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ENHANCEMENT OF BIOAVAILABILITY OF ATORVASTATIN CALCIUM THROUGH GASTRIC RESIDENT FORMULATION APPROACH

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ABSTRACT

Atorvastatin calcium (ATC), a lipid-lowering drug, is much less bioavailable because of insufficient time for absorption in the GI tract. The real issue in the development of oral drug delivery systems is to prolong the residence time of the dosage form in the stomach or upper GI tract until the drug is completely released and absorbed. Several approaches are currently used to retain the dosage form in the stomach. The principle of ATC floating tablets offers a simple and practical approach to achieve increased GRT to enhance the bioavailability and to obtain CR/SR. ATC Floating tablets are designed based on gas generating principle. Design of ATC floating tablets needs a strong matrix former. All the floating tablets prepared were evaluated for hardness, friability, floating characteristics, swelling index, in vitro drug release characteristics, stability and in vivo radiographic studies and were subjected to FTIR, DSC and p-XRD studies. Formulation TF15 obtained the desired drug release profile and floated with a lag time of 48 sec, for these reasons it was considered as the best formulation among all the formulations. Olibanum might be a promising polymer for Gastroretentive floating drug delivery systems in combination with synthetic hydrophilic polymer i.e., PEO enhanced the floating duration and help to maintain the dimensional stability at initial stage. The bioavailability studies were carried out for the optimized formulation and compared with that of reference formulation in twelve rabbits. Based on in vivo performance significant difference was observed between C_{max}, t_{max}, $t_{1/2}$, $AUC_{0-\infty}$ and MRT of TF15 and control formulation. The increased relative oral bioavailability (f_r) of test formulation (TF15) was 1.94 folds when compared to control formulation. The increased relative oral bioavailability may be due to floating (Gastroretentive) of dosage form, which is desirable for the drugs absorbed in the upper part of GIT. The developed floating tablets of ATC may be used for prolonged drug release, thereby improving the bioavailability and patient compliance.

KEY WORDS

Floating tablets, enhancement of bioavailability, sustained release, Hydrophilic polymers, Atorvastatin calcium (ATC), Olibanum gum resin.

INTRODUCTION

Oral bioavailability is a relative term used to describe the rate and extent of absorption after oral administration of a drug compared to that after its administration via a reference route, usually bolus injection. Bioavailability has no unit, often it is expressed as percentage.

For any drug to elicit its pharmacological response, it's very necessary to become available to body, that is, it has to reach the blood. For drugs orally dosed in solid dosage forms such as tablets or capsules, there are two distinctive processes during absorption:

- Dissolution of solid drug particles to drug molecules in the GI fluid

- Permeation of the drug molecules across intestinal membranes.

Orally administered drugs must pass through the intestinal wall and then the portal circulation to the liver; both are common sites of first-pass metabolism Thus, many drugs may be metabolized before adequate plasma concentrations are reached. *Insufficient time for absorption* in the GI tract is a common cause of low bioavailability. If the drug does not dissolve readily or cannot penetrate the epithelial membrane, time at the absorption site may be insufficient. Those drugs that depend on some form of facilitated transport process generally display good absorption from upper GI tract, but show poor absorption in large intestine (or colon). Hence, their oral bioavailability can be affected by the limited absorptive site. Hence, the concept of an 'Absorption Window' has become popular and need for developing promising absorption strategies for such "Narrow Absorption Window Drugs (NAW Drugs)" arises (Chawla et al., 2003). The Pharmaceutical approach or Formulation approach for bioavailability enhancement can be applied by following technique: Enhancement of *Gastrointestinal Retention (GR)*. There are mainly three approaches which are taken into consideration for formulation of *Narrow Absorption Window Drugs* (NAW Drugs).

- Modification of small intestinal transit
- Bioadhesion
- Gastroretention

Gastroretentive Drug Delivery Systems (GRDDS)

One of the most feasible approaches for achieving a prolonged and predictable drug delivery profiles in gastrointestinal tract is to control the Gastric Residence Time (GRT) using *Gastro Retentive Dosage Forms (GRDFs)* that offer a new and better option for drug therapy (Abdul et al., 2011).

Advantages of GRDDS (Patil et al., 2010)

GRDDS have numerous advantages listed below (Hwang et al, 1998):

These are summarized as follows:

- Enhanced bioavailability
- Sustained Release Drug Delivery/ reduced frequency of dosing
- Site-specific drug delivery
- Reduced fluctuations of drug concentration
- Minimized adverse activity at the colon
- Absorption enhancement

Approaches to Gastric Retention (Vyas and Khar, 2002, p.196)

Floating drug delivery systems (Ravi et al., 2011)

(A) Non-effervescent systems

(i) Layered tablets; (ii) Colloidal gel barrier system; (iii) Microporous compartment system; (iv) Alginate beads; (v) Hollow microspheres / Microballons

(B) Effervescent systems (Vinod et al, 2010).

(i) Volatile liquid containing systems; (ii) Gas generating systems

Non-Floating Systems (Talukdar and Fassihi, 2004)

(A) Swelling systems; (B) Mucoadhesive (Park and Robinson, 1984); (C) High-density systems; (D) Expandable systems.

Need for the investigation

Biotechnology is the use of living systems and organisms to develop or make products, or "any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use". Bioavailability of drugs is a very important pharmacokinetic parameter for their clinical efficacy. A careful literature study revealed lack of systematic, comprehensive and cost-effective approach to improve oral bioavailability of Atorvastatin Calcium (ATC) (oral bioavailability is about 14%).

In the present investigation, different Gastroretentive technology platforms were exploited to improve oral bioavailability of ATC. Gastroretention is a known approach to improve the oral bioavailability of drugs which exhibit absorption through a specific anatomical region of gastrointestinal tract, especially upper part of GIT. ATC has an absorption window located at the upper part of the GIT rendering it as a suitable candidate for delivery through Gastroretentive technology. The literature study also revealed negligible research undertaken to explore resinous materials from biological (plant) source in drug delivery technology for improvement of bioavailability. For the first time, I am reporting Olibanum gum resin (obtained from *Boswellia serrata*, *Roxburgh* and other species of *Boswellia)* as a functional excipient for development of dosage forms for improvement of bioavailability of ATC.

The present work is aimed at preparing gastric retentive floating formulations *viz.* floating tablets, mucoadhesive microspheres and *in situ* gel of ATC using various low density polymers and natural polymers. The composition of these formulations will be selected by using *trial and error* methods.

MATERIALS AND METHODS

Materials

Atorvastatin Calcium was a gift from Hetero Drugs Ltd, Hydroxy propyl methyl cellulose **(**HPMC K15M) and polyethylene oxide (PEO) were obtained from M/s S.S.Pharma Scientific Equipments; Olibanum was received from Krystal colloids, Mumbai, India. All other solvents and reagents were purchased from S.S.Pharma Scientific Equipments, India and were of analytical grade.

Methods

Determination of λ_{max} and preparation of calibration curve of ATC in 0.1 N HCl

Preformulation studies were conducted before starting the formulation of gastroretentive floating tablets. This section contains determination of λ_{max}, preparation of standard graphs of the drug i.e., Atorvastatin Calcium.

Formulation of floating tablets of ATC by Direct compression technique

Floating tablets containing ATC were prepared by direct compression technique using varying concentrations of different grades of synthetic and natural polymers with Sodium bicarbonate and ethyl cellulose. All the powders were accurately weighed and passed through an 80 mesh sieve (180 micrometer size). Then, except talc and magnesium stearate, all other ingredients were blended uniformly in glass mortar. After sufficient mixing of drug as well as other components, talc was added, as post lubricant, and further mixed for additional 2- 3 minutes. The blend was compressed into tablets having average weight of TF1 to TF-15 (each 350 mg) using multiple rotary tabletting machines (Riddhi's, India) fitted with an 8mm round flat punches (Tables 1 and 2). Powdered olibanum was extracted using solvent ether from *Boswellia serrata* species (Chowdary et al., 2006a).

Table 1: Formulation Chart for ATC Gastroretentive Floating Tablets (TF1 to TF9)

Ingredients (mg)	Formulation Code								
	TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8	TF9
ATC	80	80	80	80	80	80	80	80	80
HPMC K15M	75	112.5	150	-	-	-	-	-	-
PEO	-	-	-	75	112.5	150	-	-	-
Olibanum	-	-	-	-	-	-	75	112.5	150
Sodium bi carbonate	25	50	37.5	25	50	37.5	25	50	37.5
Ethyl cellulose	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5
Lactose	122.5	60	35	122.5	60	35	122.5	60	35
Talc	5	5	5	5	5	5	5	5	5
Magnesium Stearate	5	5	5	5	5	5	5	5	5
Total Weight (mg)	350	350	350	350	350	350	350	350	350

Table 2: Formulation Chart for ATC Gastroretentive floating tablets (TF10 to TF15)

Ingredients(mg)	Formulation Code					
	TF10	TF11	TF12	TF13	TF14	TF15*
Atorvastatin Calcium	80	80	80	80	80	80
HPMC K15M	75	112.5	150	-	-	-
PEO	22.5	15	7.5	22.5	15	7.5
Olibanum	-	-	-	75	112.5	150
Ethyl Cellulose	37.5	37.5	37.5	37.5	37.5	37.5
Sodium bi Carbonate	30	60	45	30	60	45
Lactose	95	35	20	95	35	20
Talc	5	5	5	5	5	5
Magnesium Stearate	5	5	5	5	5	5
Total Weight (mg)	350	350	350	350	350	350

**Lead Formulation used in Pharmacokinetic studies.*

Preformulation Studies

Preformulation studies (Drug-Excipient interaction studies i.e., FTIR, DSC and p-XRD) and flow properties) were carried out for powder blends to detect any interaction between drug and excipients and to determine the flow properties of ingredients (Havaldar et al., 2008). The flow properties (Kumar and Shiva kumar, 2006) of powder blend were studied through measuring different parameters like angle of repose, bulk density, tapped density and carr's index, Hausner's ratio were determined.

Post Compression Parameters

Prepared tablets were evaluated for post compression parameters like thickness, weight variation, hardness, friability, drug content, swelling index, *in vitro* buoyancy studies and *in vitro* drug release studies. All the studies were performed in triplicate, and results were expressed as mean ± SD (Verma et al., 2005).

***In vitro* Buoyancy Studies**

The floating characteristics of the GFDDS are essential, since they influence the *in vivo* behaviors of the drug delivery system. a) Floating Lag Time (FLT): The time taken by the tablet to emerge onto the surface of the liquid after adding to the dissolution medium simulated gastric fluid without pepsin, at pH 1.2, temperature 37 ± 0.5°C paddle rotation at 50 rpm and measured by using stopwatch. b) Total Floating Time (TFT): The time taken by the tablet to float constantly on the surface of the gastric fluid without pepsin, at pH 1.2, temperature 37 ± 0.5°C, paddle rotation at 50 rpm and measured by using stopwatch (Gambhire et al., 2007).

***In vivo* confirmation of buoyancy by using radiographic studies**

The X-ray images show the tablet residence in stomach for about 6 h clearly indicating the good floating property. The mean GRT was calculated (Bomma et al, 2009).

Determination of swelling index

The swelling behavior of a dosage unit was measured by studying its weight gain. The swelling index of tablet was determined by placing the tablets in 200 ml beaker using 0.1 N HCl. After every one hour up to 12 h, each tablet was removed and blotted with tissue paper to remove the excess water and weighed on the balance. The swelling index is expressed as a percentage.

***In vitro* dissolution studies**

Dissolution test was carried out using USP XXIV (model DISSO, M/s. Labindia) rotating paddle method (Apparatus II). The stirring rate was 50 rpm. 0.1 N Hydrochloric acid was used as dissolution medium (900 ml). It was maintained at 37 ± 5°C. The collected samples were suitably diluted with dissolution fluid analyzed for the ATC at 246 nm by using Elico Double Beam UV-Visible Spectrophotometer. Cumulative % drug release was determined (Saravanan et al, 2011).

Kinetic Analysis of Dissolution Data

To analyze the *in vitro* release data various kinetic models were used to describe the release kinetics.

Stability studies

Stability studies were carried out according to International Conference on Harmonization (ICH) guidelines. The samples were stored at 40 ± 0.5°C/ 75 ± 5% RH (Relative Humidity) and 25 ± 0.5°C/ 60 ± 5% RH for three months. The samples were withdrawn and evaluated for their drug content and other parameters (Mathews, 1999).

Pharmacokinetic and Bioavailability evaluation of ATC Optimized Formulations

Comparative Cross-over bioavailability study was conducted on 12 rabbits. The protocol for the animal study in prescribed proforma-B was approved by the *Institutional Animal Ethics Committee (IAEC) of Talla Padmavathi College of Pharmacy, Warangal, India, under Reg.No.1505/po/a/11 CPCSEA*, Dated: August 10 2011. The optimized formulations of ATC were subjected to *in vivo* pharmacokinetic studies. Healthy rabbits of both sexes weighing 1.5–2.5 kg were fasted overnight and divided into two groups (Group I and Group II). Control tablets/Microspheres/Gel formulations as reference was administered to Group I (n=6). Optimized formulations of ATC were given to Group II (n=6). After 35 days washout period, Group I received optimized formulations of ATC (Test) (from Tablets, Microspheres and Gel formulations) and Group II received control formulation (Reference). Blood samples were collected and analysed by developed HPLC method for ATC (Khan and Dehghan, 2011).

Various pharmacokinetic parameters such as C_{max}, t_{max}, AUC, half-life ($t_{1/2}$), MRT were determined. The determined pharmacokinetic parameters of floating tablets/microspheres/Gel formulations and control formulations were subjected to statistical analysis using paired *t*-

test to determine significance of difference at 0.05 level of significance. If the value is *P*<0.05, then it was considered as statistically significant.

RESULTS AND DISCUSSION

Standard Calibration curve of ATC at 246 nm in 0.1 N HCl buffer

Standard calibration curve of ATC was obtained by plotting Absorbance Vs Concentration. The standard calibration curve showed the slope of 0.009 and Correlation coefficient of 0.9980. The curve is found to be linear in the range between 10-90 µg/ml at 246 nm (Figure 1).

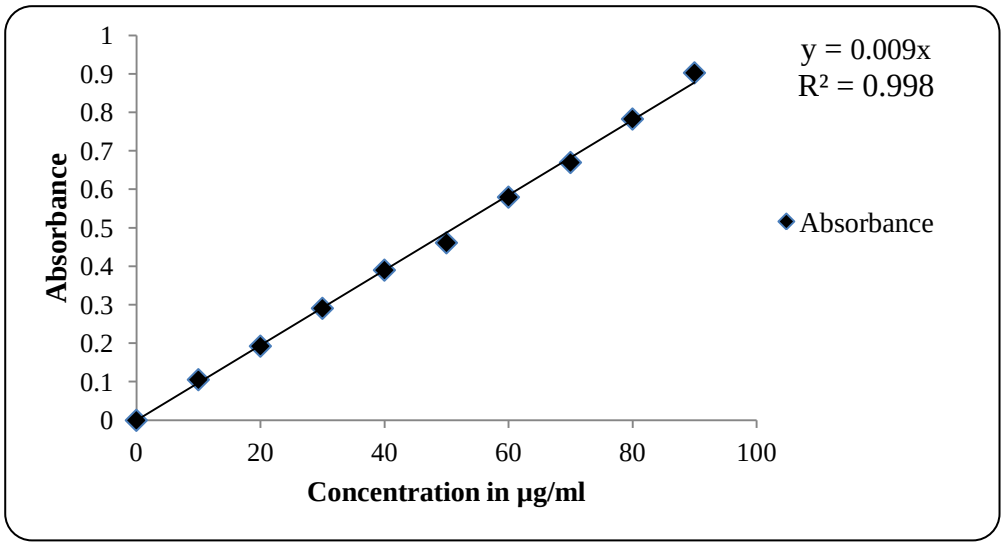


Figure 1: Calibration curve for ATC in 0.1 N HCl buffer

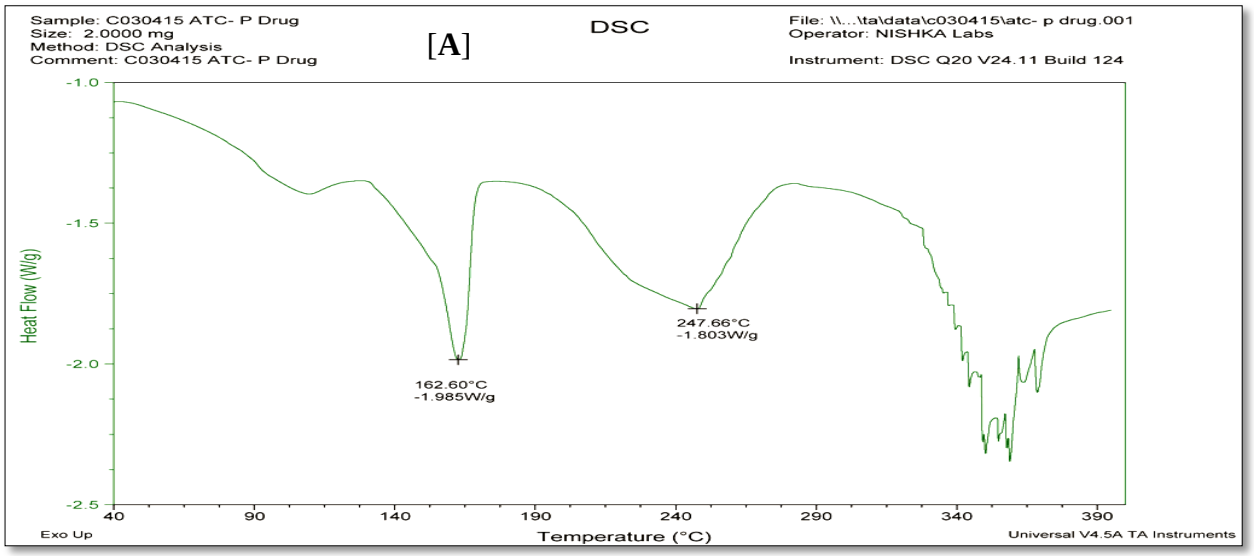
Preformulation Studies

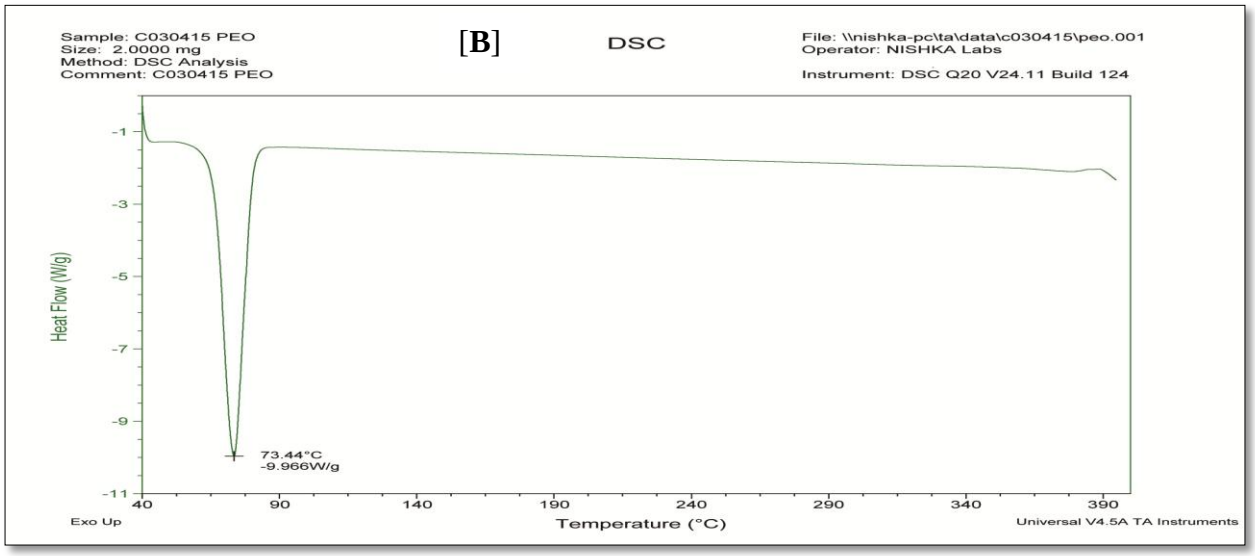
i) FT-IR Spectroscopy studies

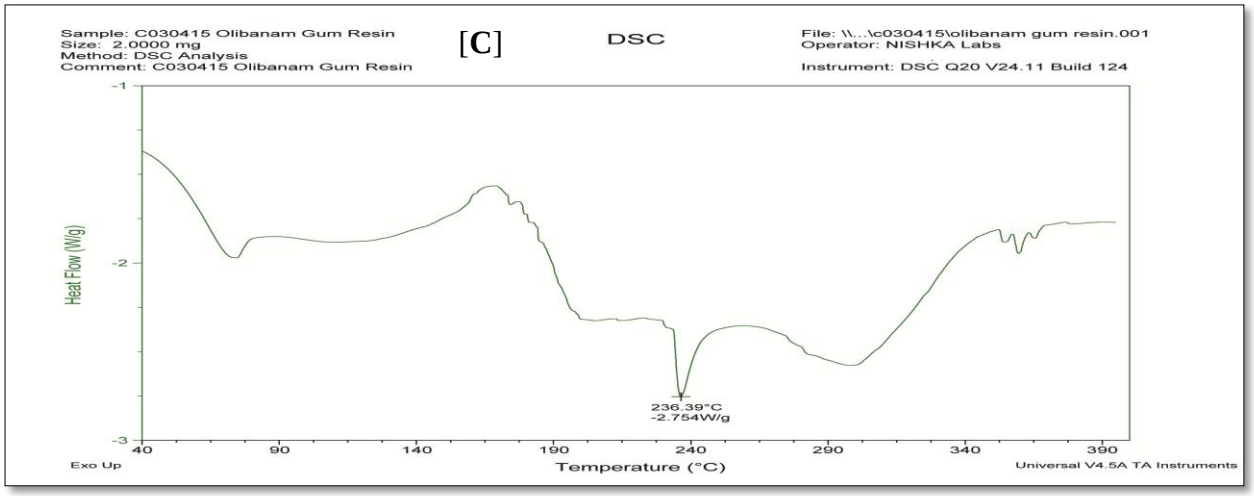
The peaks observed at 3612.36 cm^{-1} for O-H stretching and C-H stretching (Alkane) peaks at 2430.25 cm^{-1} and C-H stretching Alkene at 1721.78 cm^{-1}. Peaks present C=C stretching 1452.58 cm^{-1} for C-O stretching, C-S stretching at 790.1 cm^{-1}.

ii) Differential Scanning Calorimetry (DSC) studies

DSC thermogram A pure drug showed one sharp endothermic peak at 162.60°C and another broad peak at 247.66°C. Thermogram B HPMC K15M showed broad endothermic peaks at 111.18°C and 364.65°C, sharp endothermic peak at 368.19°C, Thermogram C PEO showed sharp endothermic peak at 73.44°C, Thermogram D Olibanum showed sharp endothermic peak at 236.39 °C, Thermogram E formulation TF15 showed endothermic peak at 193.45°C but the thermogram of formulation did not show any endothermic peak corresponding to the fusion of ATC. This suggests that there is no chemical interaction between the excipients used in the formulation and pure drug.


Sample: C030415 ATC- P Drug
Size: 2.0000 mg
Method: DSC Analysis
Comment: C030415 ATC- P Drug
[A]
DSC
File: \\...\ta\data\c030415\atc- p drug.001
Operator: NISHKA Labs
Instrument: DSC Q20 V24.11 Build 124
Heat Flow (W/g)
-1.0
-1.5
-2.0
-2.5
162.60°C
-1.985W/g
247.66°C
-1.803W/g
40
90
140
190
240
290
340
390
Exo Up
Temperature (°C)
Universal V4.5A TA Instruments


Sample: C030415 PEO
Size: 2.0000 mg
Method: DSC Analysis
Comment: C030415 PEO
[B]
DSC
File: \\nishka-pc\ta\data\c030415\peo.001
Operator: NISHKA Labs
Instrument: DSC Q20 V24.11 Build 124
Heat Flow (W/g)
-1
-3
-5
-7
-9
-11
73.44°C
-9.966W/g
40
90
140
190
240
290
340
390
Exo Up
Temperature (°C)
Universal V4.5A TA Instruments


Sample: C030415 Olibanam Gum Resin
Size: 2.0000 mg
Method: DSC Analysis
Comment: C030415 Olibanam Gum Resin
[C]
DSC
File: \\...\c030415\olibanam gum resin.001
Operator: NISHKA Labs
Instrument: DSC Q20 V24.11 Build 124
Heat Flow (W/g)
-1
-2
-3
236.39°C
-2.754W/g
40
90
140
190
240
290
340
390
Exo Up
Temperature (°C)
Universal V4.5A TA Instruments

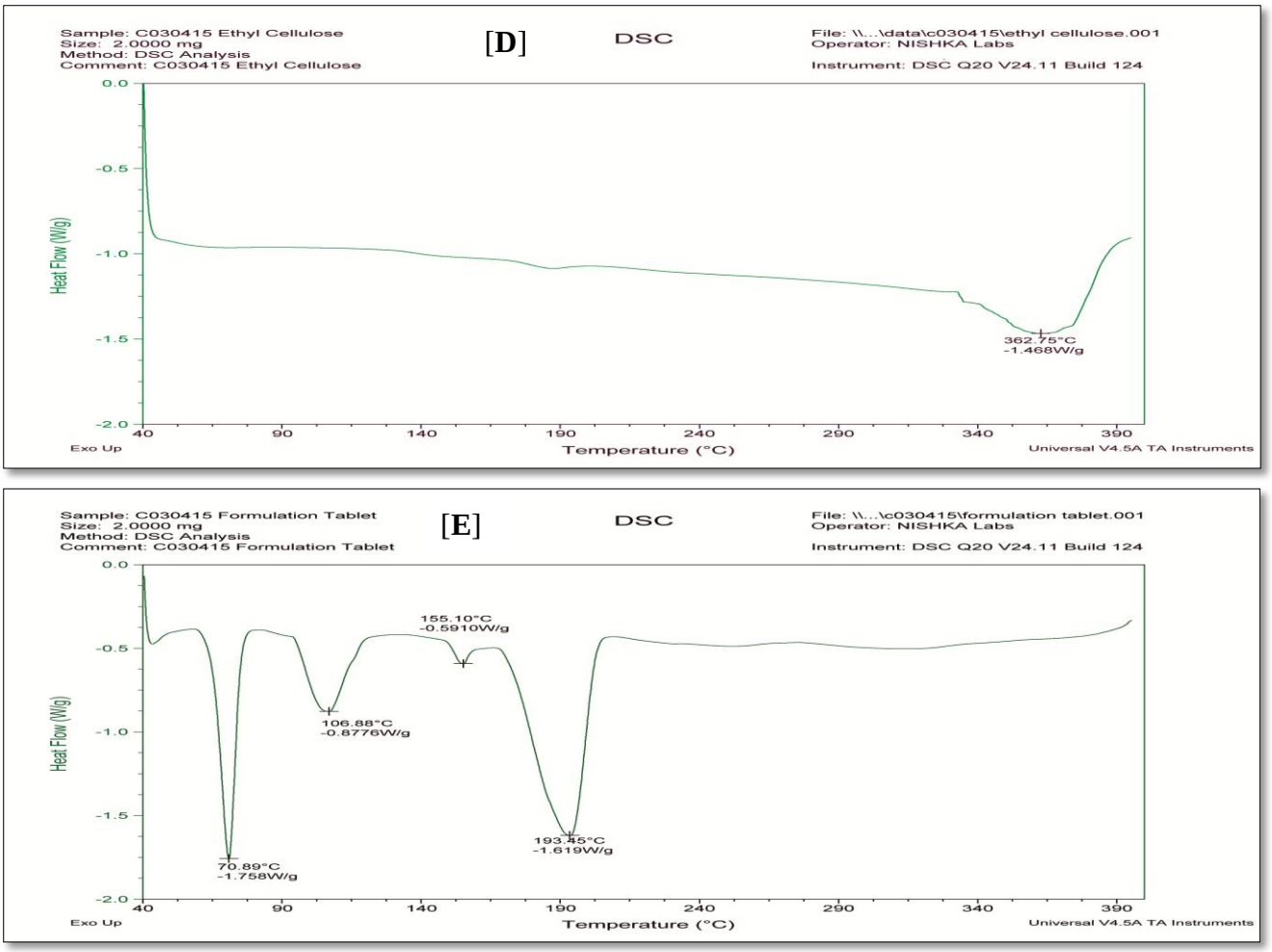


Figure 2: Differential Scanning Calorimetry thermograms of A) Pure drug (ATC); B) HPMC K15M; C) PEO; D) Olibanum; E) Tablet Formulation (TF-15).

iiii) Powder X-ray diffraction (p XRD) studies

The diffraction pattern of pure drug showed characteristic high-intensity diffraction peaks at 10.12, 11.68, 12.02, 15.02, 16.79, 18.10, 18.68, 19.24, 19.70, 21.35, 22.39, 23.14, 23.50, 24.23, 26.11, 27.25, 27.78, 28.10, 28.79, 30.23, 30.62, 31.67, 32.68, 33.04, 33.74, 36.03, 36.99, 38.02, 38.34, 39.12, 39.88, 42.92, 44.03, 64.40 and 77.52 which indicates that the drug is present in the crystalline form that is also confirmed by DSC results, whereas TF-15 Floating Tablet formulation showed reflections at 15.10, 18.14, 19.35, 21.98, 23.48, 26.78, 28.91, 30.58, 34.50, 36.37, 40.42, 44.09, 44.92, 57.20, 64.45, and 77.56. The pure drug exhibits reflections at 15,18.1, 44, 64.4 and 77.5, these strong reflections of pure drug were masked in the Floating Tablet formulation and exhibits weak reflections at 15 and 18.1 (two theta) 2θ as shown in Figures 3, 4 and 5 (Dimitra et al., 2008).

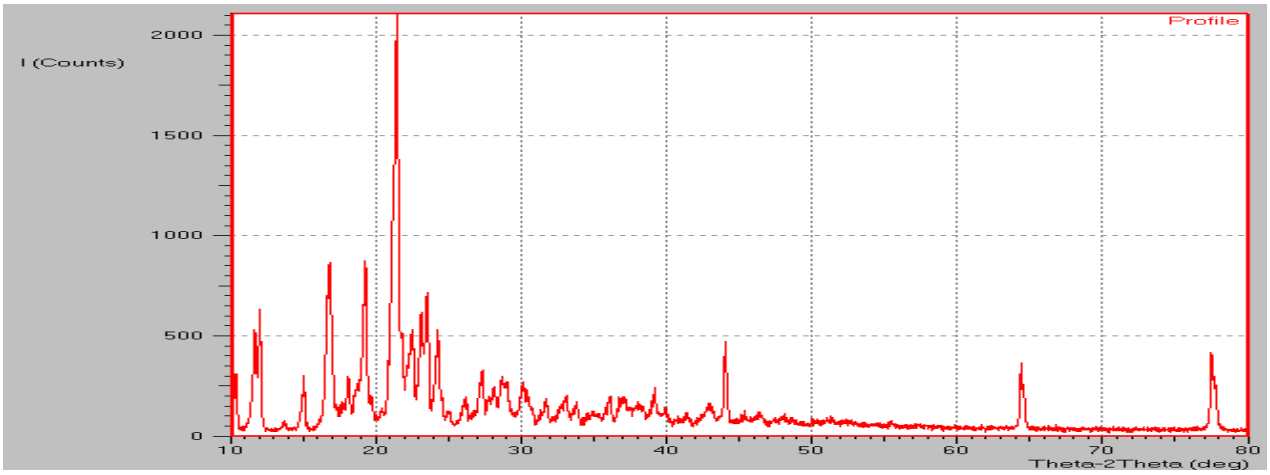


Figure 3: X- ray Diffraction graph of pure drug (ATC)

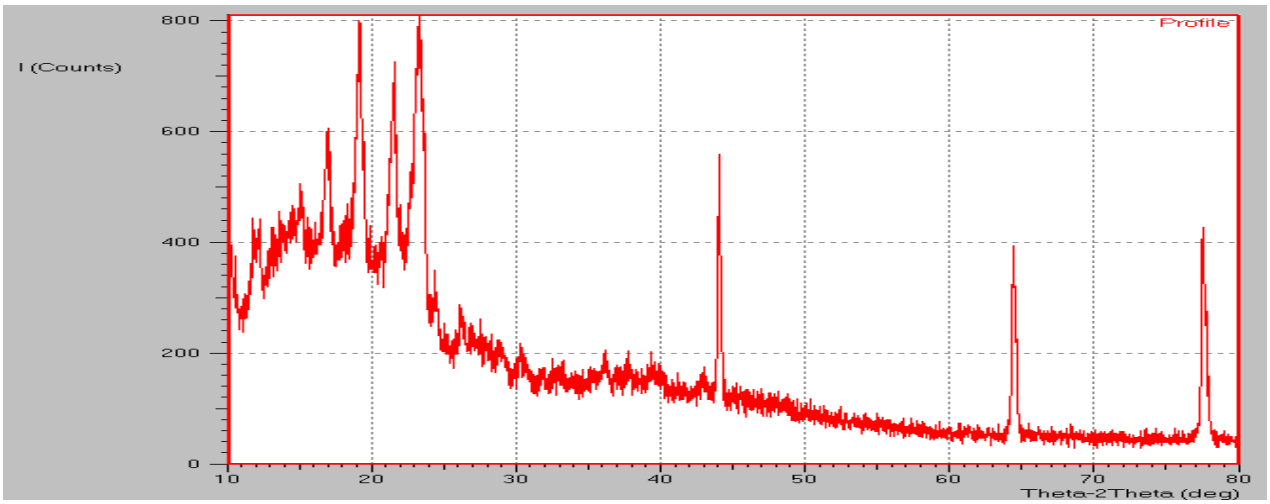


Figure 4: X- ray Diffraction graph of physical mixture of drug-excipients

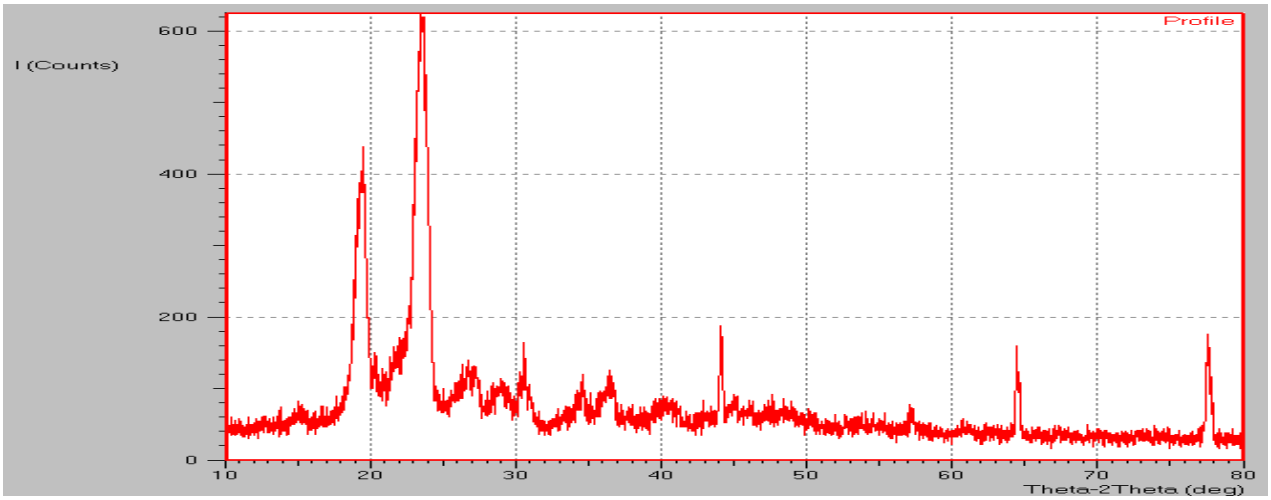


Figure 5: X- ray Diffraction graph of formulation TF-15

Precompression Parameters

Physical properties of all prepared powder blends are within the limits as per USP. The angle of repose values were found to be in the range of 23.35° to 29.86°. Carr's index lies within the range of 13.687±0.02% to 21.641±0.19%. Hausner's ratio was found to be in the range of 1.127±0.06 to 1.185±0.05. Bulk density was found to be in the range of 0.274±0.05 to 0.313±0.14 gm/cc. Tapped density was found to be in the range of 0.326±0.11 to 0.394±0.04 gm/cc (Table 3).

Table 3: Physical properties of all prepared powder blends

Formulation code	Angle of repose (°)	Carr's index (%)	Hausner's Ratio	Bulk density (gm/cc)	Tapped density (gm/cc)
TF-1	27.12 ±1.13	19.753±0.05	1.133±0.06	0.281± 0.11	0.343±0.09
TF-2	26.12 ±1.13	14.088±0.12	1.180±0.05	0.290± 0.15	0.326±0.11
TF-3	25.12 ±1.13	17.796±0.17	1.130±0.06	0.274±0.05	0.354±0.16
TF-4	29.56 ±1.46	18.844±0.11	1.127±0.06	0.283±0.08	0.360±0.06
TF-5	27.11 ±1.14	19.743±0.01	1.160±0.07	0.307±0.12	0.392±0.03
TF-6	29.56 ±1.86	21.641±0.19	1.133±0.06	0.313±0.14	0.375±0.14
TF-7	29.13 ±1.26	20.105±0.13	1.160±0.07	0.297±0.05	0.348±0.08
TF-8	24.64 ±1.35	15.225±0.09	1.173±0.05	0.285±0.17	0.339±0.12
TF-9	27.12 ±1.22	18.348±0.05	1.173±0.05	0.291±0.11	0.327±0.19
TF-10	25.45 ±1.19	13.687±0.02	1.148±0.03	0.283±0.12	0.346±0.15
TF-11	29.86 ±1.35	20.145±0.18	1.185±0.05	0.270±0.11	0.365±0.13
TF-12	23.35 ±1.46	16.389±0.17	1.150±0.07	0.295±0.05	0.394±0.04
TF-13	26.19 ±1.28	19.621±0.13	1.185±0.05	0.287±0.19	0.377±0.07
TF-14	29.64 ±1.54	15.987±0.12	1.667±0.08	0.279±0.02	0.367±0.11
TF-15	24.89 ±1.22	14.258±0.04	1.173±0.05	0.281±0.03	0.331±0.13

Data represents mean ± SD (n=3)

Post Compression Parameters

The weight variation among the each batch was found to be in the percentage deviation of 3.20 to 3.90 mg showing satisfactory results as per the U.S.P 2009. Hardness was maintained to be within 4.12 ± 0.15 kg/cm^2 to 4.40 ± 0.20 kg/cm^2. The thickness for different formulations was found in the range of 4.40 ± 0.01 to 4.54±0.01 mm. The results indicated that in all the formulation TF1 to TF15 it was found to be from 94.48±1.8 to 98.91±2.8 % (Table 4).

Table 4: Physical evaluation parameters of ATC Gastroretentive floating tablets

Formulation code	Weight variation (mg)**	Friability (%) ***	Hardness (kg/cm^2)*	Thickness (mm)*	Drug content* (%)
TF-1	300 ± 3.2	0.63	4.12 ± 0.15	4.50 ± 0.01	97.32±2.3
TF-2	310 ± 3.5	0.69	4.19 ± 0.17	4.48 ± 0.02	98.56±2.0
TF-3	320 ± 3.3	0.59	4.21 ± 0.16	4.54 ± 0.01	98.21±1.8
TF-4	330 ± 3.6	0.65	4.22 ± 0.19	4.40 ± 0.01	95.91±1.5
TF-5	340 ± 3.4	0.70	4.20 ± 0.14	4.44 ± 0.02	97.75±2.3
TF-6	350 ± 3.7	0.57	4.24 ± 0.24	4.49 ± 0.02	96.25±1.8
TF-7	360 ± 3.9	0.64	4.15 ± 0.22	4.45 ± 0.01	97.48±2.8
TF-8	300 ± 3.5	0.56	4.18± 0.15	4.46 ± 0.02	97.69±2.4
TF-9	310 ± 3.2	0.60	4.23± 0.17	4.52 ± 0.02	97.35±1.7
TF-10	320 ± 3.8	0.65	4.16± 0.12	4.49 ±0.01	96.55±2.4
TF-11	330 ± 3.4	0.63	4.20± 0.19	4.40 ±0.02	94.48±1.8
TF-12	300 ± 3.6	0.58	4.15 ± 0.14	4.43 ±0.01	95.42±.09
TF-13	310 ± 3.3	0.64	4.22 ± 0.19	4.46 ±0.01	95.99±1.3
TF-14	320 ± 3.5	0.66	4.14 ± 0.16	4.50 ±0.02	98.91±2.8
TF-15	350 ± 3.7	0.63	4.40 ± 0.20	4.52 ±0.01	98.46±3.2

*= Data represents mean ± SD (n=3); **=Data represents mean ± SD (n=20); *** =Data represents mean ± SD (n=10)

***In vitro* Buoyancy studies:**

Floating Lag Time and Total Floating Time

Further, the formulated tablets on immersion in 0.1N HCl media they remain buoyant for 12 h with FLT of 104 to 132 sec (TF-1 to TF-6, TF-8 and TF-10). Formulations TF-7 and TF-9 remain buoyant for 18 h with FLT of 145 sec and 125 sec, whereas formulations TF-11 to TF-15 floated with a lag time of 42 sec to 64 sec.

Swelling Index

The formulations with HPMC K4M, PEO, Olibanum showed the swelling and tablet integrity. The formulation TF-15 with combination of PEO and Olibanum showed highest swelling Index (345.2% at 8 h) compared to that of the formulations Olibanum alone.

Effect of hardness on Floating Lag time:

The effect of hardness on FLT for batch TF15 was studied. The results of FLT of tablets having hardness of 4.5 kg/cm^2, 5.5 kg/cm^2 and 7 kg/cm^2 were 48 sec, 110 sec and 221 sec respectively. Batch TF15 was selected for the study because it showed FLT of 110 sec at hardness of 4.5 kg/cm^2.

Table 5: Results of *in vitro* buoyancy study of ATC Gastroretentive floating tablets

Formulation code	Floating Lag Time (FLT) (Sec)	Total Floating Time (TFT) (h)
TF-1	104	>12
TF-2	108	>12
TF-3	115	>12
TF-4	121	>12
TF-5	128	>12
TF-6	136	>12
TF-7	145	>18
TF-8	117	>12
TF-9	125	>18
TF-10	132	>12
TF-11	53	>24
TF-12	64	>24
TF-13	42	>24
TF-14	44	>24
*TF-15	48	>24

**Optimized formulation used in Pharmacokinetic studies*

Table 6: Percent swelling of formulations from TF-1 to TF-15

Time (h)	Swelling index (% weight gain)														
	TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8	TF-9	TF10	TF11	TF12	TF13	TF14	TF15
1	38.15	42.56	54.23	60.2	71.26	79.56	86.59	58.25	68.25	72.5	62.15	33.15	38.56	46.23	96.5
2	78.23	82.23	94.32	105.6	118.56	129.56	135.6	128.5	142.5	156.2	95.36	63.23	78.23	84.32	182.0
3	106.3	121.5	136.5	145.6	159.6	161.6	169.5	176.6	185.6	172.0	146.9	96.13	105.5	126.5	215.6
4	145.5	156.9	171.2	189.3	199.25	209.6	218.5	182.5	216.2	235.0	185.2	125.5	136.9	161.2	265.5
6	196.5	215.6	241.2	246.5	253.56	261.3	269.5	201.5	246.5	276.3	236.5	146.5	165.6	211.2	299.3
8	212.5	246.9	286.5	291.6	301.5	316.5	326.5	256.2	296.5	301.5	261.5	172.5	196.9	246.5	**345.2**
10	156.5	145.2	136.5	142.3	156.0	166.5	172.5	176.5	185.2	174.6	149.6	136.5	145.2	156.5	168.2
12	101.5	109.0	104.3	106.9	126.3	142.5	159.2	125.5	115.0	126.0	114.6	104.5	129.3	124.3	136.5

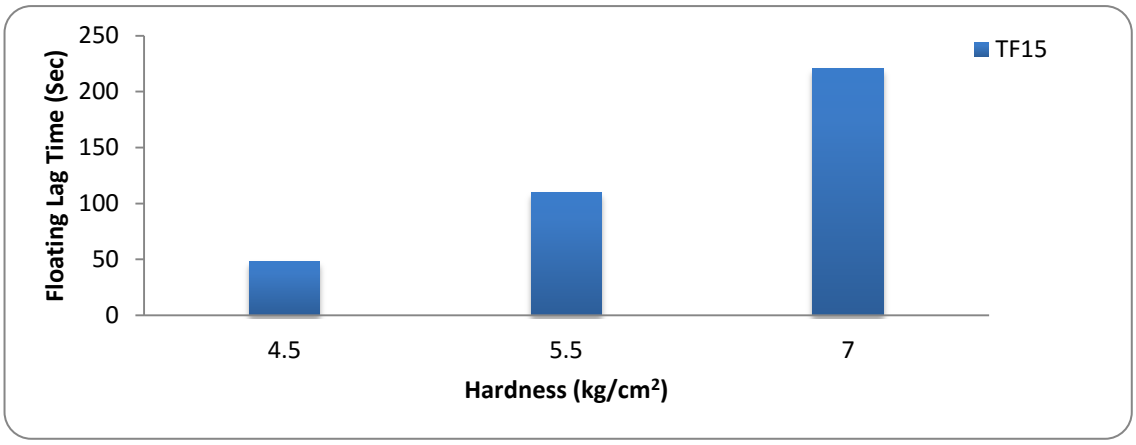


Figure 6: Plot of Floating Lag time vs. Tablet hardness

***In vitro* Dissolution Studies**

Cumulative drug release from formulations TF1, TF2 and TF3 was found to be 95.70±1.2% in 6h, 97.34±2.1% in 12h and 99.86±1.9% in 15h respectively. Formulation TF4 showed 98.97±1.4 % at 18 h and formulations TF5 and TF6 showed a drug release in 24 h with 96.01±1.6 and 89.05±1.1% respectively. The formulations made with natural hydrophobic polymer alone showed the complete release 18 to 24 h (TF7 to TF9). Formulation TF9 showed the drug release 94.53±2.3% in 24 h. The cumulative drug release from formulations TF10 and TF11 was found to be 88.53±2.3% and 84.15±1.5% in 24 h. Formulation TF12 drug released about 99.49±1.3% of cumulative amount in 12h. The order of release retarding efficiency of the polymers was PEO + Olibanum > HPMC+PEO > Olibanum > PEO > HPMC (Figure 7 to Figure 12).

Table 7: Cumulative percentage drug released from ATC floating tablets prepared with HPMC K15 M (22%, 32% and 42% in formulations (TF-1 to TF-3).

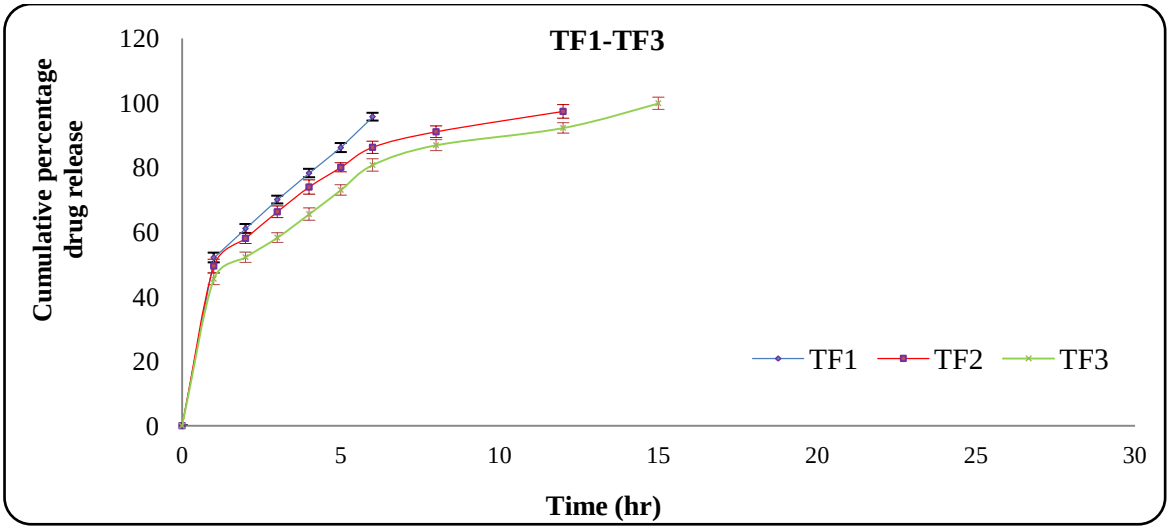


Figure 8: Cumulative percentage drug released from floating tablets of ATC prepared with HPMC K15 M (TF-1 to TF-3) (n=3).

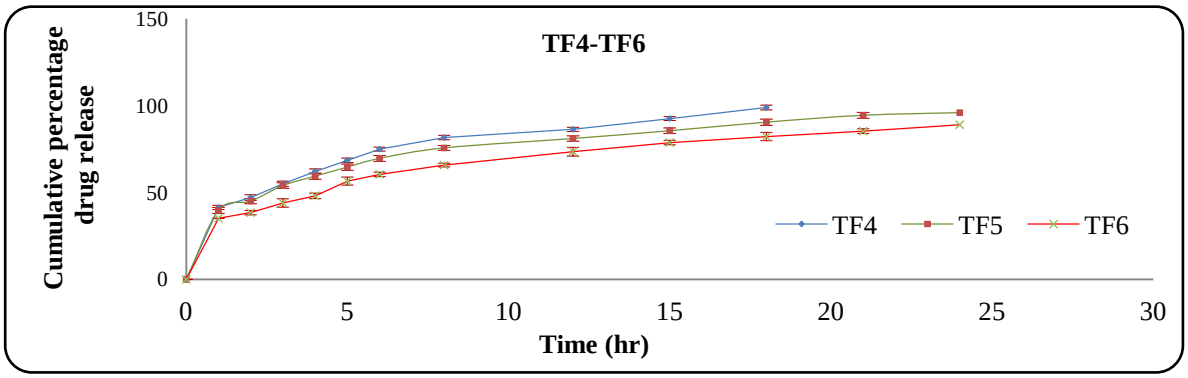


Figure 9: Cumulative percentage drug released from floating tablets of ATC prepared with PEO (TF-4 to TF-6) (n=3).

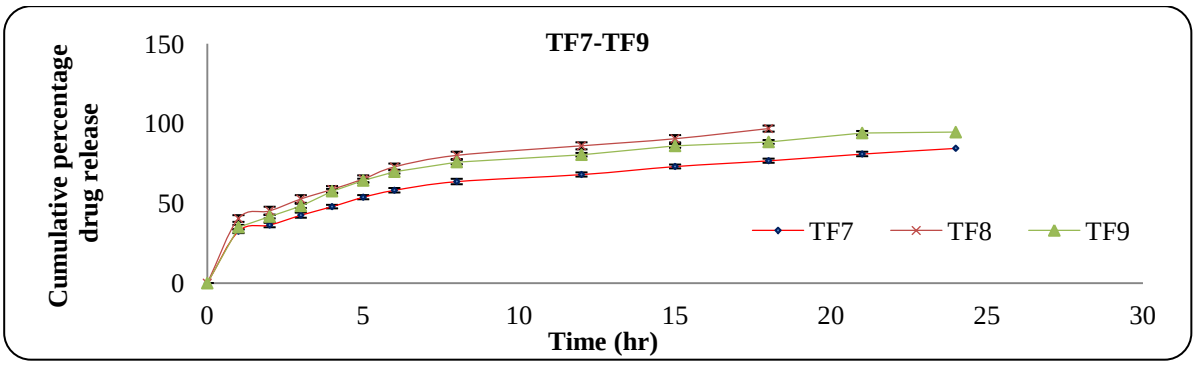


Figure 10: Cumulative percentage drug released from floating tablets of ATC prepared with Olibanum (TF-7 to TF-9) (n=3).

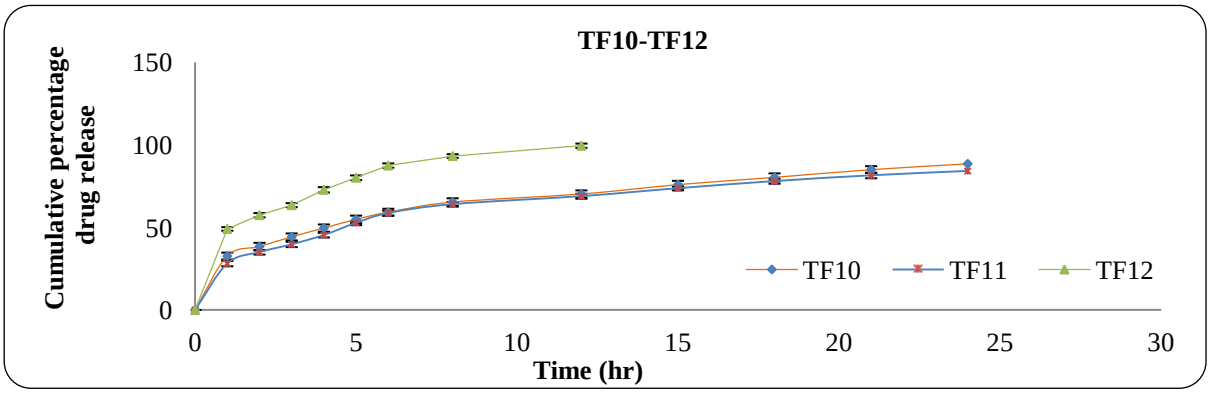


Figure 11: Cumulative percentage drug released from floating tablets of ATC prepared with Combination of HPMC K15M and PEO (TF-10 to TF-12) (n=3).

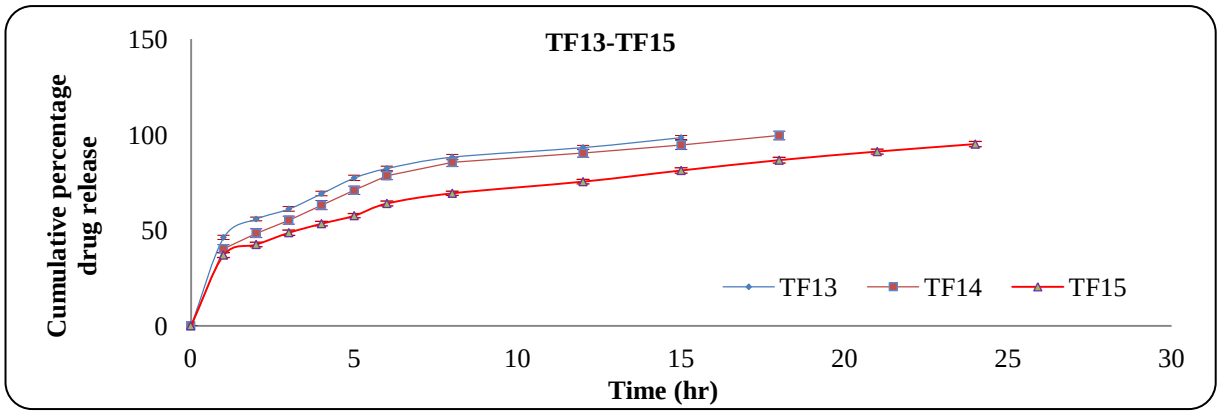


Figure 12: Cumulative percentage drug released from floating tablets of ATC prepared with Combination of PEO and Olibanum (TF-13 to TF-15) (n=3).

Kinetic Analysis of Dissolution Data

In the present study, *in vitro* release profiles could be best expressed by Higuchi's equation and was governed by anomalous (Non-Fickian) diffusion as optimized formulation (TF15) showed good linearity (R^2: 0.990) indicates that diffusion is dominant mechanism of drug release with these formulations (Figure 13).

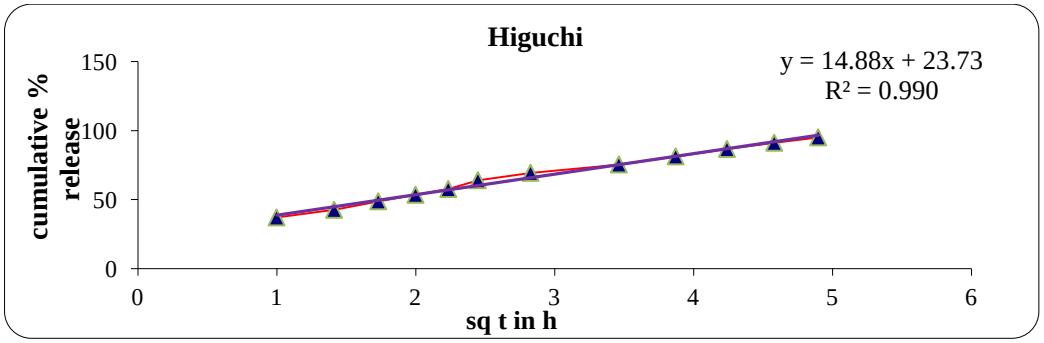


Figure 13: Higuchi matrix release kinetics of optimized formulation (TF15)

***In vivo* radiographic studies:**

The X-ray images show the tablet residence in stomach for more than 6 h clearly indicating the good floating property.

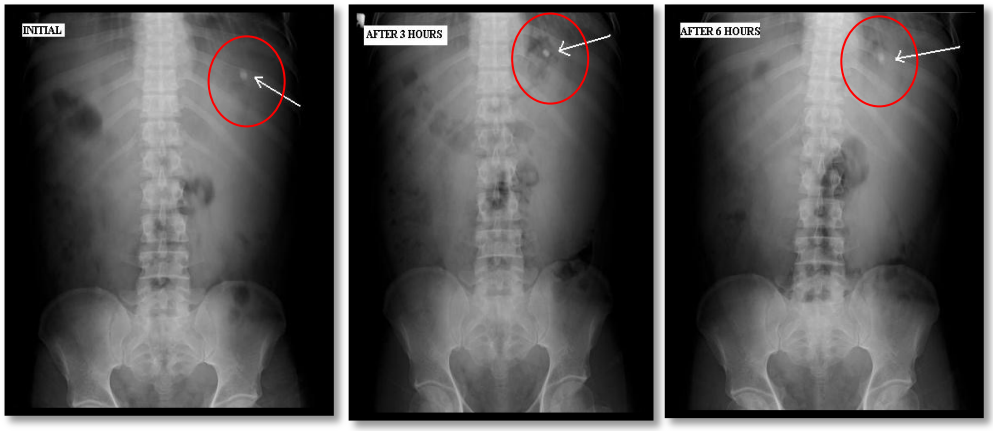


Figure 14: X–Ray image showing the BaSO4-loaded floating tablet prepared with PEO and Olibanum.

Stability studies

The optimized floating tablets (TF-15) were selected for stability study on the basis of *in vitro* buoyancy and *in vitro* drug dissolution studies. The tablets were investigated at 40°C / 75% RH for 3 months.

Table 7: Stability study (40 °C / 75 % RH) of Optimized Formulation (TF15)

Parameters	1st Month*	2nd Month*	3rd Month*
Physical appearance	Off white, smooth, Flat faced.	Off white, smooth, Flat faced.	Off white, smooth, Flat faced.
Weight variation (mg)	350 ± 3.7	350 ± 3.7	350 ± 3.7
Hardness (kg/cm^2)	5.5 ± 0.20	5.4± 0.15	5.3± 0.12
Friability (%)	0.63	0.62	0.64
Drug Content (%)	98.46	98.36	98.35
Floating Lag Time (Sec)	48	46	47
Total Floating Time (h)	>24	>24	>24
Floating on disturbing	Float	Float	Float
In vitro drug release (%) 24 h	95.02± 0.14	95.01± 0.13	95.00± 0.15

*Statistically not significant (p>0.05)

Pharmacokinetic evaluation in rabbits

In this design, pharmacokinetic evaluation was done on Optimized formulations in comparison to control formulation of ATC.

The pharmacokinetic parameters were calculated using Kinetica software. The following table contained the obtained data.

Table 8: Pharmacokinetic parameters of Atorvastatin Calcium Control formulation (Lipitor) and Gastroretentive floating tablet formulation (TF-15) in Rabbits (n=12).

Pharmcokinetic parameters	Control tablets Mean ± SD	TF-15 Mean ± SD
C_{max} (ng/ml)	401.19 ± 39.83	700.60 ± 123.79
T_{max} (h)	1.5 ± 0.13	2 ± 0.59
AUC_{0-t} (ng×h/ml)	2060.54 ± 203.37	4016.28 ± 1041.90
$AUC_{0-\alpha}$ (ng×h/ml)	2088.56 ± 208.55	4212.18 ± 273.06
$t_{1/2}$ (h)	3.70 ± 0.35	5.45 ± 2.16
MRT (h)	6.23 ± 0.41	8.07 ± 1.88
%Fr	100	194.91

By student paired t-test, p<0.05 is considered statistically significant in all the parameters.

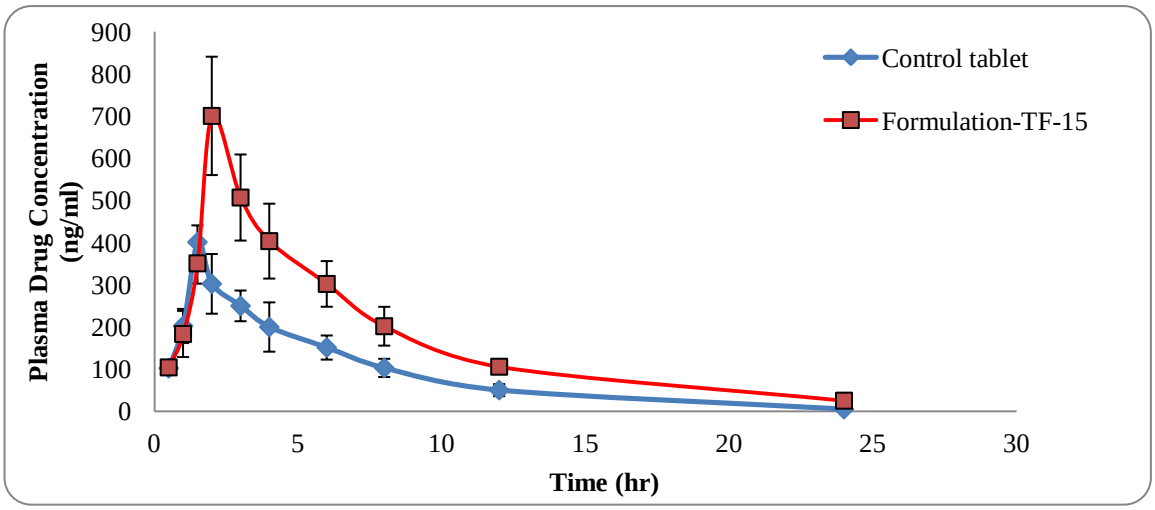


Figure 15: Mean serum concentration-time profiles of control formulation and Gastroretentive floating tablets (TF-15) in rabbits (n=12).

In all 12 rabbits the bioavailability study was successfully completed according to the protocol. The serum samples were analyzed by simple RP-HPLC method. The pharmacokinetic parameters used to access the bioavailability of test verses reference were $AUC_{0-\infty}$ for the extent of absorption and C_{max} and T_{max} for the rate of absorption. The mean (± SD) ATC serum concentration-time curves for test (TF-15) and reference (conventional formulation) are shown in the Figure 15. The C_{max} value for reference formulation was found to be 401.19±39.83 ng/ml, where as C_{max} value for test (TF-15) was found to be 700.60 ± 123.79 ng/ml. T_{max} values for both reference and test (TF-15) was found to be 1.5 ± 0.13 h and 2 ± 0.59 h respectively. $t_{1/2}$ value for reference was found to be 3.70 ± 0.35 and for test 5.45 ± 2.16 h. $AUC_{0-\infty}$ values for reference and test were 2088.56 ± 208.55 ng×h/ml and 4212.18 ± 273.06 ng×h/ml, respectively. Mean residence time (MRT) values for reference and test formulation were 6.23 ± 0.41 h and 8.07 ± 1.88 h, respectively. In the present study student's paired *t*-test showed that there was significant difference ($p< 0.05$) between two formulations in their pharmacokinetic parameters, $AUC_{0-\infty}$, C_{max}, T_{max}, and MRT. The increased Relative bioavailability of test formulation was 1.94 fold when compared to reference formulation (Table 8).

SUMMARY AND CONCLUSION

Friability and hardness were within the standard limits thus showing good mechanical strength of tablets. The drug content was well within the Pharmacopoeial limits indicating uniform distribution of drug within the SR-GRDF. The Optimized floating tablets (TF15) showed satisfactory results with short buoyancy lag time, long total buoyancy time and sustained drug released up to 24 hrs. Floating characteristics like FLT, TFT for all the formulations were studied and reported. The formulation TF15 showed highest swelling Index compared to that of the formulations Olibanum alone. Formulation TF15 obtained the desired drug release profile and floated with a lag time of 48 sec. *In vitro* release profiles could be best expressed by Higuchi's equation and was governed by anomalous (Non-Fickian) diffusion as optimized formulation (TF15) showed good linearity (R^2: 0.990) indicates that diffusion is dominant

mechanism of drug release with these formulations. *In vivo* of floatation behaviour evidences by taking the X-ray by at 0h, 3h and 6h it can be concluded the floatation behavior of tablet. Results of the stability studies showed that there were no significant changes in different properties. The increased relative bioavailability (f_r) of test formulation (TF15) was 1.94 folds, when compared to control formulation.

In the present research study, carried out on bioavailability enhancement of ATC, the preformulation studies, proven to show good compatibility between the drug and excipients. The novel oral formulations of ATC done using various polymers both synthetic and natural were Floating tables (TF1-TF15). Promising *in vitro* and *in vivo* pharmacokinetic results have been reported for Floating Tablets and the optimized formulation found to be TF15. Formulation TF15 have shown good biocompatibility, bioavailability and stability. This ensures maximal bioavailability of the drug with improved efficiency having NAW.

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