were subjected for evaluation according to various official specifications. Result of test such as Shape and color, Thickness, Hardness, Friability, Weight variation , % Drug content uniformity and in vitro disintegration time are shown in table no.4.

Wetting time:

Wetting time is closely related to the inner structure of tablets. The result of the wetting time is shown in Table No.4. All formulation showed quick wetting in the range of 24±0.816s to 28±1.731s.

Surface pH:

In an acidic or alkaline pH which may cause irritation to the sublingual mucosa, it was determined to keep the surface pH as close to neutral as possible. Surface pH of all formulation was found to be 6.3±0.1075 to 6.4±0.9968. Surface pH values for all the formulations shown in Table No. 4.

Table no.4: Evaluation of post compression parameter of tablet

Sr.no	Name of tests	F1	F2	F3	F4	F5
1.	Thickness (mm)	4.1±0.026	3.9±0.038	4.1±0.032	4.0±0.041	4.2±0.040
2.	Hardness (kg/cm^2)	3.33±1.34	3.32±1.31	3.52±1.41	3.51±1.40	3.51±1.42
3.	Friability (%)	0.91±0.005	1±0.005	0.7±0.007	0.7±0.003	0.7±0.004
4.	Weight Variation (mg)	251±2.516	249±2.081	250±3.102	249±1.000	251±2.523
5.	Drug Content (%)	98.53±0.3	98.51±0.6	98.44±1.1	98.91±0.1	98.63±0.1
6.	Disintegration Time (min)	4.29±1.70	3.55±1.30	3.35±1.16	3.05±1.72	2.40±0.95
7.	Wetting Time (sec)	24±2.0816	27±2.1453	28±1.7311	25±1.9252	25±2.1356
8.	Surface pH	6.4±1.014	6.4±0.873	6.3±0.961	6.3±0.107	6.4±0.996
9.	Mucoadhesion Time (min)	110	90	70	60	50

In vitro dissolution studies:

All the five formulations were subjected for the in vitro dissolution studies using tablet dissolution tester USP II. The samples were withdrawn at 10 min intervals and analyzed at 242 nm. The results obtained in the *in-vitro* drug release for the formulations F1 to F5 are shown in Fig. No. 6.

Formulation F1 to F5 prepared by direct compression method was found to be drug release in the range of 94.34% to 97.21%. Here in all batch of F1 to F5 the dissolution rate was found to increase linearly with increasing concentration of superdisintegrant. This was marked by increased mucoadhesion time values for tablet formulation containing higher proportions of mucoadhesive polymer i.e. chitosan. In all formulation the drug release was nearer to 100% within 110 minutes. F4 prepared by direct compression method showed good drug release (96.69%) than other formulation.

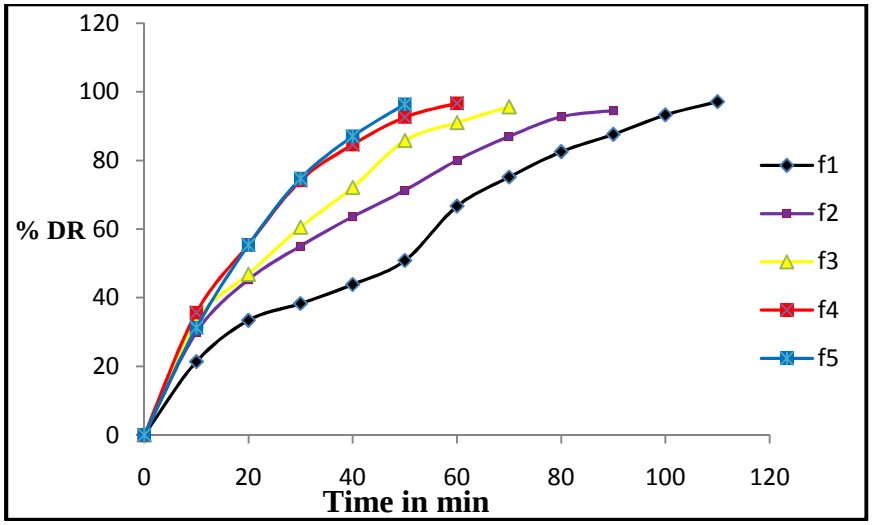

Fig. No.6. In-vitro % drug release of all formulation

Release kinetics and mechanism:
To know the release mechanism and kinetics of Rosuvastatin calcium optimized formulations (F4) were attempted to fit into mathematical models and n, r2 values for zero order, first order, matrix Korsmeyer- Peppas and Hixon-Crowel models were represented in Table No.7.7.

The Hixson-Crowell cube root law describes the release from systems where there is a change in surface area and diameter of particles or tablets. For a drug powder consisting of uniformly sized particles, it is possible to derive an equation that expresses the rate of dissolution based on the cube root of the particles.

$$Q_0^{1/3} - Q_t^{1/3} = K_{Hc}\, t$$

Where, Qt is the amount of drug released in time t,

Q0 is the initial amount of the drug in tablet and KHC is the rate constant for Hixson-Crowell rate equation. Observation of all the R^2 values indicated that the highest R^2 (0.997) value was found for Hixon-Crowel release which are shows in Table No.5. and Fig. No.7.

Table No.5. In-vitro Drug Release Kinetics of F4 formulation

Models	R^2 value	K value
Zero order	0.9090	1.9200
1st order	0.9911	-0.0507
Matrix	0.9953	12.8642
Korsmeyer- Peppas	0.9917	9.9684
Hixon- Crowel	0.9976	-0.0115

Mucoadhesion test:
The residence time for selected formulations varied from 110 to 50 min. The optimized formulation (F4) showed 60 min. The difference in the resident time could be due to the different ratios of polymer, which may affect the muco-adhesion. Residence time values were given in Table No.4. The maximum residence time (110min) was found for formulations F1 and low residence time (50 min) was found for formulations F5. As the polymer concentration in formulation increased, residence time increased.

Stability study:
From the stability study, it was proved that the evaluated formulation (F4) showed there was no influence of variety of environmental factors such as temperature, humidity and light, and during storage conditions or shelf life of drug.

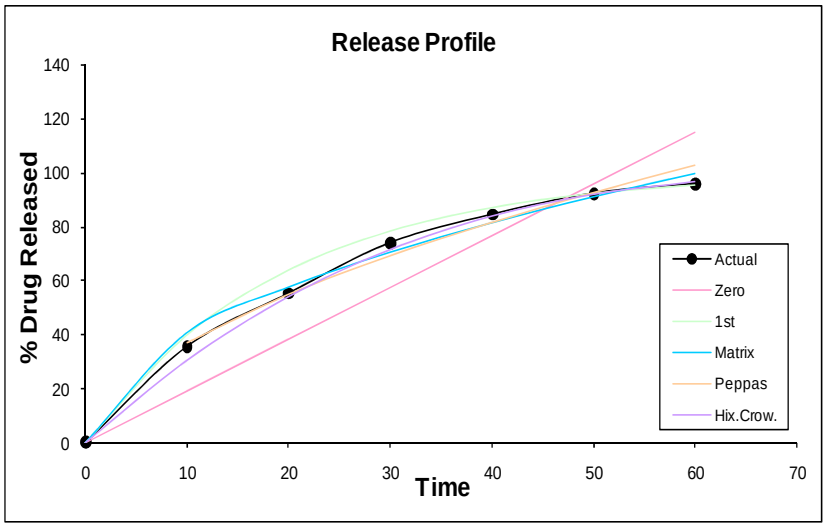

Fig. no.7. In-vitro Drug Release Kinetics of F4 formulation

Table No.6: Stability Parameters after 0, 30, 60 and 90 days

Days	Study conditions specification	% Drug Content Rosuvastatin calcium
Initial	4-8˚C	98.89±0.27
	Room Temperature	98.90±0.53
	$40^0C \pm 2^0C/75\% \pm 5\%$ RH	98.87±0.64
After 30 day	4-8˚C	98.89±0.31
	Room Temperature	98.89±0.83
	$40^0C \pm 2^0C/75\%$ RH ± 5% RH	98.86±0.18
After 60 day	4-8˚C	98.88±0.54
	Room Temperature	98.89±0.46
	$40^0C \pm 2^0C/75\%$ RH ± 5% RH	98.86±0.85
After 90 days	4-8˚C	98.86±0.38
	Room Temperature	98.88±0.88
	$40^0C \pm 2^0C/75\%$ RH ± 5% RH	98.84±0.63

CONCLUSION

Formulation of mucoadhesive sublingual tablets of Rosuvastatin calcium (anti-lipidemic drug) was successfully prepaed by direct compression method. It was concluded that the all evalution parameter of the optimized formulation (F4) was suitable for mucoadhesive sublingual drug delivery. The inclusion complexes of Rosuvastatin calcium with β-CD by kneading method showed increase in solubility. Stability constant value (67.18 M^{-1}) indicate stable complex. Hixon-Crowel models is best fit model for optimised formulation. FTIR and DSC studies concluded that there is no interaction between drug and excipients (β-CD). The mucoadhesive sublingual tablets showed a mucoadhesion time up to 120 min. similarly *in-vitro* dissolution study showed 96.1 % drug release. It can be concluded that formulation F4 could be used for sublingual mucosa without the risk of mucosal irritation.

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