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RESEARCH ARTICLE

NOVEL DRUG DELIVERY SYSTEM

FORMULATION AND EVALUATION OF DULOXETINE HYDROCHLORIDE DELAYED RELEASE ENTERIC COATED CAPSULES

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ABSTRACT

The main objective of the present study is to prepare robust and stable formulation and evaluation of duloxetine hydrochloride delayed release enteric coated pellets in capsules. Since Duloxetine hydrochloride degrades in the acidic environment, it is important to bypass the acidic pH of the stomach. Protection of drug from acidic environment is done by coating the drug with enteric polymers by using suspension layering technique in Fluidized bed processor (FBP) with different enteric polymers like PVAP (Poly vinyl Acetate phthalate), Kollicoat MAE 30 DP, Eudragit L30 D55 (Methacrylic acid copolymer) and HPMCP (Hydroxy propyl methyl cellulose phthalate). Eudragit L30 D55 is a good enteric material. Based on the vendor data and details, drug release shows after pH6.5 buffer, where as marketed preparation release starts at pH 5.5 buffer. So Eudragit was not taken for further trails. The prepared pellets were studied for their *Invitro* release studies and were analyzed by using HPLC technique. The released kinetics was analyzed using the zero-order model, first-order model and Higuchi's square root equation. FT-IR (Infrared spectroscopy) and DSC (Differential Scanning Calorimetry) studies were performed to know the compatibility of the drug with various excipients and SEM (Scanning Electron Microscopy) analysis performed to know the particle size and morphology of the pellet. The results depicted that HPMCP gave a good dissolution profile and process suitability compared to Eudragit L30 D55, Kollicoat MAE 30DP and PVAP and hence optimized based on the similarity factor (f2 value).

KEY WORDS

Dissolution, Duloxetine Hydrochloride, Enteric coated pellets

INTRODUCTION

Duloxetine hydrochloride is a selective serotonin norepinephrine reuptake inhibitor currently indicated for the treatment of major depressive disorder, generalized anxiety disorder, diabetic peripheral neuropathic pain, and fibromyalgia. Duloxetine Hydrochloride is an anti-depressant drug. The degradation of this anti-depressant drug in the acidic environment of stomach leads to sub therapeutic levels. In order to avoid this degradation and to bypass the acidic pH of the stomach, one of the proven approaches is formulation of delayed release dosage forms (single unit or multiple units) by using different enteric polymers in a Fluidized bed processor (FBP) and the method used was solution/suspension layering. Multiple unit particulate system show better *In Vitro* release behavior than other dosage forms.[1]

According to BCS classification, Duloxetine is a class –II drug and thus has limited aqueous solubility. There are instances where the rate of dissolution of a poorly soluble drug is a rate-limiting factor in its absorption by the body.

Duloxetine undergoes many degradation reactions and the most common degradation is by hydrolysis. α-Naphthol (4-(3-methylamino-1-thiophen-2-yl-propyl)-naphthalene-1-ol hydrochloride), 4-naphthol duloxetine (name 4-(3-methylamino-1-thiophen-2-yl-propyl)-naphthalene-1-ol hydrochloride) and 3-Acetyl duloxetine ((+)-N-methyl-3-(1-naphthalenyloxy)-3-mine hydrochloride) impurities are the degradants formed by hydrolysis.[2]

The type of coating technique strongly affects the film microstructure and thus affects the release mechanism and rate from pellets coated with polymer blends.[3] The characteristics of the pellets in the product are round pellets with good flow behavior, with compact structure, with good dispensability and easy to dose.[4] Pellets have gained importance over the years due to their distinctive advantages in both technological and therapeutic aspects[5]. So, in the present study, Duloxetine hydrochloride delayed release capsules containing pellets were prepared and studied.

The process involves four successive coatings of Drug, Barrier material, Enteric coating material and Top coating material were given to non pareil seeds (sugar spheres)[6].

Suitable pH dependent enteric polymers selected are HPMCP (HP-55 and HP-50), Eudragit L30 D55, Kollicoat MAE 30DP and PVAP. By varying the phthalate content, the pH for dissolution of the coating can be controlled between 5 to 5.5. And this polymer solution is sprayed on the separating layer and is then dried to obtain delayed release composition[7]. Top layer is readily dissolved in the stomach and leaves enteric layer which protects the drug layer[8], it also avoids the interaction between enteric coating and packing system by preventing the contact between them[9].

MATERIALS AND METHODS

MATERIALS:

The following chemicals were obtained from commercial suppliers and used as received: Duloxetine Hydrochloride (Hetero Drugs, Hyderabad), Sugar spheres (Werner, USA), HPMC 5cps (Dow chemicals), HPC (Aqualon, Hercules, Japan), Kollicoat MAE 30 DP (an aqueous methacrylic acid/ethyl acrylate copolymer dispersion, BASF, Ludwigshafen, Germany), Crospovidone (kollidone CL-M, BASF, Germany)HPMCP HP-50 and HP- 55 (Shin Etsu, Japan), PVAP (Colorcon, Kent, UK), sucrose (Nordzucker GmbH, Braunschweig, Germany), talc (Luzenac, Italy), triethyl citrate

(Morflex, Greensboro, USA), Titanium dioxide (Kronos), Opadry White Y-1-7000(Colorcon ltd.), IPA, Dichloromethane, Acetone and purified water. All chemicals were reagent grade or higher.

ANALYTICAL METHOD:

An in-house developed and validated HPLC method (Waters, symmetry C_8, 250×4.6 mm, 5µ) using 0.1 N HCl in first 2 hours and in 6.8 pH phosphate buffer for 90minutes at 288nm was used for the estimation of drug in bulk, formulations and in dissolution samples[10].

Method of Preparation

The pellets were prepared using Pelletization technique that converts fine powders or granules of bulk drugs and excipients into small, free-flowing, spherical or semi-spherical units, referred to as pellets. The type of coating technique strongly affects the film microstructure and thus affects the release mechanism and rate from pellets coated with polymer blends[3]. In the present study we have used a circulating fluidized bed processor with a bottom-spray of the Wurster type [Model No: Plam glatt GPCG 1.1]

Design of duloxetine hydrochloride delayed release enteric coated capsules

Process for preparing duloxetine delayed release formulation comprises of coating a core (sugar spheres of 750 -810 micron size) in succession with a drug layer containing duloxetine hydrochloride, a separating layer, an enteric layer comprising of enteric polymer and then top layer. Application of each layer is in the form of suspension or solution. Each layer is spray coated and dried prior to the application of the next successive coating.

Drug Coating (Formula-I)

Preparation of Drug Suspension

HPMC 5cps (28.8 grams) was added (batch size is for 3000 capsules) to purified water (18%w/w solution) under continuous stirring for 30minutes (or) till clear solution was formed. Then HPC (7.2 grams) was added under continuous stirring for 40 minutes (or) till clear solution was obtained. Duloxetine Hydrochloride (242 grams) was slowly added and the stirring was continued for 20 minutes (or) till it forms a uniform dispersion. Crospovidone (14.4 grams) was added to above solution under continuous stirring for 10 minutes (or) till uniform dispersion was obtained. Finally Talc (20.52 grams) was added to the above solution and continued stirring for 10 minutes (or) until it forms a uniform dispersion. The composition of the formula is seen in the Table I : Drug coating (Formula- I).

Table I
Drug Coating (Formula-I)

S. No	DRUG COATING (mg/unit)	D1	D2	D3	D4	D5
1	**Sugar spheres/non-pariel seeds**	130.3	130.3	130.3	130.3	**130.3**
2	Duloxetine Hydrochloride	67.3	67.3	67.3	67.3	**67.3**
3	Hypromellose 5cps	16	12	8	8	**8**
4	HPC	-	-	-	2	**2**
5	Crospovidone	-	-	-	-	**4**
6	Talc	5.7	5.7	5.7	5.7	**5.7**
7	Purified water	q.s	q.s	q.s	q.s	q.s
	Total	**219.3**	**215.3**	**211.3**	**213.3**	**217.3**

Barrier Coating (Formula-II)

Preparation of Barrier coating dispersion

PREPARATION OF BARRIER COATING SUSPENSION (B1)

HPMC 5CPS (10.4 grams), sucrose (7.15 grams) were added to purified water(15%w/w) under continuous stirring for 45 minutes and 10 minutes respectively. Then, talc (5.2 grams) was added to the above solution. Again, it was stirred for 10 minutes to get a homogenous dispersion.

PREPARATION OF BARRIER COATING SUSPENSION (B2)

Hydroxy propyl methyl cellulose 5CPS (10.4 grams) ,Triethyl citrate (0.78 grams) were added to purified water(10%w/w) under continuous stirring. Then, talc (5.2 grams) was added to the above solution, and it was stirred to get a homogenous dispersion.

The composition of the formula is seen in the Table II : Barrier coating (Formula – II).

Table II

Barrier Coating (Formula-II)

S.No	BARRIER COATING(mg/unit)	B1*	B2**
1	**Drug coated pellets D5**	**217.3**	**217.3**
2	Hydroxy propyl methyl cellulose 5cps	***8***	***8***
3	Talc	***4***	***4***
4	Triethyl citrate	***-***	***0.6***
5	Sucrose	***5.5***	***-***
6	Purified water	q.s	q.s
	Total	***234.8***	***229.9***

***With TEC**

****With Sucrose**

Enteric Coating (Formula-III)

Preparation of Enteric coating suspension

Polymer used was PVAP: Methanol and methylene chloride (1:1) were taken in a stainless steel vessel. PVA Phthalate was slowly added to the above solvent and the contents were mixed for 15 minutes under continuous stirring. TEC and Talc were added to solution, under continuous stirring till a homogenous dispersion was formed. The composition of the formula is seen in the Table III: Polymer- Polyvinyl acetate phthalate (PVAP).

Polymer used was KOLLICOAT MAE 30 DP: Purified water was taken in a stainless steel vessel. KOLLICOAT MAE 30 DP was slowly added to the purified water and the contents were mixed for 15 minutes under continuous stirring. NAOH solution (4% w/w) was added to the above step. TEC and Talc were added to solution, under continuous stirring till a homogenous dispersion was formed. The composition of the formula is seen in the Table IV: Polymer- Kollicoat MAE 30 DP [Poly(methacrylic acid, ethyl acrylate) (1,1)].

Polymer used was HPMCP HP-55 and HP-50: Acetone and water were taken in the ratio of 8:2 in a stainless steel vessel. HPMCP (HP-55) was slowly added to this solvent and the contents were mixed for 10 minutes under continuous stirring. Then HPMCP (HP-50) was slowly added to this solvent and the contents were mixed for 2 hours under continuous stirring. TEC and Talc were added to above solution, under continuous stirring till a homogenous dispersion was formed. The composition of the formula is seen in the Table V: Polymer-

Hydroxy propyl methyl cellulose phthalate (HPMCP).

Formula for enteric coating

Table III
Polymer: Polyvinyl acetate phthalate (PVAP)

S.No.	ENTERIC COATING(mg/unit)	E1
1	**Barrier coated pellets**	**234.8 (b1)**
2	PVA Phthalate (Sureteric)	57
3	Tri ethyl citrate	5.7
4	Talc	6.3
5	Methanol /Methylene chloride (DCM)	q.s
	Total	**303.8**

Table - IV
polymer: Kollicoat MAE 30 DP [Poly(methacrylic acid, ethyl acrylate) (1,1)]

S.No	ENTERIC COATING(mg/unit)	E2	E3
1	**Barrier coated pellets**	**234.8 (b1)**	**229.9 (b2)**
2	Kollicoat MAE 30DP	42	42
3	Sodium hydroxide (NaOH)	0.008	0.008
4	Tri ethyl citrate	4.2	4.2
5	Talc	5.8	5.8
6	Purified water	q.s	q.s
	Total	**286.8**	**281.9**

Table V
Polymer: Hydroxy propyl methyl cellulose phthalate (HPMCP)

S.No	ENTERIC COATING(mg/unit)	E4	E5	E6	E7	E8	E9	E10
1	**Barrier coated pellets**	**234.8 (b1)**	**234.8 (b1)**	**229.9 (b2)**	**229.9 (b2)**	**229.9 (b2)**	**229.9 (b2)**	**229.9 (b2)**
2	HPMCP HP-55	57	-	57	-	28.5	28.5	**28.5**
3	HPMCP HP -50	-	57	-	57	28.5	28.5	**28.5**
4	Tri ethyl citrate	5.7	5.7	5.7	5.7	5.7	5.7	**5.7**
5	Talc	6.3	6.3	6.3	6.3	6.3	6.3	**6.3**
6	IPA/DCM	q.s	q.s	q.s	q.s	q.s	-	**-**
7	IPA/Acetone	-	-	-	-	-	q.s	**-**
8	Acetone/Puri. water (8:2)	-	-	-	-	-	-	q.s
	Total	**303.8**	**303.8**	**298.9**	**298.9**	**298.9**	**298.9**	**298.9**

Top Coating (Formula-IV)

Preparation of Top coating solution: Required quantity of purified water was taken into stainless steel (SS) vessel which gives 12% w/w suspension and to this Opadry-Y-1-7000 (10.08 grams) was added slowly to the vessel under continuous stirring for 15 minutes. Finally talc (10.08 grams) was added to above mixture under constant stirring for 10 minutes till it forms uniform dispersion. The composition of the formula is seen in the Table VI: Top coating (Formula – IV). Optimization parameters of FBP can be seen in the Table VII: Optimization of process parameters.

Table VI
Top Coating (Formula-IV)

S.No	INGREDIENTS	mg/unit
	TOP COATING	**T1**
1	**Enteric coated pellets**	**298.9**
2	Opadry Y- 1- 7000	**7.2**
3	Talc	**7.2**
4	Purified water	q.s
	Total	**313.3**

Table VII
Optimization of Process parameters

S. No.	Process parameters of FBP	Drug	Barrier	Enteric	Top
1	Inlet temperature (°C)	50-55	35-40	35-40	40-46
2	Product temperature (°C)	35-45	30-40	30-40	40-45
3	Exhaust temperature (°C)	30-35	30-35	30-40	30-35
4	Drive speed (CFM)	40-65	40-50	40-50	50-55
5	Atomization (Barr)	0.8-3.5	1.0-1.5	1.0-1.5	1.0-1.8
6	Spray rate (g/min)	2-5	2-5	2-5	2-5
7	Wurster height (mm)	20-50	20-30	20-30	20-30

Analytical Method

Preparation of HPLC Diluent:

Diluent A: 1.41g disodium phosphate dissolved in 1litre water

Diluent B: Diluent (1) + Methanol (50:50)

Assay:

Assay was conducted by using HPLC technique using Waters C8 column at wavelength 288nm. Duloxetine HCl pellets equivalent to 68mg of Duloxetine were accurately weighed and transferred into a 100ml volumetric flask. About 50ml of methanol was added and sonicated for 30 minutes with vibration until pellets dissolved (sonicator bath temperature to be maintained between 20-25°C) and the volume was made with the diluent A. The liquid was centrifuged to at 5000 rpm for 10 minutes. The resultant

solution was filtered through 0.45µ filter and the first 1-2ml of filtrate was discarded. The final solution (5ml) was transferred into 50ml volumetric flask and the volume was made with the diluents B.

Acid Resistance:

Acid Resistance was conducted by HPLC technique to check the acid resistance of Duloxetine Hydrochloride enteric coated capsules with different enteric polymers. Duloxetine HCl pellets (68mg) equivalent to 1 capsule was accurately weighed and transferred into a 100ml volumetric flask. About 50ml of methanol was added and sonicated for 20 minutes with vibration until pellets dissolved (sonicator bath temperature to be maintained between 20-25^{0}C), diluted to volume with diluent A. Above mixture was centrifuged at 5000 rpm for 10 minutes. The solution was filtered through 0.45µ filter and 1-2ml of filtrate was discarded. 5ml of the remaining solution was transferred into 50ml volumetric flask and made with the diluent B.

Dissolution

Enteric coated capsules prepared from PVAP, Kollicoat MAE 30DP and HPMCP were subjected to a dissolution study in 1000mL of 0.1N HCl followed by pH 6.8 phosphate buffer using a USP Type I apparatus. Transferred 1000 ml of 0.1 N HCl into each vessel and allowed the medium to equilibrate to the temp of 37±0.5°C. One capsule is placed in each of the baskets, and the baskets were fixed to shafts and operated dissolution apparatus at 100 rpm for 2 hours. After 2 hrs run in acid, 0.1 N HCl was carefully drained and the fresh solution of 1000 ml of pH 6.8 phosphate buffer was taken into the vessel. The process continued to operate the apparatus for specified time. Then, 10 ml of the samples were taken from each vessel at specified interval of time. The amount of samples of aliquots taken was replaced with equal volume of dissolution and medium was maintained at 37±0.5°C. The collected sample was filtered through 0.45 µ membrane filter.

FTIR spectroscopy

FTIR spectra of drug and optimized formulation were obtained. Sample about 5 mg was mixed thoroughly with 100 mg potassium bromide IR powder and compacted under vacuum at a pressure of about 12 Psi for 3 minutes. The resultant disc was mounted in a suitable holder in Perkin Elmer IR spectrophotometer and the IR spectrum was recorded from 4000 cm^{-1} to 625 cm^{-1} in a scan time of 12 minutes. The resultant spectra were compared for any *spectral changes.*

Differential Scanning Calorimetry (DSC)

DSC analysis of drug, placebo and optimized formulation were obtained on a Perkin Elmer Thermal Analyzer. The instrument was calibrated with indium standard. Accurately weighed about 3.5mg of sample was placed in an open, flat bottom, aluminium sample pans. Thermograms were obtained by heating the sample at a constant rate 10.00°C/min A dry purge of nitrogen gas (20ml/min) was used for all runs. Samples were heated from 32.00°C to 350.00°C with a hold time for 1.0 min at 32.00°C. The melting point, peak maxima, appearance of any new peak and peak shape was noted.

Scanning Electron Microscopy (SEM)

The surface of drug pellets and coated pellets were examined under a scanning electron microscope (Leica Cambridge S-360, UK). The pellets were mounted onto stubs using double sided adhesive tape. The mounted samples were sputter coated (Polron Sc 515, UK) under an argon atmosphere with gold palladium and examined at 15 KV accelerating voltage.

Evaluation of Pellets

The flow properties of the enteric coated pellets for optimized formulation (E10) were measured. The flow properties were studied by measuring the quality parameters like Bulk density, Tapped density, Compressibility Index, Hausner's ratio, weight variation and friability[11].

Kinetic models of Reference and Optimized formulation

Kinetic studies were conducted for Reference and Optimized formulation. Zero order plot, first order plot and Higuchi plots were plotted for Reference standard and optimized formulation, based on the regression coefficient values obtained kinetics of Reference and Optimized formulation were studied.

Stability on Storage

Stability studies were conducted for the optimized enteric coated formulation at 40ºC / 75% RH for about 3 months in stability chamber (Thermo lab). Samples were analyzed for assay, acid resistance and dissolution at the end of 1st, 2nd and 3rd month.

RESULTS

Assay

The enteric coated pellets prepared are complied with the In-house specifications mentioned in the Table VIII: Assay results of enteric coated pellets (n=6).

TABLE VIII

Assay results of enteric coated pellets (n = 6)

Formulation	Assay Specifications :98-102%
Reference	**101±1.02**
E1	98.8±0.68
E2	99.9±1.28
E3	100.1±0.91
E4	99.7±1.60
E5	98.8±0.53
E6	98.5±0.69
E7	99.4±0.69
E8	100±1.23
E9	98.8±0.46
E10	**99.35 ±0.74**

Each value represents mean ± SD (n=6)

Acid Resistance

From the above results, the enteric coated pellets prepared are complied with the In-house specifications mentioned in the Table IX: Acid Resistance results of enteric coated pellets (n=6).

TABLE IX
Acid Resistance results of enteric coated pellets (n = 6)

Formulation	Acid Resistance Specifications :98-102%
Reference	**99±0.86**
E1	99.5±1.47
E2	99±0.36
E3	100±0.79
E4	99±0.76
E5	98.9±0.72
E6	98.9±0.58
E7	100.4±0.8
E8	99.8±0.89
E9	99.8±1.0
E10	**98.75±0.86**

Each value represents mean ± SD (n=6)

Invitro studies in 6.8 pH phosphate buffer
The results in the *invitro* graphs indicates that the dissolution profile of E10 formulation is found to be similar to that of the reference standard. The percentages of duloxetine released from E10 and the reference in 0.1 N HCl at 120 min are 1.0% and 1.3% respectively.

Figure 1
Invitro dissolution profiles of E1-E10 formulations and Reference

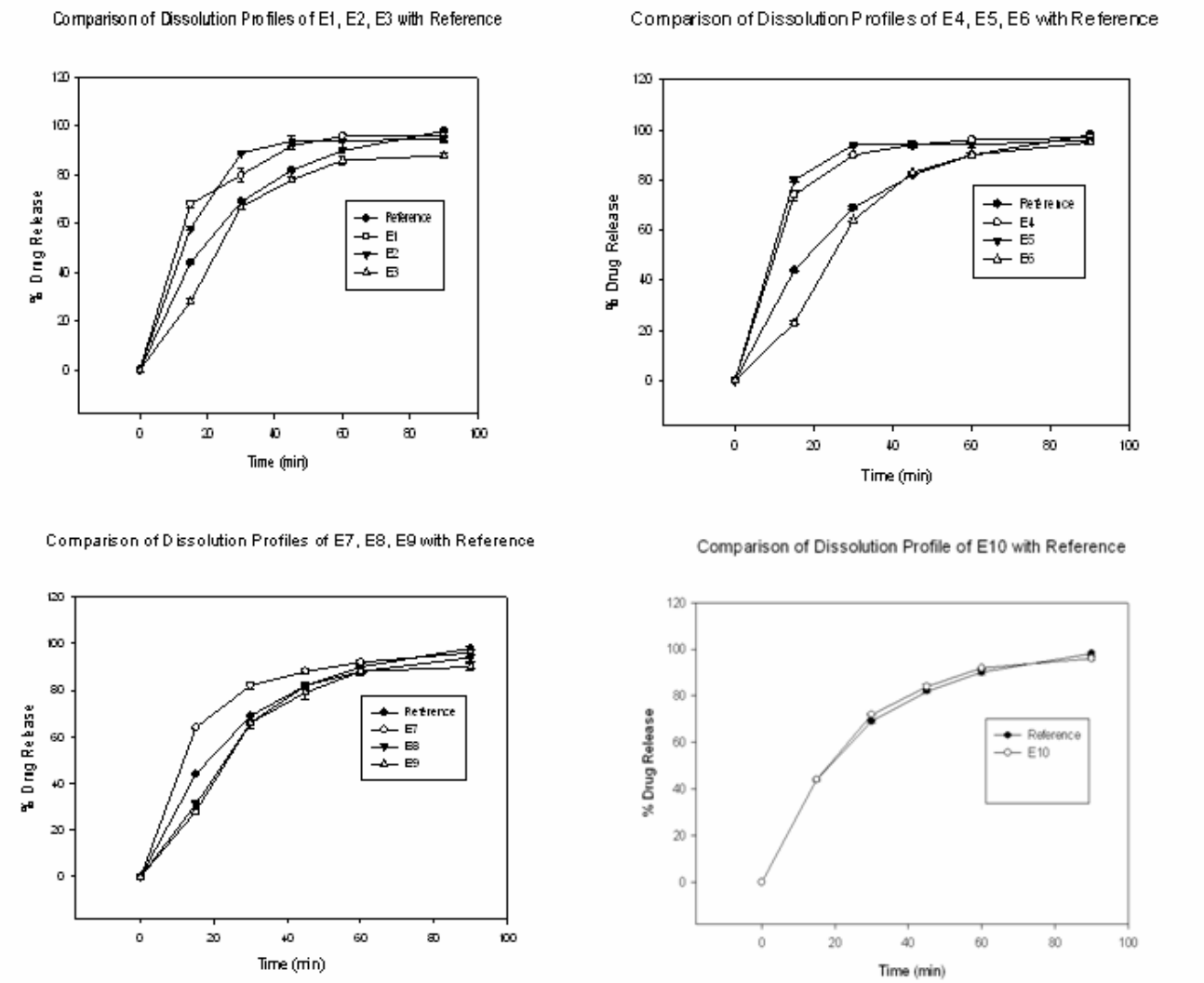
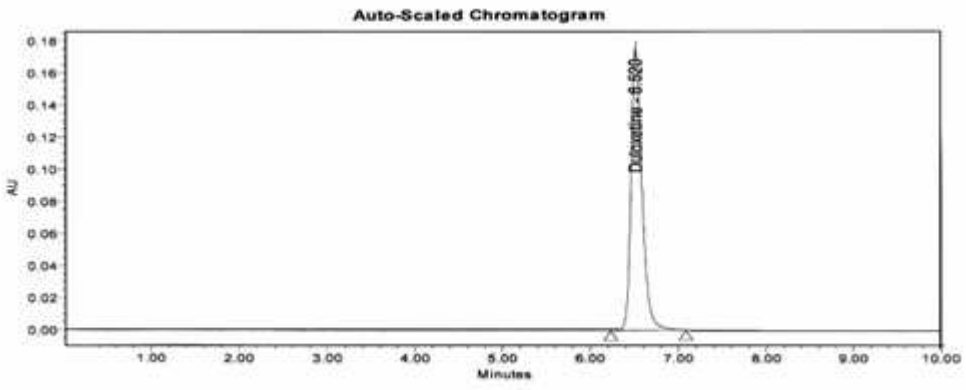


Figure 2
Chromatogram for optimized formulation (E10)



Dissolution Profile Comparison Using Similarity Factor, f2:

The factor f2 measures the closeness between the two profiles. Where n is the number of dissolution time points, and R_t and T_t are the reference and test dissolution values (mean of at least 6 dosage units) at time t. The f1 and f2 values for all formulations (E1-E10) are given below in the Table X: Dissolution profile comparison of Test and Reference (n=6).

TABLE X

Dissolution profile comparison of Test and Reference (n=6)

Formulation	f1 (difference factor)	f2 (similarity factor) (%)
E1	0.13	44.3
E2	0.13	45.2
E3	0.09	52.4
E4	0.182	37.86
E5	0.206	34.46
E6	0.078	50.40
E7	0.112	47.68
E8	0.057	59.78
E9	0.083	53.95
E10	**2.34**	**82.0**

FTIR

The FT-IR spectrum of the formulation showed the presence of the drug in its active form without alteration of its chemical structure.

Figure 3.1

FT-IR of Pure drug

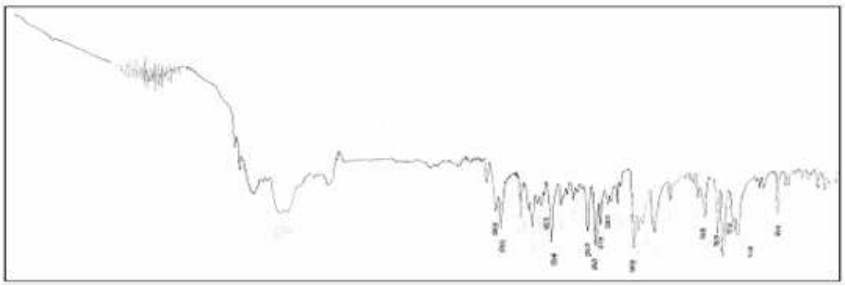
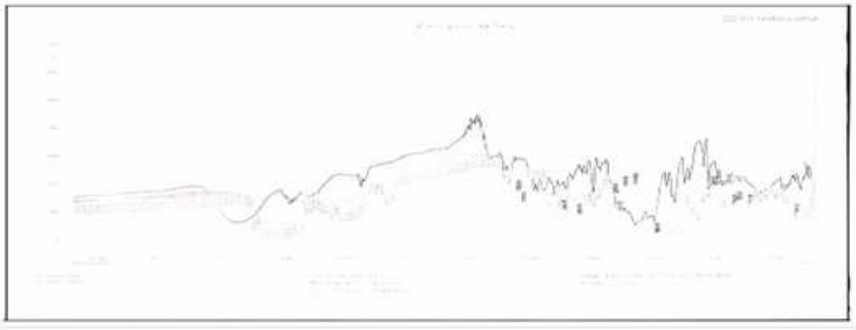


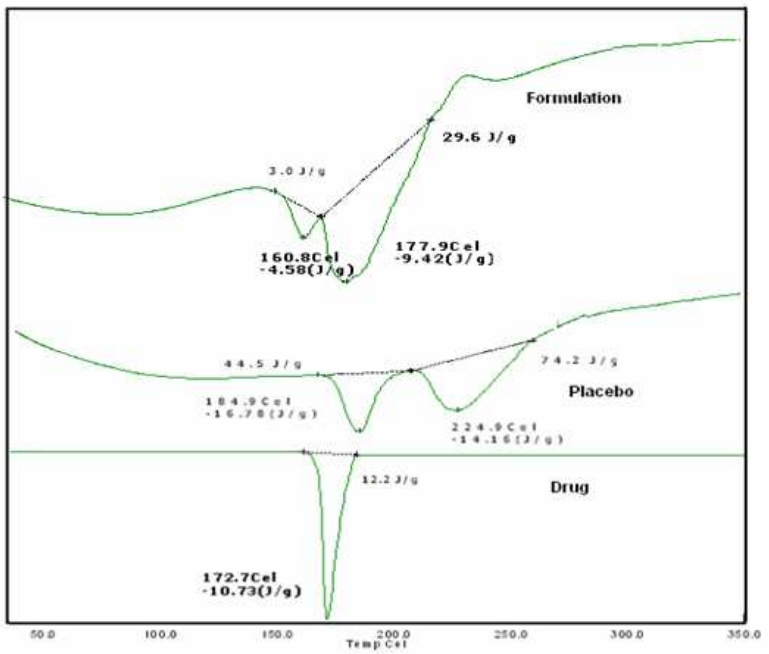
Figure 3.2

FT-IR of Formulation



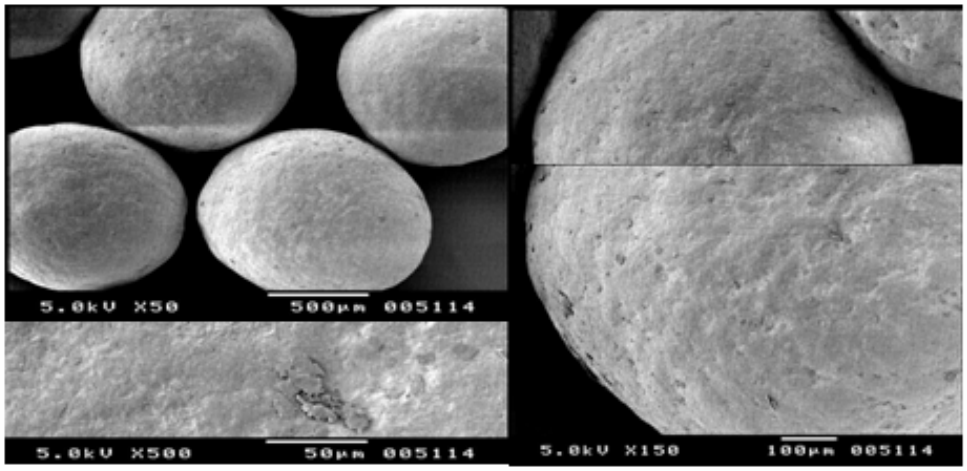
Differential Scanning Calorimetery:
In Placebo thermogram, it showed peaks of excipients at 184.9°C and 224.9°C. There was no change in the peak temperature of the optimized formulation, when compared to the pure drug, which indicates no interaction between drug and excipients.

Figure 4
DSC of formulation, placebo and pure drug



Scanning Electron Microscopy: Enteric coated pellets were examined under scanning electron microscopy at (x50), (x100) and (x500) magnifications. The electron photomicrographs are shown in figures.

Figure 5
Scanning Electron Microscopy figures of optimized formulation (E10)



Evaluation of enteric coated pellets (n=6)

The flow properties of the enteric coated pellets for optimized formulation (E10) were measured. The flow properties were studied by measuring the quality parameters like Bulk density, Tapped density, Compressibility Index, Hausner's ratio, and friability. Also the weight variation is checked for this formulation. The results are shown in Table XI: Evaluation of enteric coated pellets (n=6).

TABLE XI

Evaluation of enteric coated pellets (n = 6)

S.NO	Parameters	Results
1	Bulk density	0.849 ±0.01
2	Tapped density	0.927 ±0.014
3	Compressibility Index	8.43 ±1.62
4	Hausner's Ratio	1.09 ±0.01
5	Weight variation	410.45 ± 1.2 (409-413)
6	Friability (%)	0.283 ±0.08

Kinetic release studies for Reference and optimized formulation

The different kinetic models were applied to the optimized enteric coated formula and the results are shown in Table XII : Release kinetics of optimized formulation and the graphs are shown in figures, for both optimized formulation and the reference.

TABLE XII

Release kinetics of optimized formulation

Release kinetics	Correlation coefficient (r^2) for Reference	Correlation coefficient (r^2) for optimized formulation E10
Zero order equation	0.8552	0.7842
First order equation	0.9907	0.9784
Higuchi (diffusion)co-efficient	0.9361	0.8833

Kinetic models

Figure 6: Kinetic models for Optimized formulation

Figure 6.1

Zero order plot for optimized formulation

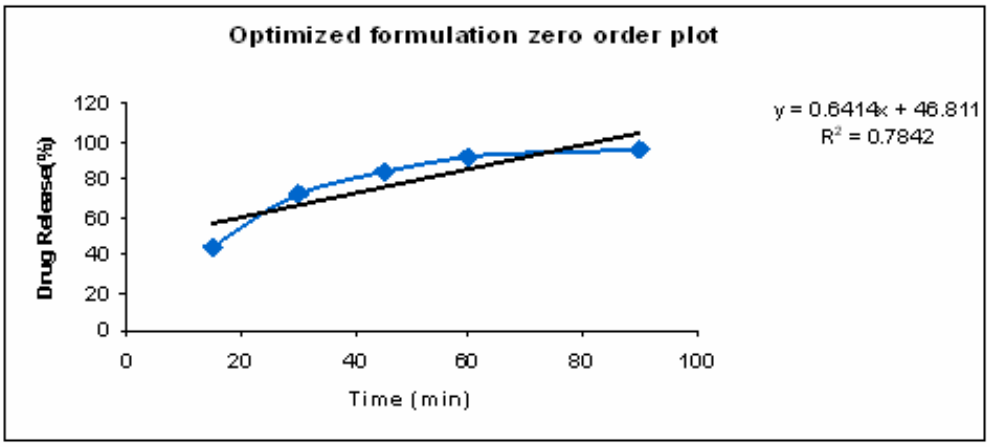


Fig 6.2

First order plot for optimized formulation.

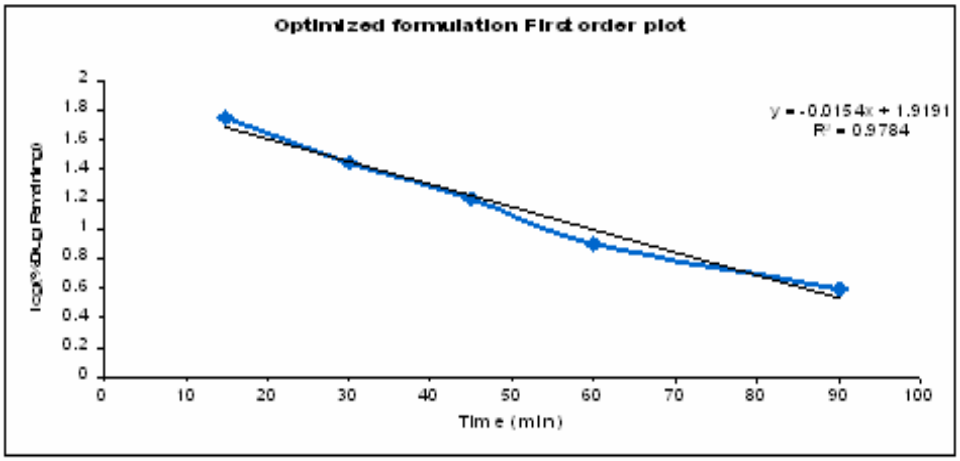


Fig: 6.3

Higuchi plot for optimized formulation

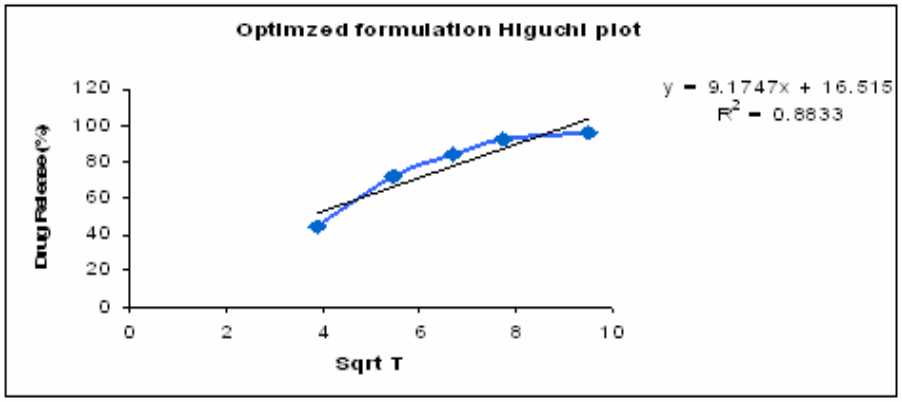


Figure 7: Kinetic models for Reference capsules

Figure 7.1
Zero order plot for reference

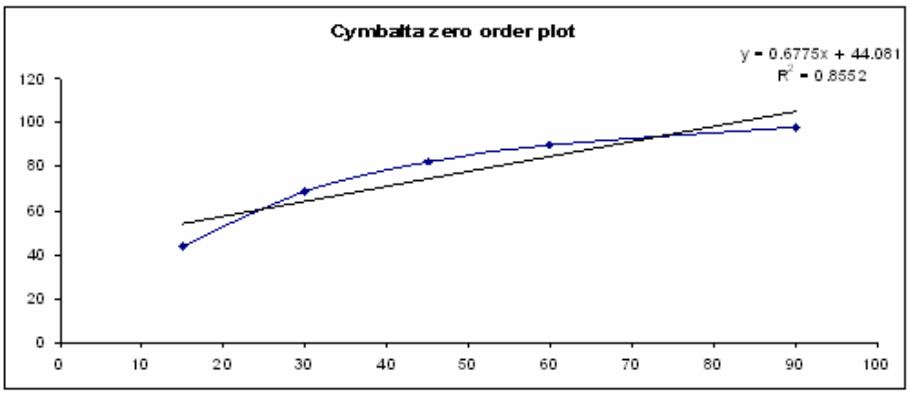


Fig 7.2
First order plot for reference

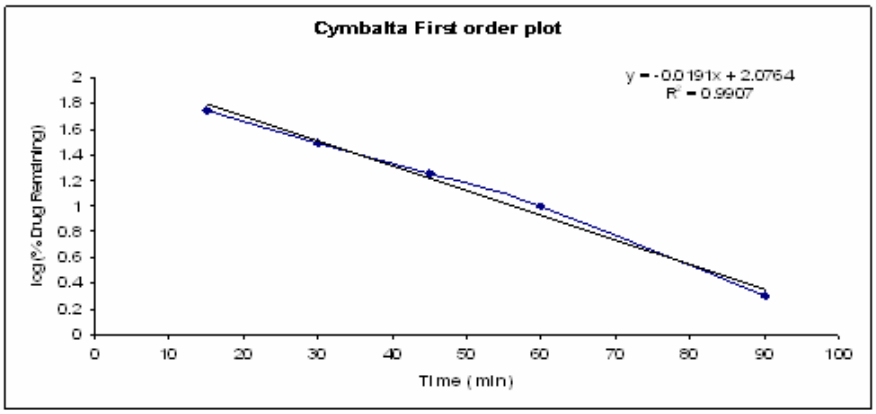
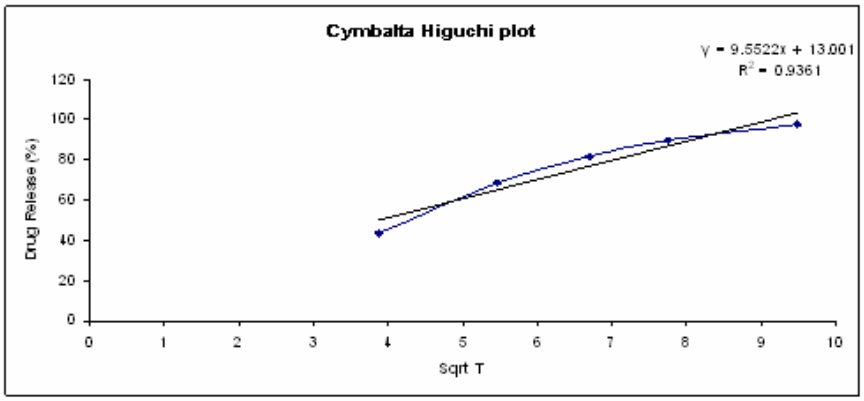


Fig 7.3
Higuchi plot for reference



Stability data of formulation

Stability studies for Optimized enteric coated formulation were conducted at 40ºC / 75% RH for about 3 months in stability chamber (Thermo lab) and observed for assay, acid resistance and dissolution profile after 1, 2 and 3 months. Results are mentioned in Table XIII: Stability data of optimized formulation.

TABLE XIII

Stability data of optimized formulation

Time	Test (%)	40ºC / 75% RH
1 month	**Assay**	99.6
	Acid resistance	99.4
2 months	**Assay**	99.6
	Acid resistance	99.4
3 months	**Assay**	99.5
	Acid resistance	99.3

Dissolution profile for stability samples at different storage conditions

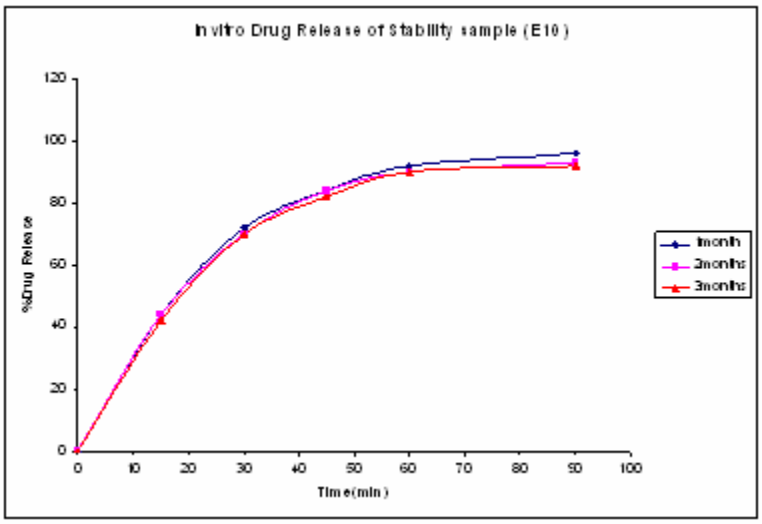
The dissolution studies for the samples of optimized formulation (E10) stored at 40ºC/75% RH for stability studies were carried out using 0.1N HCl for 2hrs and in pH 6.8 phosphate buffer for the next 90 minutes for the samples collected at the end of 1st, 2nd and 3rd months. The dissolution profile of samples collected at the end of 1st, 2nd and3rd months are shown in the figure below. The details are given below:

Acid stage: 0.1 N HCl, 1000ml, USP basket apparatus, 100rpm, 120 minutes.

Buffer stage: pH 6.8 phosphate buffer, 1000ml, USP basket apparatus, 100rpm,

Sampling points 15, 30, 45, 60 and 90 minutes.

Figure 8
Invitro dissolution profile of optimized formulation at 40ºC / 75% RH



DISCUSSION

The enteric coated pellets prepared are compiled with the In-house specifications mentioned.These values of percentage of drug content indicated that the drug was uniform and resistant to acidic environment in the batch of enteric coated pellets in all cases.

From the results above, the enteric coated pellets prepared are compiled with the In-house specifications mentioned. These values of percentage of drug content indicated that the drug was uniform and resistant to acidic environment in the batch of enteric coated pellets in all cases.

Drug coating: Drug coating was performed on the sugar spheres by using suspension layering technique. The lab scale batches (n=5) with batch size of 3000 capsules were developed using different binders, namely HPMC 5cps and HPC and varying binder concentrations. The drug coated pellets were analyzed for their *invitro* drug release in buffer (6.8pH phosphate). As per In- house specifications, the *invitro* drug release of 90% or above in 15 minutes is considered as the optimized formula. About 72% of drug release was seen in D1 formulation in 15 minutes, but this formulation was not taken up for further experiment because of processing problems and aggregate formation, which led to decreased drug release. Thus problem was addressed by optimizing the binder concentration. The concentration of binder was then reduced to 12% from16% in D2 formulation to address the processing problems encountered in D1. Although the percentage drug release was improved compared to D1 formulation, the processing problems were not overcome. When the binder concentration was further reduced to 8% in formulation D3, it was observed that the lump formation could be avoided but the required binding capacity was not observed in the pellets. In these formulations the percentage drug release was found to be 86% in 15 minutes. D4 formulation was found to achieve 88% drug release by using combination of binders. No significant change in drug release was observed compared to D3 formulation. Further trials were planned to improve the drug release in order to match the specifications by incorporating 2% of crospovidone as a superdisintegrant. The D_5

formulation using crospovidone was found to have achieved a drug release of 95% in 15 minutes and the desired drug release profile was achieved. Hence this formulation was chosen as the optimized formulation to be taken up for further coating stages, i.e. barrier coating. The dissolution readings of drug coating formulation are shown in Table XIV: Dissolution readings of Drug coated pellets.

Table XIV
Dissolution readings of Drug coated pellets

Time(min)	D1	D2	D3	D4	D5
0	0	0	0	0	0
10	42	68	72	70	92
15	72	77	86	88	95
30	88	88	92	92	97
45	90	92	92	92	97
60	90	92	92	92	97

Barrier coating: Barrier coating was applied to protect the drug coated pellets from reacting with the enteric coating and other environmental conditions. In B1 and B2 formulations HPMC 5CPS was used as binder, along with different release modifiers like Sucrose and TEC. Sucrose and TEC are used as release modifiers in B1 and B2 formulations respectively. Drug release patterns were compared. B1 formulation showed better drug release as sucrose acts as wicking agent. B2 formulation showed better film formation as TEC forms a rigid film. These formulations were further coated using different enteric polymers. The dissolution readings of drug coating formulation are shown in Table XV: Dissolution readings of Barrier coated pellet.

Table XV
Dissolution readings of Barrier coated pellets

Time (min)	B1	B2
0	0	0
10	91	82
15	94	91
30	97	97
45	97	97
60	97	97

Enteric coating: Enteric coating was optimized by comparing the parameters like assay, acid resistance and dissolution of the enteric coated pellets with that of reference. Enteric coating formulations were optimized based on the above results mentioned in Table 4.4.

Effect of Enteric Coating: PVA Phthalate was used as enteric polymer in E1 formulation which showed very rapid drug release compared to the reference. So in E2 (with B1) and E3 (with B2) formulations another enteric polymer Kollicoat MAE 30 DP was used. The drug release was found to be rapid in E2 due to the

presence of sucrose while E3 formulations showed very slow drug release pattern. In E4 (with B1) and E6 (with B2) another enteric polymer HPMC Phthalate (HP-55) was used with solvent IPA/DCM (1:1). Again E4 (with B1) showed rapid release compared to E6 (with B2). The formulations E5 (with B1) and E7 (with B2) another enteric polymer HPMC Phthalate (HP-50) with solvent IPA: DCM (1:1) also behaved in the similar manner. Rapid release was observed with barrier coating B1 and slow release was observed with barrier coating B2 in case of both HPMC Phthalate (HP-55) and (HP-50). Further trials with combination of both HP-50 and HP-55 were taken up to get a desired drug release profile matching with that of the reference.

Effect of Solvents: In E8 formulation, combination of HP-50 and HP-55 in 1:1 ratio and solvent IPA:DCM (1:1) and barrier coating B2 was taken. Here the drug release profile obtained was close to reference. In E9 formulation, the same formula with another solvent IPA: Acetone (1:1) was taken and checked for drug release profile. Reduced drug release was observed. In E10 formulation, acetone: purified water (8:2) was taken as solvent. The drug release was found to match with the reference and this formulation was optimized further for top coating.

Top coating*:* Top coating was given to pellets to get enough mechanical strength to the pellets during coating process. It comprises of 2-10% by weight of the composition. Top coat improves the elegance of the product, as well as its handling, storage, stability and machinability. It also provides stability against hygroscopicity and protects from mechanical damage. So the concentrations of Top coating were optimized. Opadry Y-1-7000, a readymade coating material from colorcon was used (composition: PEG400, HPMC, Titanium dioxide) for this purpose.

From the figure 1: *Invitro* dissolution profiles of E1-E10 formulations and Reference, the results indicated that the dissolution profile of E10 formulation is found to be similar to that of the reference standard and therefore chosen as the optimized formulation. The percentages of duloxetine released from E10 and the reference in 0.1 N HCl at 120 min are 1.0% and 1.3% respectively. The chromatogram peak for the dissolution is seen in the figure 2: Chromatogram for optimized formulation (E10).

From the results, it is clearly evident that E10 formulation has shown highest similarity factor f2, compared to other formulations, which indicates that the closeness between reference and test is more and hence this formulation (E10) is considered as Optimized formulation.

From the figure 3: FT-IR of pure drug and Formulation, the FT-IR spectrum of the formulation showed the presence of the drug in its active form without alteration of its chemical structure. The following important FTIR bands of the drug remain intact in both the spectra of the drug and formulation.

1490 cm-1 : for thiophene ring
3000-3001 cm-1: for aromatic alkene proton (C=C-H)
1400-1600 cm-1: for aromatic alkene (C=C)
1000-1300 cm-1: ether (C-O)
1080-1360 cm-1: for C-N bond.

These characteristic bands are present in both the FTIR spectrum, confirming the presence of the drug in its original structure in the formulation and preserving drug efficacy.

Differential scanning calorimetry enables the quantitative detection of all processes in which energy is required or produced (i.e. endothermic or exothermic phase transformations). The thermogram of pure Duloxetine showed a sharp peak at about 172.7^{o}C; ΔH=-10.73 J/gm in figure 4: DSC of formulation, placebo and pure drug. Peak of duloxetine hydrochloride at 177.9°C was almost present at the same position in the optimized formulation and no peaks of duloxetine hydrochloride were found at this temperature for placebo formulation. In

Placebo thermogram, showed peaks of excipients at 184.9°C and 224.9°C. There was no change in the peak temperature of the optimized formulation, when compared to the pure drug, which indicates no interaction between drug and excipients. This confirmed the physicochemical stability of drug with the formulation excipient used in the study.

From the figure 5: Scanning Electron Microscopy figures of optimized formulation (E10), the coated pellets appeared to exist as spherical discrete units whilst the surface morphology of the pellets was compact, continuous and uniform and is porous in nature. SEM demonstrated the spherical nature of the pellets. The average size of the pellets was found to be 1080±5 µm.
Results of measurements such as bulk density, tapped density, compressibility index and Hausner's ratio are represented in the table below. From the results Optimized formulation E10 showed good flowable properties.

From the figure 6: Kinetic models for Optimized formulation and figure 7: Kinetic models for Reference capsules, it was observed that formulation was following First order kinetics and it complies with reference as mentioned in the table below. The formulation shows highest R^2 for first order kinetics.

From the results, no significant change was observed in figure 8: *Invitro* dissolution profile of optimized formulation at 40°C/ 75% RH and also there was no significant change observed in case of Assay and Acid resistance when compared with Initial samples. Thus, it means that formulation E10 was found to be stable.

CONCLUSION

Duloxetine Hydrochloride is an acid labile drug which degrades at acidic pH of stomach. In order to delay the release in the stomach and promote the drug release in the intestine, enteric coating of the drug was attempted. Drug loading on the sugar spheres was done by using different binders i.e., HPC and HPMC 5cps with different concentrations. The amount of drug bound to sugar spheres increased with increase in the concentration of HPMC. E10 enteric coated formulation [HPMCP (HP-50 and HP-55, 1:1)] was found to be optimized because of similar release profile when compared to reference and is confirmed by the similarity factor. Based on the results, it was concluded that the optimized formulation of Duloxetine Hydrochloride (MUPS) delayed release pellets in capsules 60mg (E10) is found to achieve the effective drug levels in the intestine.

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