AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Formulation Development of Pulsatile Drug Delivery of Tiotropium Bromide

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ABSTRACT

A Pulsatile drug delivery system delivers drug in rapid and burst manner within a short time after a lag time. There are many situations where drug is needed to be released immediately (after bursting the delaying film coat) at specific site. The aim of present work is to develop Pulsatile drug delivery of Tiotropium Bromide using press coating method. All the formulations have shown satisfactory results for various physicochemical parameters like hardness, friability, thickness, weight variation. Ethyl cellulose has predominant effect on the lag time, while also shows significant effect on drug release. Press coated tablet shows a delayed release pattern. Among all the core tablet formulations T7 was selected based on drug release within a given period of time. In-vitro release rate studies showed that the P3T7 was optimized based on less amount of drug release during lag time. Formulations P3T7 found to be stable at 45^{o} C and 75% RH for a period of 6 months. FT-IR studies revealed that there was no interaction between Tiotropium bromide and the polymers.

Keywords: Pulsatile drug delivery, circadian rhythms, Tiotropium Bromide, Lag time, Press coated tablets.

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Received 01 January 2019, Accepted 03 June 2019

Please cite this article as: Goud CM *et al.*, Formulation Development of Pulsatile Drug Delivery of Tiotropium Bromide. American Journal of PharmTech Research 2019.

INTRODUCTION

A Pulsatile drug delivery system delivers drug in rapid and burst manner within a short time after a lag time. There are many situations where drug is needed to be released immediately (after bursting the delaying film coat) at specific site. These systems are mainly appropriate for drugs that are metabolized to pharmacological active compounds, drugs which have long in vivo half lives showing an inherently prolonged duration of action, and drugs which are required in very low dose. Additionally a delayed burst release can also be utilized for enhancing absorption, reducing side effects, increasing and decreasing

Advantage of PDDS

1. Increases absorption and bioavailability than conventional immediate release or sustained release drug due to its ability to release drug in a burst manner, at target site of absorption.
2. Site targeting allows delivery of poorly bioavailable drugs that would get destroyed in higher GI tract environment e.g. (peptide and protein molecules)
3. Reduces dose of drug without decrease in therapeutic effects.
4. Decreases side effects.
5. Decreases drug interaction due to lower cytochrome P450 isoenzymes.
6. Decreases food effect (change occurring in bioavailability of drug when given with food).
7. Improved compliance.
8. Chronotherapy, programmed delayed release provides optimal treatment of diseases.
9. Pulse release allows multiple dosing in a single dosage form.
10. Allows site specific release for local treatment of diseases.

Disadvantage of pulsatile drug delivery system

1. Low drug loading capacity and incomplete release of drug.
2. Higher cost of production.
3. Large no. of process variables.
4. Lack of manufacturing reproducibility and efficacy.
5. Batch manufacturing process.
6. Unpredictable IVIVC.

MATERIALS AND METHOD

Tiotropium bromide was obtained as a free sample from Chandra labs, Hyd, Micro crystalline cellulose was obtained from Degussa India Pvt. Ltd., Mumbai L.R, Cross povidone, Sodium starch glycolate, Starch, Magnesium stearate & Cross Caramellose sodium were obtained from S.D. Fine

Chem. Ltd., Mumbai. L.R, HPMC & Ethyl cellulose were obtained from L.R. Sisco Research Lab.Pvt. Mumbai, Xanthum gum & Guar gum were obtained from ESSEL fine chem. Mumbai.

Methods [1,2,3,4]:

PRE-FORMULATION STUDIES

Preformulation testing was an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. It was the first step in the rational development of dosage forms.

Objective /Purpose of Preformulation study:

Pre-formulation studies on active pharmaceutical ingredients (API), inactive ingredients (Excipients), and their combinations were carried out to finalize specifications of active pharmaceutical ingredients (API), **S**tudy the compatibility between active and inactive ingredient, **C**haracterization of reference product

Scope:

The use of preformulation parameters maximizes the chances in formulating an acceptable, safe, efficacious and stable product[41].

Class:

Preformulation study can divided into two subclasses:

API characterization

Compatibility study

Active pharmaceutical ingredient (API) characterization:

Organoleptic evaluation:

These are preliminary characteristics of any substance which is useful in identification of specific material. Following physical properties of API were studied.

Table 1: Organoleptic Evaluation

Parameter	Properties of Tiotropium bromide
Organoleptic Evaluation	White or off-white powder.
Solubility Analysis	Sparingly soluble in water , soluble in methanol

DRUG - EXCIPIENT COMPATIBILITY STUDY

Fourier Transform infra-red (FTIR) spectroscopy

Infrared spectroscopy is a useful analytical technique utilized to check the chemical interaction between the drug and excipients used in the formulation.1-2 mg of solid fine powder of Tiotropium bromide and 200-300 mg of dry powder of KBr (IR grade) were taken in a mortar and mixed well with the help of a spatula. Spectrum measurement was carried out using KBr disk

method in the wavelength region of 4000-400 cm^{-1} by FTIR spectrophotometer. The IR spectrum of the physical mixture was compared with that of the pure drug to check any possible drug-excipient interaction.

PREPARATION OF CALIBRATION CURVE FOR TIOTROPIUM BROMIDE

STANDARD CURVE IN pH 6.8 PHOSPHATE BUFFER

Stock Sample Preparation:

Accurately weighed 100 mg of drug (Tiotropium bromide) was first dissolved in100 mL of pH 6.8 phosphate buffer in 100 mL of volumetric flask to make a concentration of 1000 µg/mL (primary stock solution). 5 mL of primary stock solution was pipetted out into 50 mL of volumetric flask and volume was adjusted with pH 6.8 phosphate buffer to make a concentration of 100µg/mL (secondary stock solution).

Sample Preparation:

From the secondary stock solution pipette out 0.2, 0.4, 0.6,0.8, and 1 in to 10ml of volumetric flask and volume made up to with 6.8pH Phosphate buffer to give various concentrations such as 2,4,6, 8and 10µg/mL were prepared for calibration curve. Standard curve was plotted by taking absorbance of secondary stock solutions in UV double beam spectrophotometer at 235nm.

FORMULATION DEVELOPMENT

Formulation of core tablets by direct compression:

The inner core tablets were prepared by using direct compression method. As shown in Table powder mixtures of Tiotropium bromide, microcrystalline cellulose (MCC, Avicel PH-102), cross-carmellose sodium (Ac-Di-Sol) ,SSG, crospovidone, starch ingredients were dry blended for 20 min, followed by addition of Magnesium Stearate. The mixtures were then further blended for 10 min., 180mg of resultant powder blend was manually compressed using KBr hydraulic press at a pressure of 1 ton, with a 8mm punch and die to obtain the core tablet.

Table 2: Composition of core tablets

Ingredients(mg)	T1	T2	T 3	T4	T5	T6	T7	T8	T9	T10	T11	T12
Drug	18	18	18	18	18	18	18	18	18	18	18	18
Cross povidone	7.5	--	--	15	--	--	18.75	--	--	22.5	--	--
Cross caramellose sodium	--	7.5	--	--	15	--	--	18.75	--	--	22.5	--
SSG	--	--	7.5	--	--	15	--	--	18.75	--	--	22.5
Magnesium stearate	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
PVP	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
MCC	117	117	117	109.5	109.5	109.5	105.75	105.75	105.75	102	102	102

Total wt (mg)	150	150	150	150	150	150	150	150	150	150	150	150

Drug: Tiotropium bromide, **MCC:** Micro crystalline cellulose, SSG: Sodium starch glycolate, **PVP: Poly vinyl pyrrolidone**

Formulation of mixed blend for barrier layer:

The various formulation compositions containing HPMC, Ethyl cellulose, Xanthum gum and Guar gum. Different compositions were weighed dry blended at about 10 min. and used as press-coating material to prepare press-coated pulsatile tablets respectively by direct compression method.

Preparation of press-coated tablets:

The core tablets were press-coated with 400mg of mixed blend as given in Table. 200mg of barrier layer material was weighed and transferred into a 12mm die then the core tablet was placed manually at the center. The remaining 200mg of the barrier layer material was added into the die and compressed at a pressure of 5 tons for 3min using KBr hydraulic press.

Table 3: Composition of Press coat tablets

Press coat	P1(mg)	P2(mg)	P3(mg)	P4(mg)	P5(mg)
HPMC	200	300	--	400	--
Ethyl cellulose	200	100	--	--	--
Xanthum gum	--	--	300	--	200
Guar gum	--	--	100	--	200
Total wt(mg)	400	400	400	400	400

EVALUATION PARAMETERS FOR PRE COMPRESSION BLEND

Bulk density:

Bulk density of a compound varies substantially with the method of crystallization, milling or formulation. Bulk density is determined by pouring pre sieved blend into a graduated cylinder via a large funnel and measure the volume and weight. Bulk density was expressed in g/cc.

Bulk density = Weight of blend/ Bulk volume of blend

Tapped density:

Tapped density is determined by placing a graduated cylinder containing a known mass of blend and mechanical tapper apparatus, which is operated for a fixed number of taps until the powder bed volume has reached a minimum volume. Using the weight of the drug in the cylinder and this minimum volume, the taped density may be computed.

Tapped density = Weight of blend/ Tapped volume of blend

Carr's Index (CI):

Carr's index is measured using the values of bulk density and tapped density. The following equation is used to find the Carr's index.

CI = (TD-BD) x100/ TD

Where TD = Tapped density

BD = Bulk density

Table 4: Flow properties and corresponding Carr's Index values

Excellent	<10
Good	11 – 15
Fair	16 – 20
Possible	21 – 25
Poor	26 – 31
Very poor	32 – 37
Very very poor	>38

Hausner's Ratio:

It indicates the flow properties of the powder and ratio of Tapped density to the Bulk density of the powder or blend.

Hausner's Ratio = Tapped density/Bulk density

Table 5: Flow Properties and Corresponding Hausner's ratio

Excellent	1.00 – 1.11
Good	1.1 – 1.18
Fair	1.19 – 1.25
Possible	1.26 -1.34
Very poor	1.35 -1.45
Very very poor	>1.60

Angle of repose:

The manner in which stresses are transmitted through a bead and the beads response to applied stress are reflected in the various angles of friction and response. The method used to find the angle of repose is to pour the powder ion a conical heat on a level, flat surface and measure the included angle with the horizontal.

Tanθ = h/r

Where,

h= height of the heap

r= Radius of the heap

Table 6: Flow Properties and Corresponding Angle of Repose

Angle of repose	Powder flow
< 25	Excellent
25 – 30	Good

30 – 40	Passable
> 40	Very poor

EVALUATION OF PRESS COAT TABLETS

Evaluation of rapid release core (RRCT) and press-coated tablets Of Tiotropium bromide

Weight variation:

Twenty tablets were randomly selected from each batch weighed individually. The average weight and standard deviation was calculated .

Thickness:

Three tablets from each batch of formulation were collected and the thickness of the tablets were measured with the help of Vernier calipers. The average thickness was calculated.

Hardness:

Hardness was measured using Monsanto tablet hardness tester. The hardness of five tablets in each batch was measured and the average hardness was calculated in terms of kg/cm^2 .

Friability (F):

Friability of the tablet determined using Roche friabilator. Pre-weighted sample of tablets were placed in the friabilator and were subjected to the 100 revolutions. Tablets were dusted using a soft muslin cloth and reweighed.

Disintegration time:

LABINDIA DT 1000 USP disintegration test apparatus. To test the disintegration time of tablets, one tablet was placed in each tube and the basket rack was positioned in a 1 liter beaker containing water at 37°C ± 1°C such that the tablet remains 2.5 cm below the surface of the liquid. The time taken for the complete disintegration of the tablets was noted.

***In-vitro* Dissolution methods for press-coated tablets:**

In –vitro Dissolution studies of Pulsatile delivery systems was done with the conventional paddle method of press coated tablets were performed at 37 ± 0.5 °C using 6.8 phosphate buffer in USP II paddle method at 50 rpm. 5 ml of filtered aliquot was manually withdrawn at pre-determined time intervals and replaced with 5 ml of fresh buffer maintained at the same temperature. The samples were analysed at 235nm using a UV spectrophotometer. The lag time and percentage release was determined for each formulation.

Kinetic Analysis of Dissolution Data:

To analyze the in vitro release data various kinetic models were used to describe the release kinetics. The zero order rate Eq. (1) describes the systems where the drug release rate is independent of its concentration (Hadjiioannou et al., 1993). The first order Eq. (2) describes the

release from system where release rate is concentration dependent (Bourne, 2002). Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion Eq. (3). The Hixson-Crowell cube root law Eq. (4) describes the release from systems where there is a change in surface area and diameter of particles or tablets (Hixson and Crowell, 1931).

$C = K_0 t$ (1)

Where, K_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

$LogC = LogC_0 - K_1 t / 2.303$ (2)

Where, C_0 is the initial concentration of drug and K_1 is first order constant.

$Q = K_H t^{1/2}$ (3)

Where, K_H is the constant reflecting the design variables of the system.

$Q_0^{1/3} – Q_t^{1/3} = K_{HC} t$ (4)

Where, Q_t is the amount of drug remained in time t, Q_0 is the initial amount of the drug in tablet and K_{HC} is the rate constant for Hixson-Crowell rate equation.

The following plots were made using the in-vitro drug release data

Cumulative % drug release vs. time (Zero order kinetic model);

Log cumulative of % drug remaining vs. time (First order kinetic model);

Cumulative % drug release vs. square root of time (Higuchi model);

And cube root of initial concentration minus the cube root of percentage of drug remaining in the matrix vs. time (Hixson-Crowell cube root law).

Mechanism of drug release:

Korsmeyer et al (1983) derived a simple relationship which described drug release from a polymeric system Eq. (5). To find out the mechanism of drug release, first 60% drug release data was fitted in Korsmeyer–Peppas model.

$M_t / M_\infty = Kt^n$ (5)

where $M_t / M\infty$ is fraction of drug released at time t, K is the release rate constant incorporating structural and geometric characteristics of the tablet, and n is the release exponent. The n value is used to characterize different release mechanisms.

A plot of log cumulative % drug release vs. log time was made. Slope of the line was n. The n value is used to characterize different release mechanisms as given in Table16, for the cylindrical shaped matrices. Case-II generally refers to the erosion of the polymeric chain and anomalous transport (Non-Fickian) refers to a combination of both diffusion and erosion controlled-drug release (Peppas, 1985).

Table 7: Diffusion Exponent and Solute Release Mechanism for Cylindrical Shape

Diffusion exponent (n)	Overall solute diffusion mechanism
0.45	Fickian diffusion
0.45 < n < 0.89	Anomalous (non-Fickian) diffusion
0.89	Case-II transport
n > 0.89	Super case-II transport

Stability Studies:

The stability study of the formulations was carried out according to ICH guidelines at 40 ± 2° C/75 ± 5 % RH for three months by storing the samples in stability chamber (Lab-care, Mumbai). The purpose of stability testing is to provide evidence of the quality of the drug substance or drug product, and how it varies with time under the influence of a variety of environmental conditions (heat, humidity, light, air etc).

The final formulation was packed in suitable packing like blister and strip packs and then they will be kept at different temperature, humidity conditions and the samples will be analyzed for their physical and chemical properties.

STABILITY STUDIES STORAGE CONDITIONS:

Table 8: Stability studies Storage conditions

Study	Storage conditions	Minimum time period covered by data at submission.
Long term	25 ± 2°c / 60 ± 5% RH or 30 ± 2°c / 75 ± 5% RH	12 months
Intermediate	30 ± 2°c / 65 ± 5% RH	6 months
Accelerated	40± 2°c / 75 ± 5% RH	6 months

RESULTS AND DISCUSSIOIN

PRE-FORMULATION STUDIES

Description: These test results were illustrated below:

Table 9: Description of TIOTROPIUM BROMIDE (API)

Test	Description
Colour	white to Off-White powder
Odour	Free of odour

Solubility:

These tests results were illustrated below:

Table 10: Solubility of Tiotropium bromide (API) in various solvents.

Solvents	Solubility

Water	Slightly soluble
Methylene chloride	Insoluble
acetone	Soluble
Methanol	Soluble

Melting Point:

The tests results were illustrated below:

Table 11: Table showing the melting point of API's

Material	Melting Point Range
Tiotropium bromide	218-220 °C

Standard Calibration Curve of Tiotropium bromide

Table 12: Standard Calibration Curve of Tiotropium bromide in 6.8pH phosphate buffer

S.NO	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.128
3	4	0.234
4	6	0.348
5	8	0.487
6	10	0.604

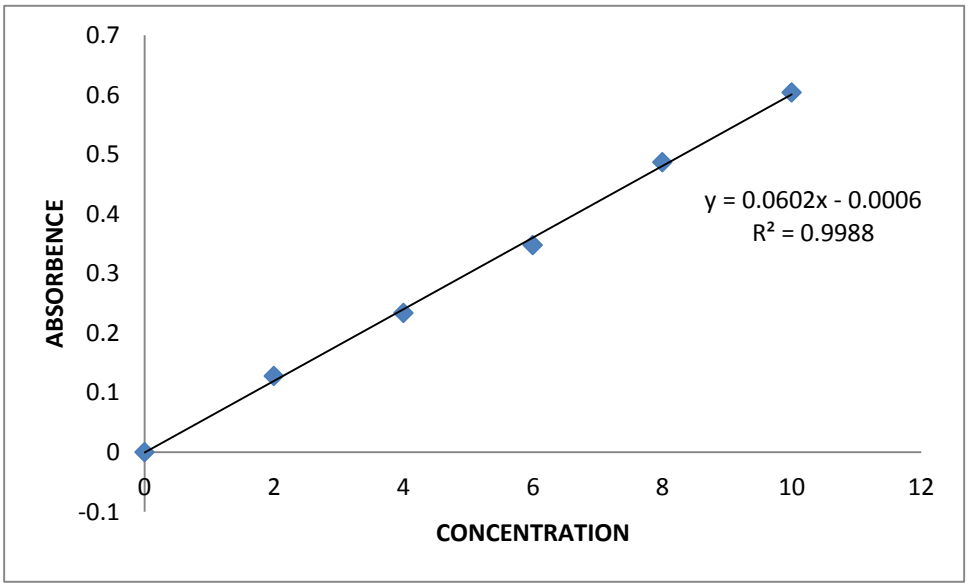

Figure 1: Standard calibration curve of Tiotropium bromide

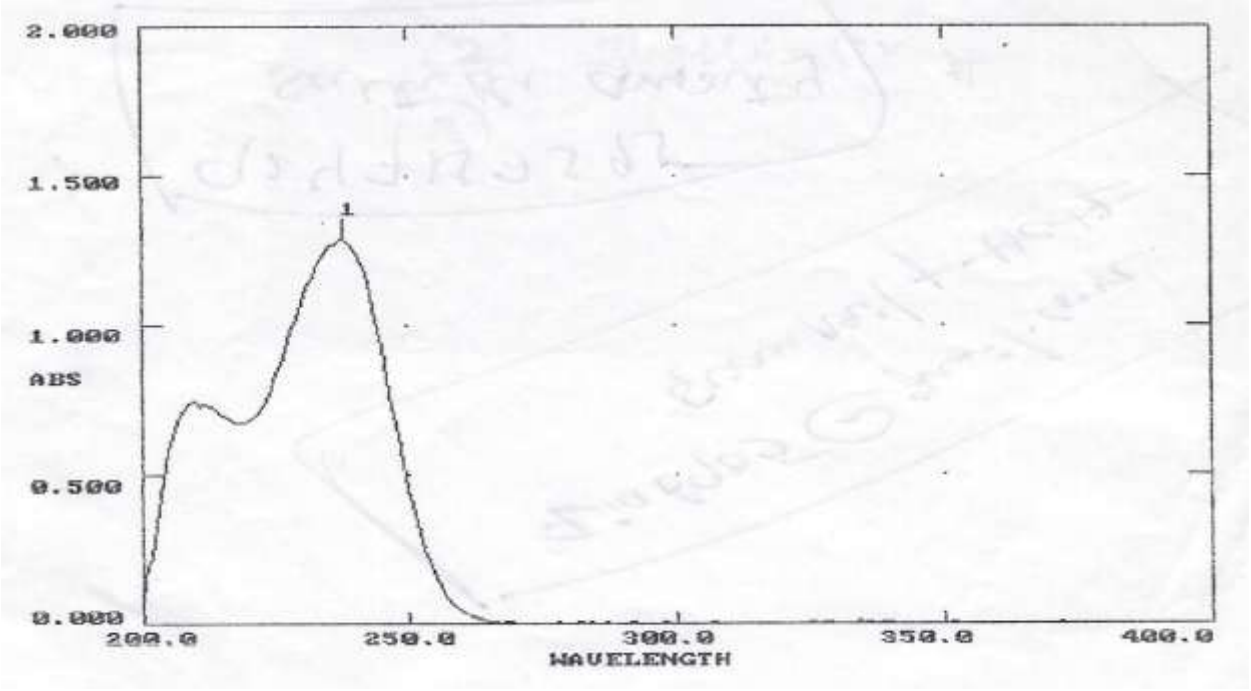

Figure 2: SPECTOPHOTOMETRY FOR Tiotropium bromide

DRUG - EXCIPIENT COMPATIBILITY STUDY

Fourier Transform infra-red (FTIR) spectroscopy

Infrared spectroscopy is a useful analytical technique utilized to check the chemical interaction between the drug and excipients used in the formulation.1-2 mg of solid fine powder of Tiotropium bromide and 200-300 mg of dry powder of KBr (IR grade) were taken in a mortar and mixed well with the help of a spatula. Spectrum measurement was carried out using KBr disk method in the wavelength region of 4000-400 cm^{-1} by FTIR spectrophotometer. The IR spectrum of the physical mixture was compared with that of the pure drug to check any possible drug-excipient interaction.

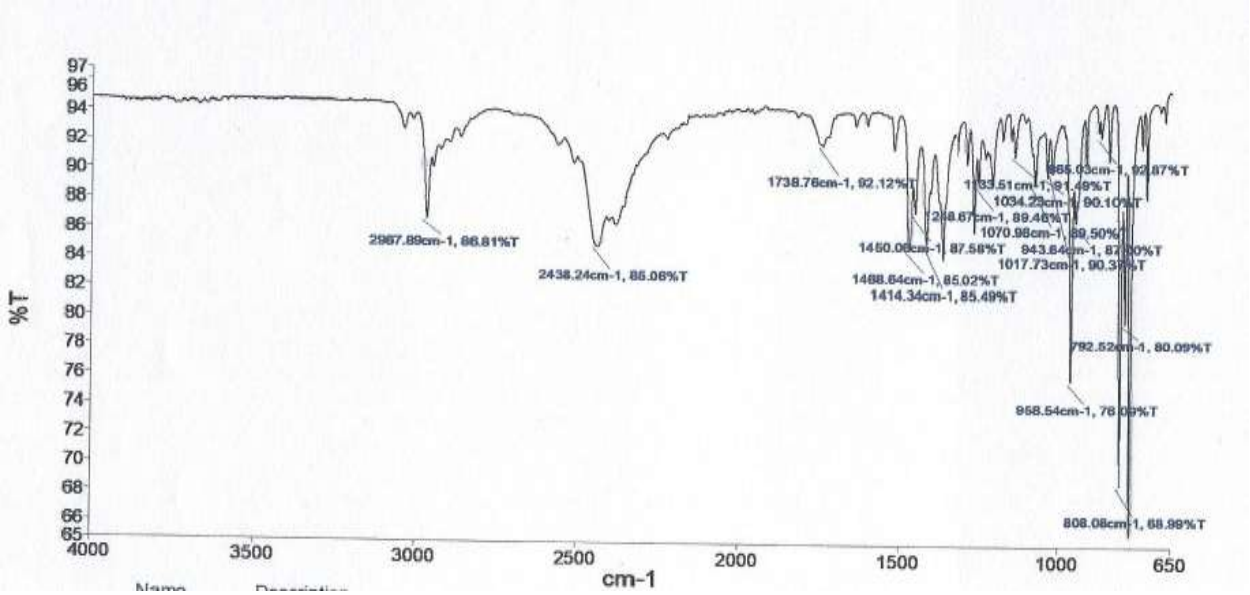

Figure 3: FTIR spectra of Tiotropium bromide pure drug

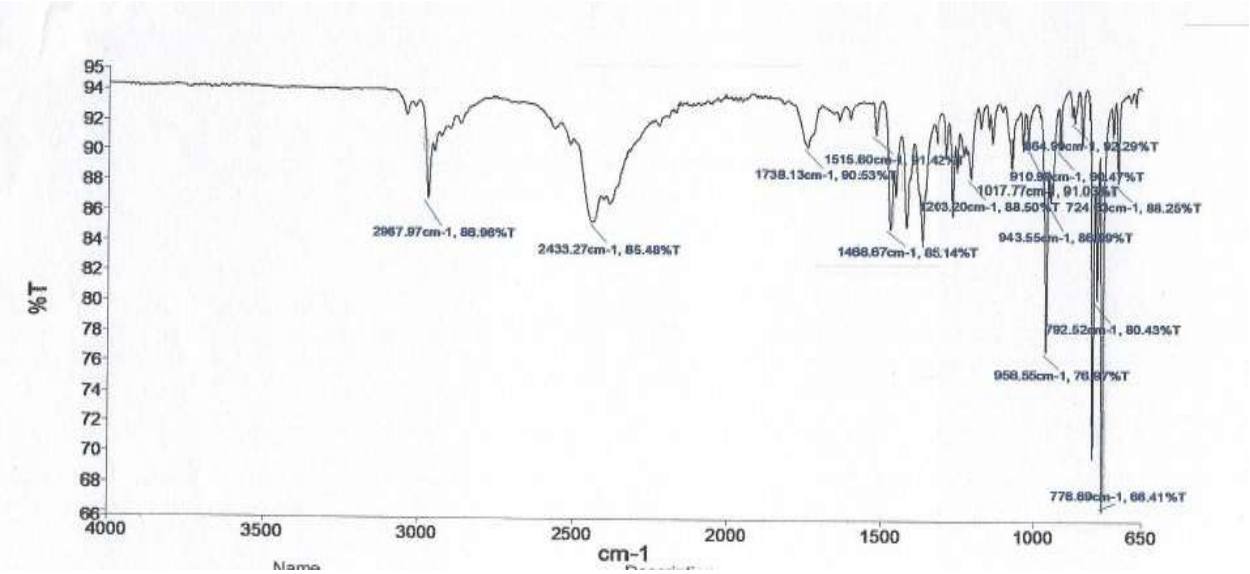

Figure 4: FTIR spectra of Tiotropium bromide Optimized formulation

PRE FORMULATION PARAMETERS

Table 13: Pre-compression parameters for formulation batches

Formulation code	Bulk density (gm/mL)	Tapped density(gm/mL)	Compressibility index (%)	Hausner's ratio	Angle of repose
T1	0.435	0.500	13.0	1.14	25^0 16'
T2	0.427	0.496	13.9	1.16	24^0 14'
T3	0.445	0.505	11.8	1.13	24^0 10'
T4	0.400	0.471	15.0	1.17	23^0 02'
T5	0.430	0.553	22.2	1.28	$26^0$25'
T6	0.461	0.521	11.5	1.13	25^0 05'
T7	0.470	0.57	17.5	1.21	$24^0$11'

T8	0.458	0.54	15.1	1.17	20^0 05’
T9	0.430	0.553	22.1	1.27	$26^0$25’
T10	0.426	0.496	13.7	1.17	24^0 14’
T11	0.430	0.551	22.0	1.25	$26^0$22’
T12	0.445	0.505	11.8	1.13	24^0 10’

Evaluation studies:

Table 14: Physical Evaluation Parameters for Core Tablets

S. No	Physical parameter	T 1	T28	T3	T4	T 5	T 6	T 7	T 8	T 9	T 10	T 11	T 12
1	Weight (mg)	152	150	148	149	148	150	151	151	150	148	150	149
2	Hardness (Kg/cm^2)	3.3	3.9	3.5	3.2	3.4	3.8	3.5	3.4	3.3	3.4	3.8	3.2
3	Thickness (mm)	3.28	3.16	3.82	3.74	3.44	3.25	3.11	3.15	3.10	3.44	3.14	3.74
4	Friability %	0.2	0.4	0.2	0.4	0.3	0.5	0.4	0.5	0.4	0.3	0.4	0.4
5	Disintegration time (min)	2min 10sec	3mins	3mins 40sec	2mins	2 min 3 sec	3mins	1min 30 sec	1 min	1min 45 sec	2 min 3 sec	3mins 30sec	2mins 45sec

Table 15: Dissolution for core tablet

Time (Min)	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
5	22.6	16.4	15.9	25	15.7	20.8	32.0	28.6	21.6	26	**0**	15.9
10	30.80	31.7	28.6	34	31.7	34.3	47.43	46.14	33.8	35	14.6	28.6
15	48.2	38.2	40.1	48	42.6	49.25	62.3	52.22	49.2	50	29.7	40.1
20	55.6	43.8	49.7	60	50.31	55.33	79.5	71.74	56.6	65	38.2	48.7
30	69.1	56.5	59.6	81	74.44	62.8	96.5	80.5	69.4	87	42.8	59.6
45	86.3	76.8	68.6	96.0	89.35	79.5	--	95.5	86.8	94	56.5	67.6
60	95.4	88.7	79.6	--	--	--	--	--	95.1		78.8	78.4

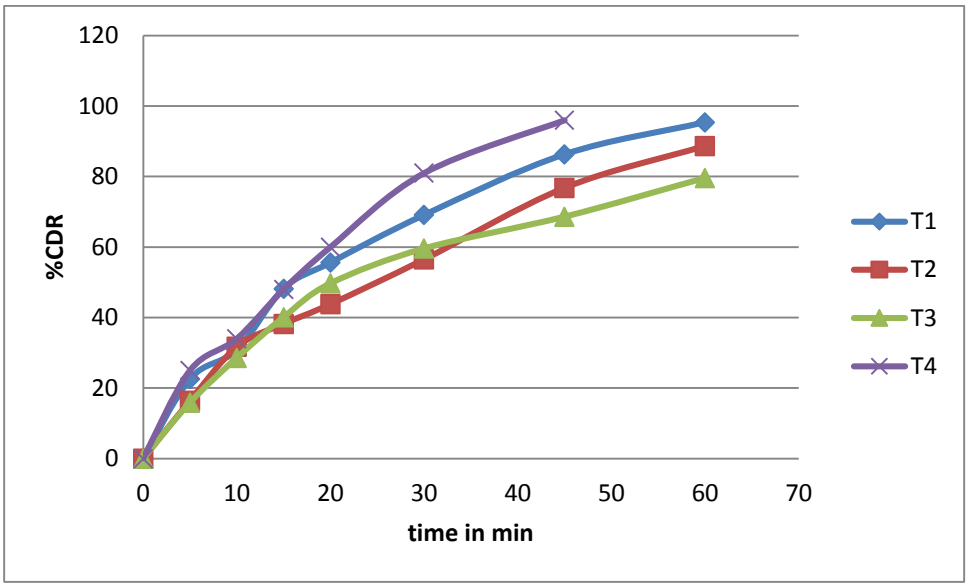

Figure 5: Dissolution for core tablet T1-T4 formulations

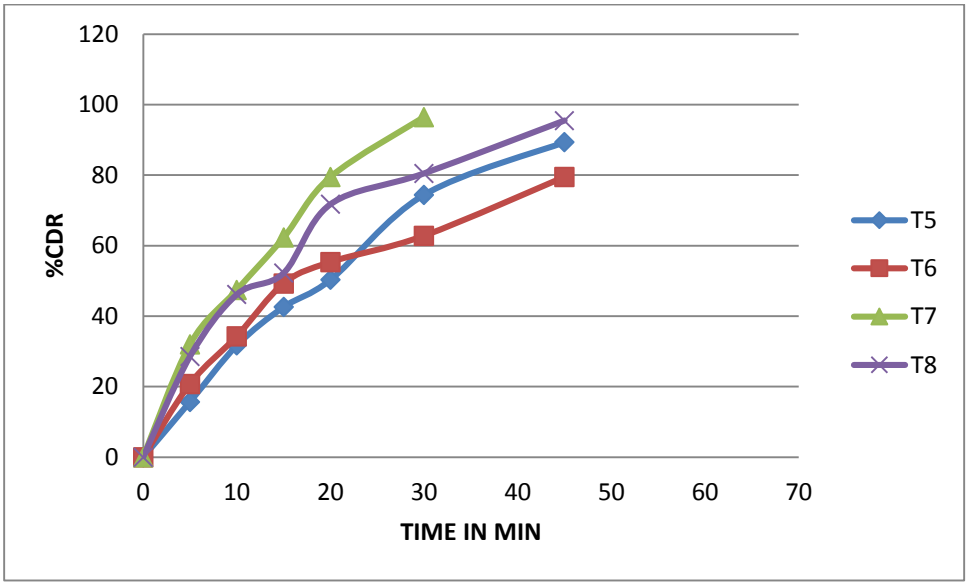

Figure 6: Dissolution for core tablet T5-T8 formulations

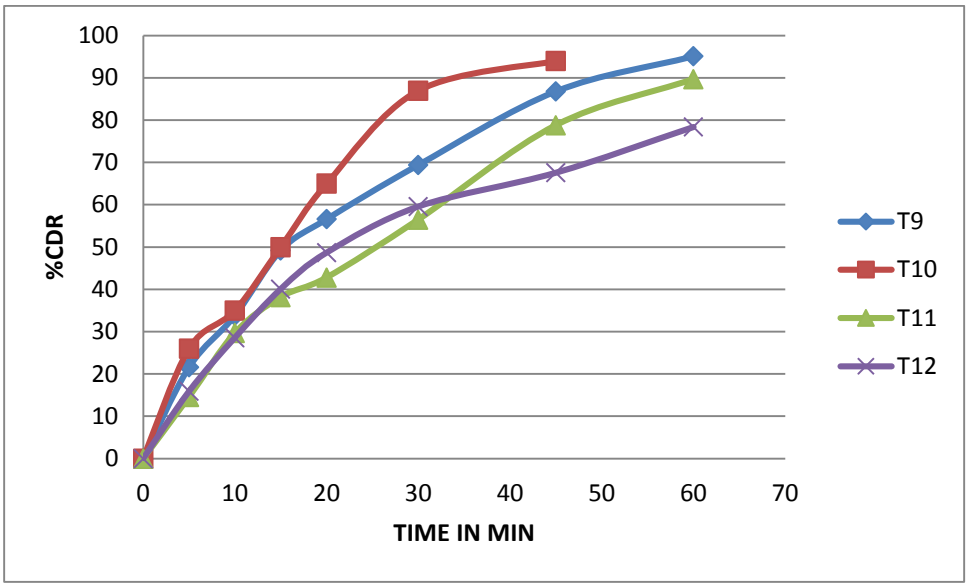

Figure 7: Dissolution for core tablet T9-T12formulations

Based on the drug release within the required time period T7 was optimized and further formulated for press coating.

KINETIC RELEASE MODELS:

Table 16: Release kinetics for T 7 formulation for Pre coated tablet

	ZERO ORDER % CDR Vs T	FIRST ORDER Log % Remain Vs T
Slope	1.46043632	-0.02728534
Intercept	30.076711	1.90134844
R 2	0.72580817	0.847827116

Figure 8: zero order release graph for T7 formulation

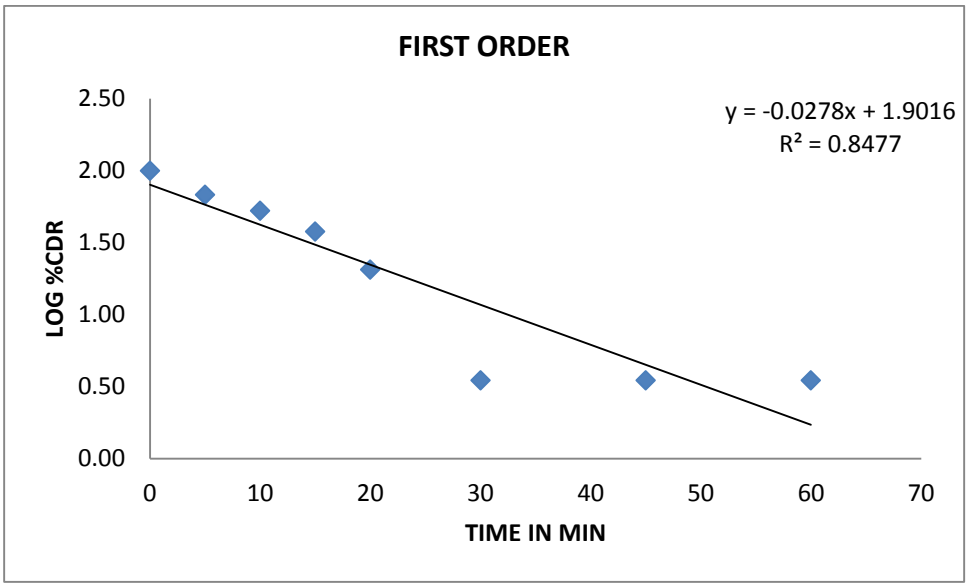

Figure 9: First order release graph for T7 formulation

From the above kinetics of drug release the Tiopropium bromide pulsatile tablets follows first order kinetic drug release.

Table 17: Evaluation Parameters for Press Coated Tablets

S. No	Physical parameter	P1T7	P2T7	P3T7	P4T7	P5T7
1	Weight (mg)	502	501	500	501	500
2	Hardness (Kg/cm^2)	6.5	6.7	6.8	6.2	6.4
3	Thickness(mm)	4.5	4.4	4.3	4.7	4.6
4	Friability %	0.56	0.55	0.62	0.54	0.52

Table 18: Dissolution data for press coated tablets

Time in hrs	Press coat Formulation code				
	P1T7	P2T7	P3T7	P4T7	P5T7
1	2.89	1.62	0	16.2	0
2	15.45	18.74	0	33.5	0
3	40.32	45.98	2.5	99.8	8.95
4	51.21	56.12	6.5	-	15.65
5	74.22	78.66	8.9	-	42.98
6	98.1	89.90	12.2	-	60.45
7	-	95.55	85.64	-	82.80
8	-	--	99.65	-	95.64

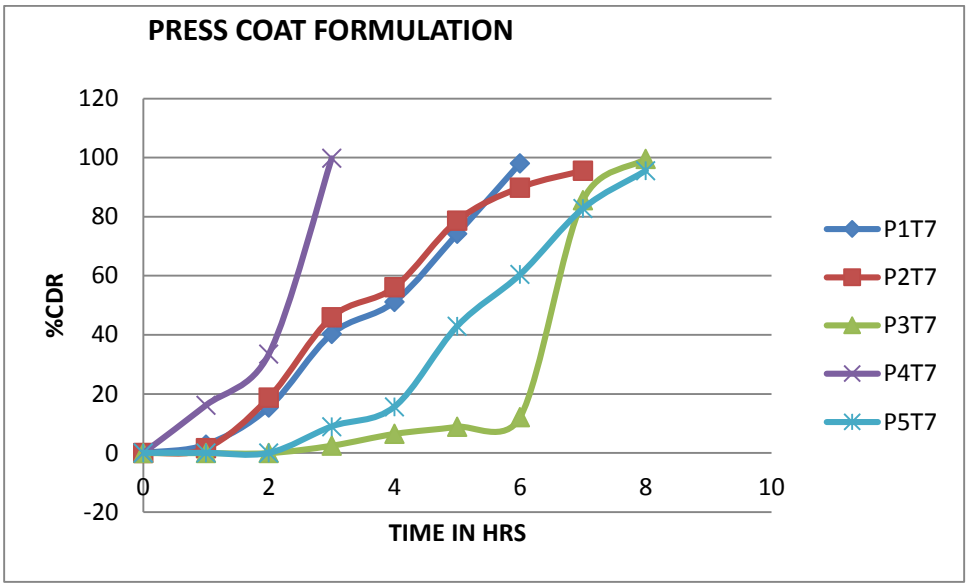

Figure 10: Dissolution for Press coat formulations

From the above core formulations P3T7 was selected for press coat by using XANTHUM GUM AND GUAR GUM was optimized based on the lag time (12.2% in 6 hours) and percent of drug release and also further evaluated.

Stability studies:

Table 19: Stability Studies of formulation P3T 7

Sampling interval	25^0C/60%RH	30^0C/65%RH	40^0C/75%RH
0 Days	99.8	99.8	99.8
30Days	99.0	99.1	99.1
60 Days	98.2	98.5	98.8
90 Days	97.8	97.8	97.8

Stability studies of the formulation **P3T 7** of Tiotropium Bromide press-coated were carried out to determine the effect of formulation additives on the stability of the drug and also to determine the physical stability of the formulation. The stability studies were carried out at 25^OC/60%RH, 30 ºC/65% RH and 40 ºC/75% RH for 90 days. There was no significant change in the physical property and percent of drug release was within the limits ±4 during 6 hours during the stability period.

CONCLUSION

From the above investigation of work results it can be concluded that, Formulation developed tablets gave acceptable results for no.of physicochemical parameters like hardness, friability, thickness, weight variation. Hydroxy propyl methyl cellulose and EC has major effect on the lag time, while also shows significant effect on drug release. Among all the core tablet formulations T7 was selected based on drug release within a given period of time. In-vitro release rate studies

showed that the P3T7 was optimized based on less amount of drug release during lag time. Formulations P3T7 found to be stable at 45^{o} C and 75% RH for a period of 6 months.

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