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Formulation and In vitro/In vivo evaluation of controlled release Entacapone trilayer matrix tablets by geomatrix

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Abstract

The purpose of the present study was to develop and optimize controlled release (CR) matrix tablets of Entacapone trilayer tablets to achieve zero-order drug release for sustained plasma concentration. Entacapone tablets were prepared by direct compression and consist of middle active layer with different grades of hydroxypropylmethylcellulose (HPMC), xanthan gum, ethyl cellulose; upper and lower layers were prepared with Carnauba wax, xanthan gum, sodium CMC and DCP. The tablets were also evaluated for physicochemical characteristics and release kinetics. The physicochemical characteristics of the prepared tablets were satisfactory. The developed drug delivery systems showed prolonged drug release rates over a period of 24 h. The release profile of the optimized formulation (HF14) was described by the Zero-order and Higuchi model. *In-vivo* (Rabbit) bioavailability studies were carried out on the optimized formulation (HF14) as marketed conventional release product as a reference standard. Entacapone was available in plasma within an hour after oral administration of reference product. The T_{max} of the optimized formulation was significantly different ($p < 0.05$) from that of the marketed product. Low T_{max} value for the reference product (1.00±0.01h) indicates rapid absorption while the higher T_{max} of the optimized (4.01±0.04h) suggests slower absorption. This delayed absorption of test preparation is most likely due to the sustained release of the drug. A fair correlation between the *in vitro* dissolution profile and *in vivo* pharmacokinetic profile of the optimized formulation was observed. The results indicate that the approach used could lead to a successful development of a controlled release formulation of the drug. These results also demonstrated the suitability of three-layered tablet formulation of Entacapone to provide controlled release for prolonged period of time and improved linearity for Entacapone in comparison to marketed product with conventional drug release profile.

Keywords: Entacapone, Trilayer matrix tablet, HPMC, Xanthan gum, Geomatrix, In-vivo bioavailability studies.

Introduction

Controlled release pharmaceutical systems have been developed and studied to improve the performance of drugs and in particular to increase their pharmacological effect and reduce any side effects [1]. The basic characteristic of the systems is that the rate of drug absorption may be adjusted through a controlled rate of drug release from the dosage forms. A number of design options are available to control or modulate drug release from a drug delivery system. Most oral controlled release dosage forms fall in the category of matrix, reservoir or multi-layer systems. Lately, multi-layer matrix systems are gaining importance in the design of oral sustained drug delivery systems. A multi-layer system consists, usually, of a hydrophilic matrix core containing the active ingredient and one or two impermeable or semi-permeable polymeric coatings (barrier-layer) applied on one or both faces of the core during tabletting [2, 3, & 4].

The barrier layers delay the interaction of active solute with dissolution medium, by limiting the surface available for the solute release and at the same time controlling solvent penetration rate [5,6]. In the device, the coat layers prevent the water penetration through the protected core for some duration. After this phase during the subsequent dissolution process, the swollen barriers erode and the surface available for drug release slowly increases. In this way the decrease of delivery rate due to the increase in diffusion path length is counter balanced by the simultaneous increase of the area available for drug release [7,8].

The use of naturally occurring biocompatible gums has been the focus of recent research activity in the design of dosage forms for oral controlled release administration, and hydrophilic polymers matrix systems are widely used because of their flexibility to provide a desirable drug release profile, cost effectiveness, and broad regulatory acceptance [9]. Xanthan gum (XG) is soluble in water, anionic hetero polysaccharide and to be sensitive to pH and ionic strengths. It swells in gastric fluid to produce a highly viscous layer around the tablet through which the drug can slowly diffuse[10] and is used for the fabrication of matrices with uniform drug release characteristics [11&12].

Geomatrix technology: There have been different approaches to achieve zero-order drug release from dosage forms for sustained plasma concentration. Among different approaches to achieve zero-order release from hydrophilic matrix technologies, multilayer matrices have been widely evaluated and developed for commercial products under the trade name of Geomatrix. The technology makes use of bilayer or trilayer tablets to modulate the release and to achieve constant release [13].

Entacapone is a selective, reversible catechol-O-methyl transferase (COMT) inhibitor, used in the treatment of Parkinson's disease. It is a member of the class of nitrocatechols. The principal therapeutic action of the COMT inhibitors is to block this peripheral conversion of levodopa to 3-O-methyl DOPA, increasing both the plasma half-life of levodopa as well as the fraction of the dose that reaches the CNS [14].

The short half life of Entacapone necessitated for fabricating extended release matrix tablets to provide a therapeutic amount of drug and maintain the desired drug concentration i.e. the drug-delivery system should deliver drug at a rate dictated by the needs of the body over a specific period of time. Sustained release tablets are intended to take once or twice daily, when compared with conventional dosage forms that may have to take three or four times daily to achieve the same therapeutic effect. The objective of the present study was to develop a trilayered tablet of Entacapone with different hydrophobic and hydrophilic polymers. The results indicate that the optimized trilayered Entacapone tablet can be successfully used for treatment of Parkinson's disease.

Materials and methods

Materials

Entacapone pure drug was generous gift from Aurobindo Pharma Limited, Hyderabad, India. Sodium carboxyl methyl cellulose, HPMC K 4 M, HPMC K 15 M & HPMC K 100 M were obtained from Rubicon labs, Mumbai. Xanthan gum and Carnauba wax were gifted from MSN Labs Ltd. Hyderabad. Entacapone (200mg) film coated tablets were purchased from Orion Pharma Ltd, Mumbai. All other chemicals used were of analytical grade.

Methods

Pre-compression parameters

Angle of Repose: In powder frictional forces can be measured with the help of angle of repose. Angle of repose is the maximum angle which is possible between surface of pile of powder and horizontal plane i.e. height.

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

Where = Angle of repose
h= height of pile
r = radius of pile [15].

Carr's compressibility Index: The propensity of the powder to be compressed is measured by compressibility index and it also helps in measurement of settling property and inter particulate interaction.

$$\text{Carr's index } (\%) = \rho t - \rho o^{*} 100 / \rho t$$

Where ρt = Tapped density gram/ml
ρo = Bulk density gram/ml

Bulk Dentistry: It is denoted by ρb and is defined as mass of powder divided by bulk volume (The United States Pharmacopeial Convention Stage 6 Harmonization Official December 1, 2012, 616.).

Tapped Density: An increased in bulk density which is attained after mechanical tapping in measuring cylinder is called as tapped density.

Tapped density= Weight of powder taken/ Tapped Volume.

Hausner Ratio: The propensity of the powder to be compressed is measured by Hausner ratio. Interparticulate interaction and settling property can be measured by Hausner ratio.

Hausner ratio= Tapped density/ Bulk density

Hausner ratio= Vo/Vf

Where, Vo= Unsettled apparent volume

Vf= Final tapped volume [16] .

Formulation of controlled release Entacapone trilayer matrix tablets

The trilayered matrix tablets of Entacapone were prepared by direct compression method. The first step in the formulation was to develop the middle active layer so as to give at least 90%drug release during 12hours. The release profile of this layer might not be of constant rate type but would be preferably of constantly falling rate type. This layer would then be sandwiched between barrier layers (Upper & Lower layers) so as to continue the drug release for 24 h.

Preparation of middle active layer

Sixteen formulations (F1-F16) for active layer were prepared by direct compression method using polymers like different HPMC grades, Xanthan gum, Sodium CMC, EC. All the formulations were varied in concentration of polymers, talc (1.5mg) & magnesium stearate (1.5mg) constituted in all the formulations. These materials were screened through 60 and mixed together in motor by using pestle. Final mixtures were compressed by using 12mm

Table 1: Formulation trails for middle active layer (F1-F16)

INGREDIENTS (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16
Entacapone	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200
HPMC K 4M	50	55	60	---	---	----	25	30	----	----	----	----	----	----	----	----
HPMC K 15M	---	----	----	50	55	60	45	40	----	----	----	----	----	----	----	----
HPMC K 100M	---	---	---	----	---	----	----	----	40	45	50	55	60	62.5	65	67.5
Ethyl cellulose	22	30	32	22	30	32	15	15	40	35	22	30	32	22.5	22.5	22.5
Xanthan gum	20	22	25	20	22	25	30	32	15	17.5	20	22	25	30	30	30
Sodium carboxy methyl cellulose	25	15	10	25	15	10	15	15	27	21.5	25	15	10	12	10	12
Dibasic calcium phosphate	30	25	20	30	25	20	17	15	25	27	30	25	20	20	19.5	15

diameter flat punches on a sixteen station rotary tablet press. Formulation of active layer was depicted in Table 1. The prepared tablets were subjected to dissolution studies.

Preparation of upper and lower layers

The barrier layers was formulated employing hydrophobic swellable polymer natural wax i.e. carnauba wax the swelling erosion modelling fillers which include water soluble DCP, EC and Xanthan gum. The procedure adopted to make the compacts was via direct compressions. For the first procedure the wax, xanthan gum and the filler was mixed in mortar and lubricated with magnesium stearate. Formulation of upper and lower layers was depicted in Table 2.

Formulation of Entacapone tryilayer tablets

The powder mixtures required for active and barrier layers were weighed accurately and thoroughly mixed using mortor and pestle for about 20 minutes. Initially, the volume of die cavity; (12mm, round) was adjusted equivalence to the weight of trilayered matrix tablets (600mg). Then the pre weighed amount of powder equivalent to bottom layer (125mg) was taken and placed in the die cavity and slightly compressed for uniform spreading. The upper punch was lifted up and the granules equivalent to 200mg of the drug was placed over the bottom layer in the die cavity and again slightly compressed. The remaining volume of the die cavity was filled with pre weighed (125mg) amount of powder equivalent to top layer and compressed with the full force of compression on rotary tablets press to obtain tri-layered tablets. Tri-layered matrix tablets of each composition were compressed and tested for their friability, Hardness, drug content and drug release characteristics with a suitable number of tablets for each test.

Table 2: Composition of Entacapone trilayer matrix tablet

INGREDIENTS	AF14	BF14	CF14	DF14	EF14	FF14	GF14	HF14
MIDDILE ACTIVE LAYAER (F14) (mg)								
Entacapone	200	200	200	200	200	200	200	200
HPMC K 100M	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
Ethyl cellulose	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5
Xanthan gum	30	30	30	30	30	30	30	30
Sodium CMC	12	12	12	12	12	12	12	12
Dibasic calcium phosphate	20	20	20	20	20	20	20	20
UPPER AND LOWER LAYER(mg)								
Carnauba wax	20	25	30	35	40	42.5	45	50
Xanthan gum	40	40	38	35	35	32.5	30	30
Ethyl cellulose	12	10	14	12	15	12	12	12
Dibasic calcium phosphate	50	47	40	40	32	35	35	30
Magnesium stearate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Talc	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

Evaluation of trilayer matrix tablets of Entacapone

Hardness

Hardness of ten randomly picked tablets was determined using Monsanto hardness tester.

Friability

A sample of twenty randomly selected tablets were accurately weighed and placed in a Roche Friabilator. The Friabilator was operated for 4 min at a speed of 25 rpm. The tablets were removed from the Friabilator, de-dusted and reweighed. The percent loss in weight due to abrasion and impact was calculated as,

% Friability = (Loss in weight/ Initial weight) X 100.

Weight variation

The weight variation test was performed as per the USP. Twenty randomly taken tablets were weighed together and the average weight was determined. Each tablet was then weighed individually and deviation from average weight was calculated.

Drug content / Assay

Five tablets were weighed individually and powdered. Then the powder of tablet equivalent to 200mg was weighed and dissolved in phosphate buffer pH 5.5, the solution was filtered and diluted using phosphate buffer pH 5.5 and then the drug content was analyzed using UV spectrophotometer at 377nm.

Swelling & Erosion studies

Swelling experiment was conducted on the prepared tablets using USP dissolution apparatus II at rotational speed of 50 rpm. The medium used was 900ml phosphate buffer pH 5.5 at 37^0C. The swelling study was done upto 10h. The tablets were removed using a small basket and swollen weight of each tablet was determined. The percentage of swelling was calculated according to the formula:

Percentage of swelling = (S/R) X 100

In-vitro drug release profile

In vitro drug release studies for developed trilayer matrix tablets were carried out by using dissolution apparatus II paddle type (Electrolab TDL-08L). The drug release profile was studied in 500ml Phosphate buffer pH 5.5 at 37± 0.5^0C temperature. The amount of drug release was determined by UV visible spectrophotometer (Shimadzu UV 1800) at 377nm.

Drug release kinetics

To describe the kinetics of the drug release from matrix tablet, mathematical models such as Zero-order, First order and Higuchi, models were used. The criterion for selecting the most appropriate model was chosen on the basis of the goodness-or fit test.

Drug-excipient compatibility studies

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra for pure drug, physical mixture and optimized formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR Affinity 1 Spectrophotometer. The samples were dispersed in KBr and compressed into disc/pellet by application of pressure. The pellets were placed in the light path for recording the IR spectra. The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Samples were accurately weighed and heated in sealed aluminium pans at a rate of 10 C/min between 25 and 350 C temperature rang under nitrogen atmosphere. Empty aluminium pan was used as a reference.

Stability studies

The stability study of the formulated trilayer tablets were carried out under different conditions according to ICH guidelines using stability chamber (REMI make). Accelerated Stability studies were carried out at 40 ^{0}C / 75 % RH for the best formulations for 6 months. The tablets were characterized for the hardness, friability, drug content and cumulative % drug release during the stability study period.

Pharmacokinetic studies

Animal Preparation

Male rabbits (weighing 2-3 kg) were selected for this study. Animals were maintained at room temperature 25^0C, Relative Humidity 45% and 12 h alternate light and dark cycle with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply and rabbits were fed with standard diet and *water ad libitum*. The animals that were healthy during the period of quarantine were used for experiment. The protocol of animal study was approved by the Institutional Animal Ethics Committee (IAEC NO: P34/VCP/IAEC/2015/2/DBP/AE12).

In vivo study design

The rabbits were randomly divided into two groups each group contains three animals. The group A was administered with prepared Entacapone matrix tablets (200mg), Marketed product was administered group B with equivalent dose of animal body weight. Blood samples (approximately 0.5ml) were obtained with syringes by marginal ear vein at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 20 and 24hrs post dose. During collection, blood sample has been mixed thoroughly with heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5min to 10 minutes and stored frozen at 20 C until analysis.

Preparation of Plasma Samples for HPLC Analysis

Rabbit plasma (0.5 ml) samples were prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was

transferred into a clean tube. The precipitate was re suspended with 1 ml of Acetonitrile by vortexing for 1 min. After centrifugation (5000 – 6000 rpm for 10 min), the Acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. For HPLC C18 column with 5 μm particle size and the Mobile Phase consisting of 30mM phosphate buffer (pH 2.75) : acetonitrile :: 62:38. The flow rate was 1.0 ml/min and the effluents were monitored at 315 nm with rofecoxib as internal standard. The retention time was 8.3 min and 10.7min for Entacapone and rofecoxib respectively.

Pharmacokinetic analysis

The pharmacokinetic parameters, peak plasma concentrations (C_{max}) and time to reach peak concentration (t_{max}) were directly obtained from concentration time data. In the present study, AUC_{0-t} refers to the AUC from 0 to 24 hrs, which was determined by linear trapezoidal rule and $AUC_{0-\alpha}$ refers to the AUC from time at zero hours to infinity.

The $AUC_{0-\alpha}$ was calculated using the formula $AUC_{0-t} + [C_{last}/K]$ where C_{last} is the concentration in μg/ml at the last time point and K is the elimination rate constant. Various pharmacokinetic parameters like area under the curve [AUC], elimination half life (t½). Volume of distribution (V_d), total clearance (Cl_T) and mean residence time for each subject using a non compartmental pharmacokinetic programme. The pharmacokinetic parameters were performed by a non compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean ±SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was considered statistically significant.

Results and Discussion

Pre-compression parameters

All the powder mixture belonging to different formulations was tested for micrometrics studies in order to determine the flow properties. All the formulations AF14 to HF14 showed good flow properties, the results are summarized in Table 3.

Table 3: Powder flow properties of Entacapone, powder blends of active layer and barrier layer polymers

Powder properties	AF14	BF14	CF14	DF14	EF14	FF14	GF14	HF14
Bulk density (g/cc)	0.7151±0.04	0.7121±0.46	0.512±0.02	0.7050±0.14	0.714±0.56	0.684±0.78	0.695±0.02	0.704±0.56
Tapped density(g/cc)	0.787±0.10	0.790±0.93	0.629±0.17	0.767±0.2	0.795±0.93	0.746±0.82	0.781±0.048	0.796±0.93
Angle of repose (o)	33.69±0.63	34.93±0.66	33.12±0.63	31.89±0.43	24.39±0.66	33.09±0.27	28.15±0.02	26.39±0.66
Carr's index	8.09±0.91	8.02±0.93	9.49±0.51	8.29±0.91	8.35±0.94	7.62±0.58	7.28±0.33	8.15±0.94

Preparation of middle active layer

The matrix tablets of Entacapone were prepared without the barrier layers. All the formulation trails were subjected to *in vitro* dissolution to determine the release profiles.

Table 4: Dissolution profile of different formulations Entacapone active layer (F1-F8)

TIME (h)	F1	F2	F3	F4	F5	F6	F7	F8
1	27.12± 0.04	27.56± 0.02	38.2± 0.01	19.12± 0.01	15.4± 0.01	25.4± 0.01	28.7± 0.01	10.3± 0.01
2	42.6± 0.01	39.12± 0.02	48.2± 0.04	39.2± 0.02	38.3± 0.02	35.2± 0.02	37.4± 0.02	25.1± 0.02
4	63.6± 0.02	42.7± 0.01	59.4± 0.02	55.12± 0.01	42.7± 0.02	47.6± 0.01	64.5± 0.04	35.3± 0.04
6	76.12± 0.01	57.2± 0.05	75.11± 0.01	64.4± 0.02	54.4± 0.01	60.4± 0.02	72.8± 0.04	55.9± 0.04
8	83.02± 0.04	75.11± 0.03	86.2± 0.04	72.1± 0.04	79.11± 0.04	65.3± 0.03	82.6± 0.02	68.3± 0.05
10	89.2± 0.05	89.12± 0.01	88.4± 0.03	79.3± 0.04	89.2± 0.02	72.7± 0.01	88.2± 0.01	69.6± 0.01
12	94.9± 0.01	95.2± 0.02	92.1± 0.02	89.6± 0.03	94.3± 0.01	86.9± 0.02	92.4± 0.01	70.8± 0.03

Table 5: Dissolution profile of different formulations Entacapone active layer (F9-F16)

TIME (h)	F9	F10	F11	F12	F13	F14	F15	F16
1	13.8±0.01	15.7±0.01	25.5±0.02	17.5±0.01	31.8±0.01	30.5±0.01	14.8±0.04	14.5±0.02
2	35.4±0.03	40.4±0.02	39.4±0.01	25.8±0.02	42.4±0.01	40.7±0.02	32.7±0.04	32.2±0.02
4	55.6±0.05	58.3±0.04	52.6±0.04	45.3±0.04	62.8±0.02	72.4±0.01	55.6±0.01	55.4±0.01
6	65.2±0.04	67.7±0.04	65.2±0.04	50.4±0.01	79.1±0.04	80.4±0.04	65.4±0.02	75.4±0.04
8	69.6±0.01	75.2±0.01	72.7±0.02	57.8±0.02	86.6±0.02	88.2±0.01	82.2±0.01	85.3±0.04
10	72.8±0.04	86.8±0.02	87.4±0.01	82.6±0.02	92.8±0.04	93.2±0.01	88.1±0.03	89.2±0.03
12	85.3±0.01	92.7±0.01	96.5±0.02	96.2±0.01	96.5±0.01	99.6±0.04	93.7±0.01	95.2±0.01

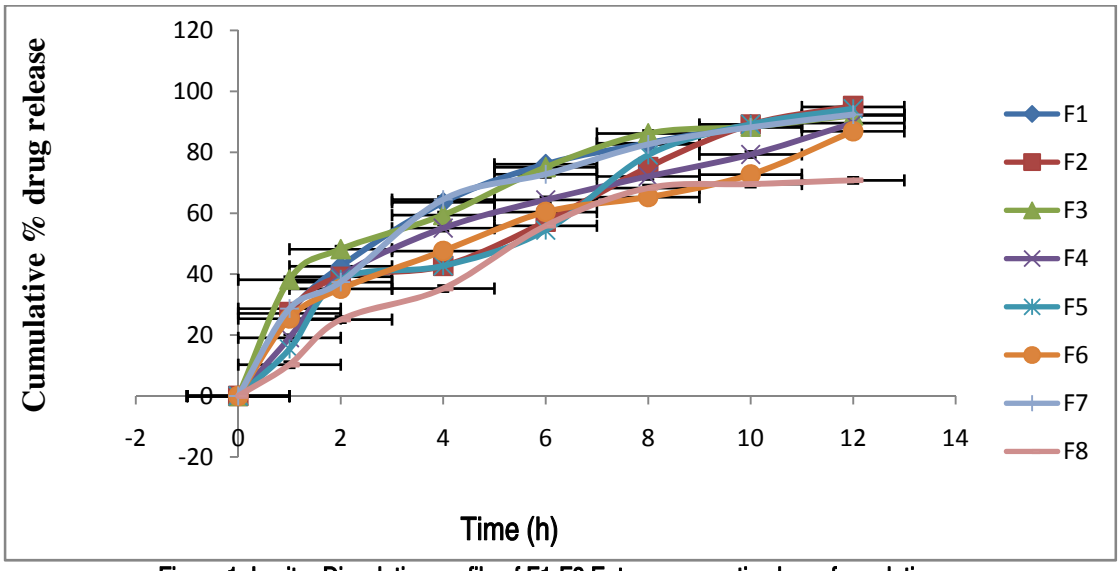

Figure 1: In vitro Dissolution profile of F1-F8 Entacapone active layer formulations

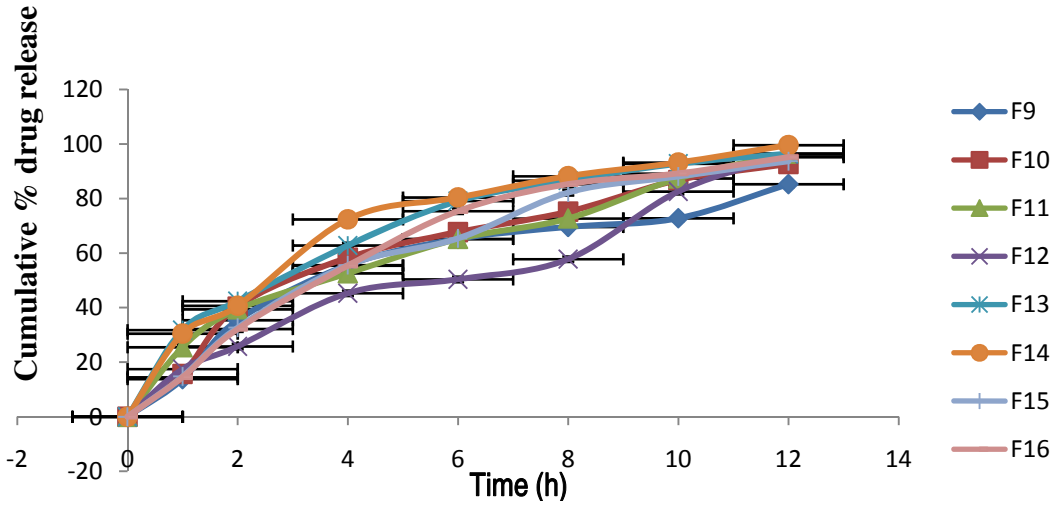

Figure 2: In vitro Dissolution profile of F9-F16 Entacapone active layer formulations

From the above results, among all the formulations the formulation F14 was decided as optimized formulation for active layer based on the highest drug release i.e. 99.6±0.04 within 12hrs when compared with other preparations (Table 4&5; Figure 1 & 2). Formulation F14 was choosen as active layer for further studies.

Evaluation of trilayer matrix tablets of Entacapone

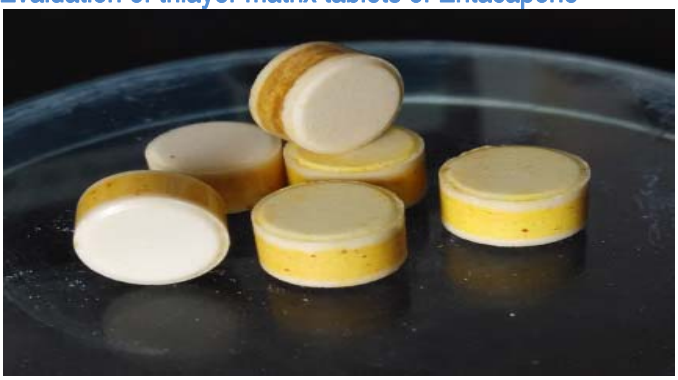

Figure 3: Entacapone trilayer matrix tablets

The Entacapone trilayer matrix tablets are shown in Figure 3. Sustained release tablets generally have hardness in the range of 7-10 kg/cm^2. In case of trilayer tablets the hardness of the tablets was found to be 7.2 to 8.4 kg/cm^2. The friability of the formulations was found to be less than 1% and hence the tablets with lower friability may not break during handling on machines and or shipping. All the batches of the tablets complied with the weight variation limits as per the IP. The drug content in different formulation was highly uniform and the results are depicted in Table 6.

Table 6: Physical evaluation of Entacapone trilayer tablets

Formulation code	Hardness(kg/cm^2)	Friability(%)	Weight variation (mg)	% Drug content
AF14	7.4±0.23	0.35	596±20	98.1
BF14	7.9±0.45	0.27	599±20	97.8
CF14	8.4±0.47	0.45	595±20	98.4
DF14	8.1±0.12	0.16	594±20	96.6
EF14	7.2±0.49	0.32	597±20	98.4
FF14	7.8±0.28	0.46	595±20	97.9
GF14	7.6±0.15	0.37	596±20	97.1
HF14	7.2±0.85	0.22	598±20	98.9

Swelling studies

In phosphate buffer pH 5.5, HPMC showed good swelling property. In trilayer tablets of Entacapone, HF14 showed highest degree of swelling index 219.43%, where as in AH14 showed leased swelling with a swelling index of 134.68%.

In vitro dissolution studies of Entacapone Trilayer tablets

The release of Entacapone from different formulations was carried out in phosphate buffer pH 5.5 and the results are depicted in Table. The trilayer tablets extended the drug release upto 24 hrs. The highest drug release was found in the formulation HF14 i.e 98.29% within 24 h. HF14 was found to be optimized formulation based on the dissolution and other evaluation parameters. The results are shown in Table 7 & Figure 4. The comparison of marketed product Entacapone (200mg) film coated tablet and optimized formulation HF14 was shown in Figure 5. The drug release from marketed product was 99% within 60min.

Table 7: In-vitro cumulative % drug release studies of Entacapone trilayer tablets

TIME (h)	AF14	BF14	CF14	DF14	EF14	FF14	GF14	HF14
1	12.34±0.01	14.22±0.04	16.21±0.04	18.47±0.05	6.16±0.01	7.85±0.04	13.22±0.04	12.49±0.04
2	22.11±0.02	18.21±0.05	18.23±0.05	24.54±0.05	12.28±0.02	12.25±0.05	25.32±0.02	24.52±0.04
4	37.15±0.03	36.32±0.04	21.28±0.05	30.43±0.05	36.38±0.02	20.23±0.05	36.35±0.03	33.35±0.03
6	45.25±0.05	48.15±0.04	33.79±0.05	55.25±0.04	42.45±0.03	27.54±0.05	49.62±0.04	49.53±0.05
8	54.16±0.09	56.42±0.08	48.53±0.05	69.45±0.02	58.98±0.04	48.15±0.04	63.32±0.05	55.54±0.07
12	60.34±0.05	74.12±0.05	56.75±0.06	70.74±0.03	69.99±0.05	50.75±0.05	74.46±0.05	62.59±0.05
16	69.75±0.03	82.24±0.04	74.68±0.06	81.65±0.03	75.55±0.04	72.32±0.01	84.53±0.04	72.63±0.05
20	80.24±0.05	90.21±0.05	82.95±0.07	90.85±0.02	80.20±0.05	87.22±0.05	89.15±0.06	86.53±0.06
24	86.12±0.04	91.22±0.02	93.45±0.01	95.25±0.02	90.24±0.03	94.24±0.04	93.55±0.04	98.29±0.09

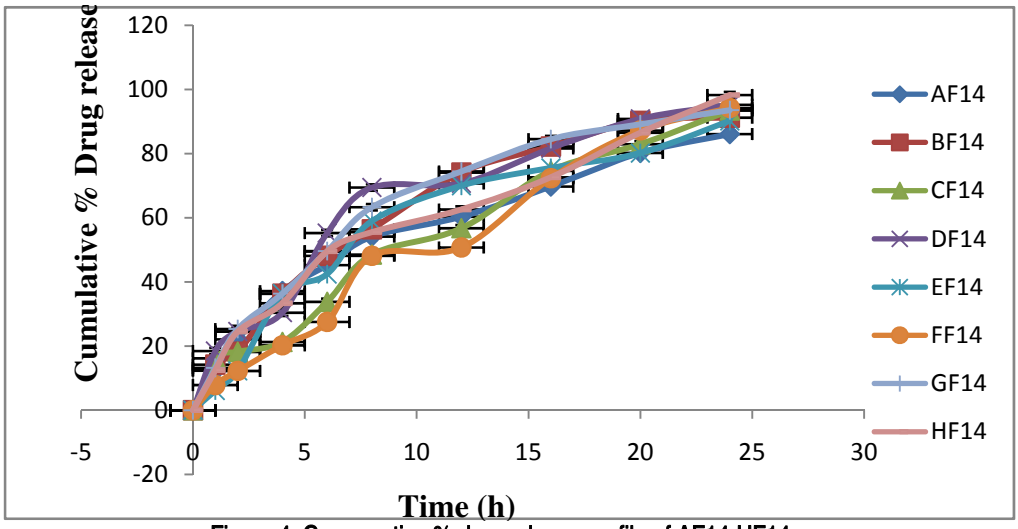

Figure 4: Comparative % drug release profile of AF14-HF14

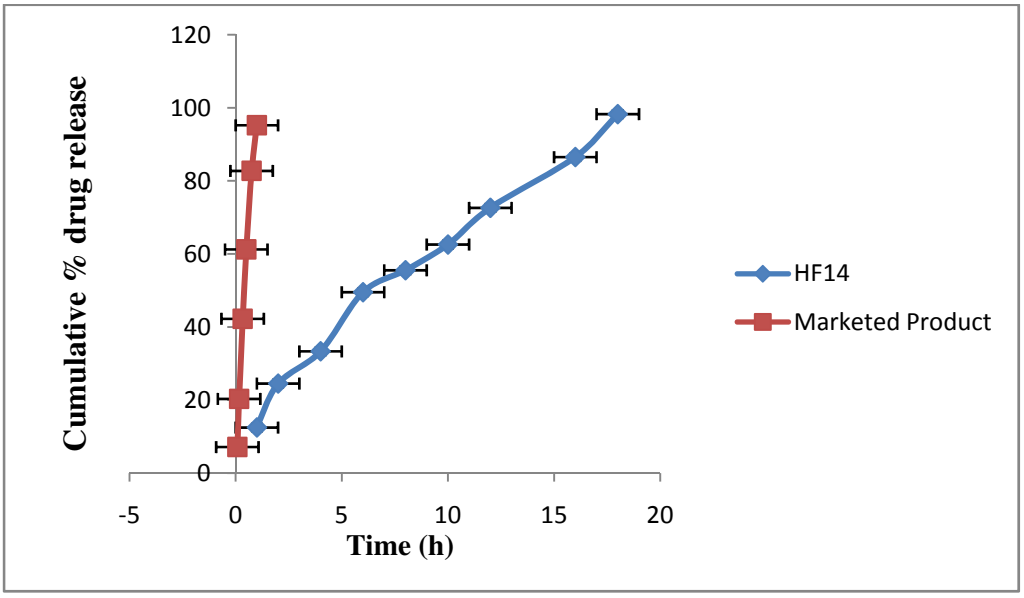

Figure 5: Cumulative percentage drug release of Entacapone from marketed product and optimized formulation HF14

Table 8: Drug release kinetics of optimized (HF14) and Marketed product

Formulation code	Zero order	First order	Higuchi model
HF14	0.979	0.717	0.913
Marketed product	0.806	0.968	------

In the present investigation drug release mechanism is best fitting to zero order and Higuchi model because regression coefficient was seen closest to 1 in these models which conforms diffusion assisted mechanism of release. The marketed product Entacapone (200mg) was explained by first order kinetics as the plot showed highest linearity (r =0.968) as the drug release was best fitted in first order kinetics.

Characterization

FT-IR:

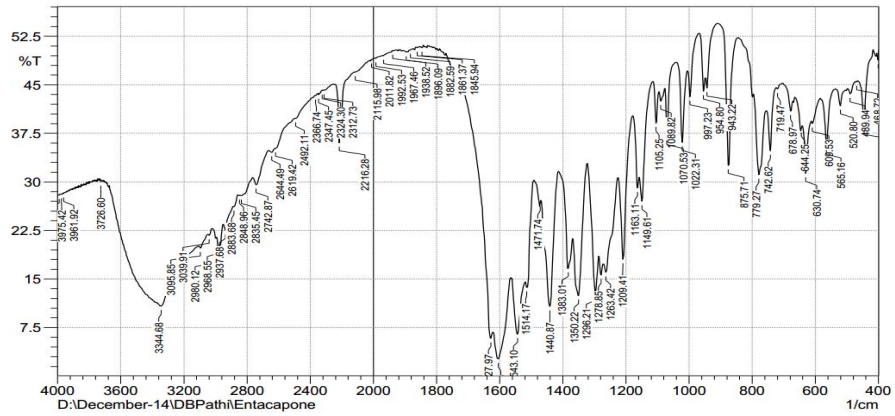

Figure 6: FT-IR spectrum of pure drug Entacapone

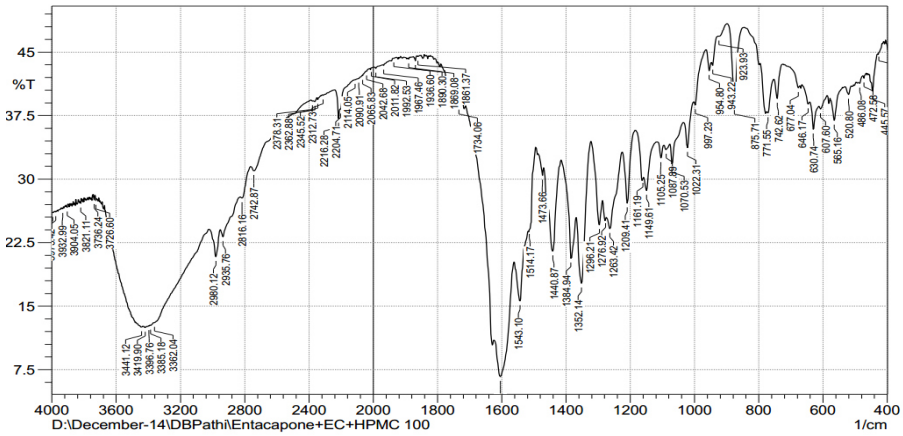

Figure 7: FT-IR spectrum of pure drug and other polymers

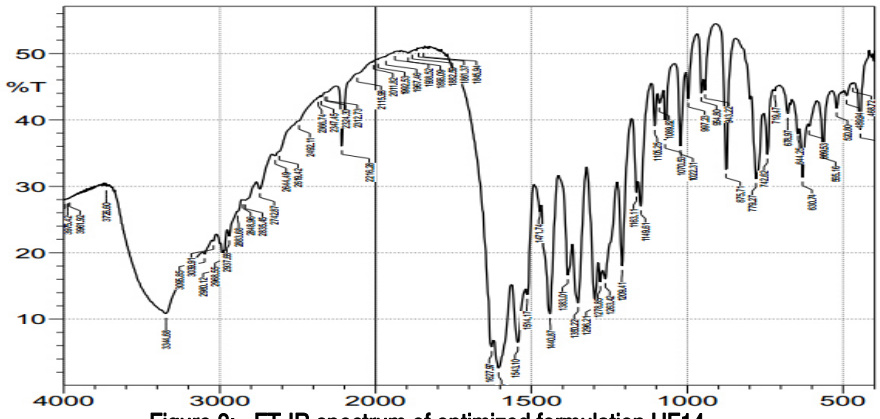

Figure 8: FT-IR spectrum of optimized formulation HF14

Overall there was no alteration in peaks of Entacapone pure drug (Figure 6) and optimized formulation (Figure 7), suggesting that there was no interaction between drug & excipients. FT-IR spectrum of pure drug and other polymers are shown in (Figure 8).

There is additional peaks appeared or disappeared hence no significant changes in peaks of optimized formulation was observed when compared to pure drug indicating absence of any interaction.

DSC studies

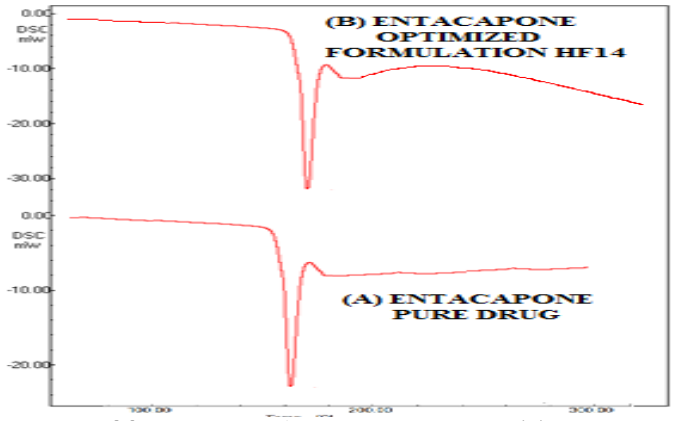

Figure 9: DSC thermogram of Entacapone pure drug (A) and optimized formulatin HF14 (B)

DSC was used to detect interaction between Entacapone and excipients. The thermogram of Entacapone exhibited a sharp endotherm melting point at 161^{0}C. The thermo gram of optimized formulation of Entacapone exhibited a sharp endotherm melting point at 164 ^{0}C.The DSC thermo gram retained properties of Entacapone, as well as polymer properties. There is no considerable change observed in melting endotherm of drug in optimized formulation (Figure 9). It indicates that there is no interaction between drug & excipients used in the formulation.

Stability studies

Optimized formulation was selected for stability studies on the basis of high cumulative % drug release. Stability studies were conducted for 6 months according to ICH guidelines. From these results it was concluded that, optimized formulation is stable and retained their original properties with minor differences which depicted in Table 9.

Table 9: Physico-chemical characteristics of optimized formulation HF14

Retest Time For Optimized formulation		Friability (%)	Hardness (kg/cm^2)	% Drug content	*In-vitro* drug release profile (%)
Initial		0.22	7.2	98.9	98.29
40 C/75%RH	1st month	0.25	7.3	98.04	97.10
	2nd month	0.28	7.3	97.25	96.65
	3rd month	0.31	7.4	96.15	96.5
	6th month	0.34	7.5	95.18	95.5
25 C/60%RH	3rd month	0.26	7.2	98.8	96.2
	6th month	0.29	7.3	98.7	95.8

Pharmacokinetic studies

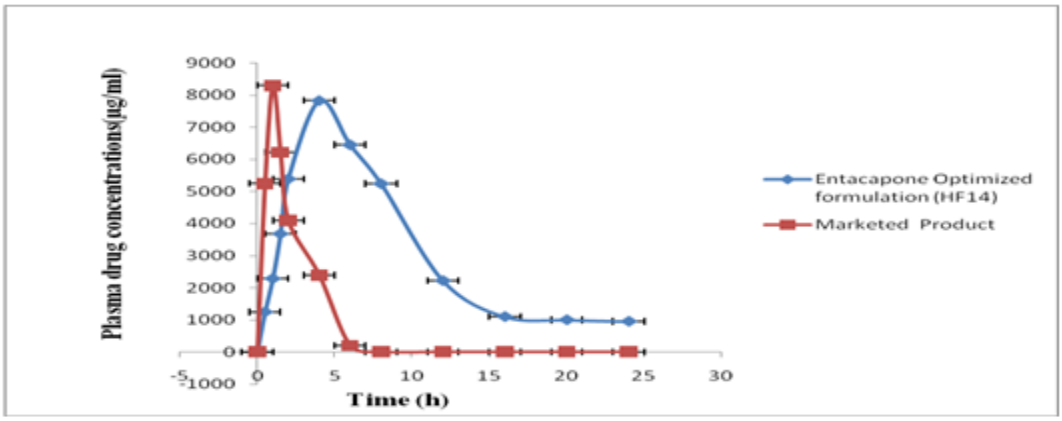

Figure 10: Plasma Concentrations of Entacapone optimized formulation (HF14) and marketed product at different time intervals

Table 10: Comparison of pharmacokinetic parameters of Entacapone Optimized formulation (HF 14) and marketed product

Parameters	Entacapone optimized formulation (HF14)	Marketed product
C_{max} (µg/ml)	7826.21±0.03	8308.29±0.03
AUC $_{0-t}$ (µg h/ml)	37386.12±0.01	30458.19±0.01
AUC_{0-} (µg h/ml)	41528.12±0.02	34589.18±0.02
T_{max} (h)	4.01±0.04	1.00±0.01
$t_{1/2}$ (h)	6.25±0.004	3.125±0.05

Bioavailability Parameters

Mean plasma concentration profiles of prepared Entacapone optimized formulation and Marketed product are presented in Figure 10. Entacapone optimized formulation exhibited as sustained release in vivo when compared with Marketed product. All the pharmacokinetics parameters displayed in Table 10. Entacapone reference drug was available in plasma within an hour after its oral administration. The T_{max} of the optimized formulation was significantly different ($p < 0.05$) from that of the marketed product. Low T_{max} value for the reference drug (1.00±0.01h) indicates rapid absorption while the higher T_{max} of the test drug (4.01±0.04h) suggests slower absorption. This delayed absorption of test preparation is most likely due to the sustained release of the drug. On the other hand, the C_{max} of reference formulation was significantly different from the test preparation. The half-life of the reference preparation was low which indicates rapid removal of the drug from plasma. This was also supported by the high elimination rate constant value. On the other hand, the test formulation exhibited higher half-life and low elimination rate constant values indicating slower drug disposition and prolonged effect. However, the AUC_{0-} values for the two formulations were significantly different. This suggests that the Entacapone contained in the test product was completely absorbed.

Summary and conclusion

It was concluded that trilayer matrix tablets of Entacapone can be successfully prepared by direct compression technique using different polymers combination. Based on the evaluation parameters, drug dissolution profile and release drug kinetics HF14 was found to be optimized formulation. The drug release from HF14 was found to fit Zero order of concentration independent and best fitted to Higuchi's model confirming to be diffusion assisted mechanism. The marketed product release was explained by first order kinetics by concentration dependent. *In vivo* bioavailability studies were conducted for optimized Entacapone trilayer tablets and marketed product, the results were indicating that the optimized Entacapone formulation was shown sustained release patterns where marketed product was shown immediate release. So the optimized formulation was shown significant plasma concentrations with controlled release and maintained for 24 hrs.

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