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Application of beeswax and cetyl alcohol as an excipients in hot-melt coating agents in controlled release metoprolol tartrate plus hydrochlorothiazide capsule formulations

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Abstract
The objective of the present study was to design metoprolol tartrate and hydrochlorothiazide controlled release pellets using beeswax and cetyl alcohol as an important hot melt coating (HMC) agents. The pellets were coated by HMC technique using beeswax and cetyl alcohol by using conventional coating pan without the use of spray system. The prepared metoprolol tartrate and hydrochlorothiazide pellets were characterized for drug content, photomicrography, in vitro- dissolution studies, flow properties of pellets. Stability studies were performed for a period of 6 months at $40^0 \pm 2\ ^0$C and 75% ± 5% relative humidity. HMC technique is easy rapid and simple method with no agglomeration seen during coating. In – *vitro* release from pellets at a given level of coating and for present pellets size was dependent upon the physico-chemical properties of the drug. HMC pellets were stable during the course of stability study. Metoprolol tartrate and hydrochlorothiazide pellets using beeswax and cetyl alcohol by HMC technique was employed successfully and capsule formulations were prepared.

Keywords: Metoprolol tartrate, hydrochlorothiazide, beeswax, cetyl alcohol, hot- melt coating agent, controlled release capsule formulations

Introduction
Metoprolol is effective and safe antihypertensive drug with short half-life is completely absorbed from the upper part of GIT and exhibits a degree β-blockade that is directly related with plasma concentrations and this is reason that it is suitable to design as a modified release formulation. Varieties of modified release metoprolol formulations are available in the international market [1]. Several formulations includes Betaloc, Betaloc SA, Dutoprol, Durules, Lapressor HCT, Lopressor SR, Meprolol H, Seloken and Toprol XL. Many of them are matrix-based products. Newer developments include the use of osmotic type systems and divisible tablets where pellets are embedded in an inert tablet. These formulations were designed for once daily dosing of metoprolol. Once daily modified release formulations of metoprolol were reported equally or more effective than twice daily conventional metoprolol tablet dosage form [2, 3].

Multi-component dosage form of metoprolol tartrate and hydrochlorothiazide has been developed, e.g. Selekomb is usually formulated which contain 100 mg of metoprolol and 12.5 mg of hydrochlorothiazide. It deliver metoprolol at a controlled rate and the hydrochlorothiazide is immediately release, so that there is an initial immediate reduction in blood pressure, which is maintained throughout the day by sustained metoprolol release [4, 5].

Although, a number of formulations already present in the international market, they are comparatively more costly than immediate release or single drug dosage forms. This makes such products unavailable to developing countries in both public and private sectors, hence the development of low cost, yet effective generic products would allow for more widespread use of such products in the developing world. This would be particularly important in the country such as India, Australia and South Africa where the majority of hypertensives are either black or elderly patients [6, 7]. Till today no formulation in the market that containing both drugs that are releasing the drugs in controlled manner which will give better controlled treatment against hypertension. Therefore an exclusive attempt was made by reducing dosing frequency, improving patient compliance and prolongs release of drugs for the treatment of hypertension.

Materials and methods

Materials

Metoprolol tartrate was obtained as a gift sample from Lincoln Pharma ltd, Ahmedabad. Hydrochlorothiazide was received from Li-taka Pharmaceuticals, Pune. Microcrystalline cellulose from Themis Laboratories, Mumbai. Beeswax, Cetyl alcohol, Sodium bicarbonate, Diethyl phthalate were received from S.D. Fine Chemicals, Mumbai. Hydroxypropylmethyl cellulose was received from Colorcon Asia Ltd., Mumbai & PVP K-30 from the Leben Laboratories, Akola.

Methods

Analysis of drugs

Organoleptic characterization: The organoleptic characteristics like colour, odour and taste of metoprolol tartrate and hydrochlorothiazide were determined.

Melting point: Melting points of metoprolol tartrate and hydrochlorothiazide were determined by using melting point apparatus.

Standard graph of metoprolol tartrate: The absorption maxima of metoprolol tartrate in 0.1N hydrochloric acid was obtained by preparing appropriate dilution of metoprolol tartrate solution and scanned between 200-400 nm. The stock solution of metoprolol tartrate in the volumetric flask (100µg/ml) was prepared. Suitable dilutions were made to prepare a range of six solutions with well-known concentrations from 5-30 µg/ml in 0.1N HCl. The absorbance of the above dilutions was measured at λmax by using the UV-Visible spectrophotometer. A graph was plotted by taking concentration (µg/ml) on X-axis and absorbance on Y-axis [8].

Standard graph of hydrochlorothiazide: Accurately weighed 10 mg of hydrochlorothiazide was dissolved in 100 ml of 0.1 N HCl (stock solution 100 µg/ml). The above solution was subsequently diluted with 0.1N HCl to obtain the series of dilutions containing 1, 2, 4, 6, 8, and 10 µg/ml of hydrochlorothiazide solution. The absorbance of the above dilutions was measured at λmax by using the UV-Visible spectrophotometer. A graph was plotted by taking concentration on X-axis and absorbance on Y-axis [9].

Simultaneous estimation of metoprolol tartrate and hydrochlorothiazide using UV spectrophotometric method: The UV spectrophotometric method for simultaneous estimation of metoprolol tartrate and hydrochlorothiazide reported by Kasture *et al* was used for the present study. Both the drugs were weighed in the ratio as in marketed formulation i.e. 4:1, MPTA (~100 mg) and HCTZ (~25 mg) and were transferred to a volumetric flask and the mixture dissolved in methanol and volume made up to mark. A 25 ml portion of this solution was transferred to 50 ml volumetric flask and volume adjusted up to mark with distilled water. A 5 ml portion of this solution was further diluted to 50 ml with distilled water. Similarly five different samples were weighed in the ratio of 4:1 and same procedure was followed as above to get nearly same concentration as in standard solution. The absorbances of the final diluted standard and samples were read at 221, 270 and 315 nm [10].

Solubility analysis: Excess amount of drug was suspended in a series of different dissolution medium of varying pH or solvents and shaken for 24 hrs using wrist action shaker at room temperature. Solutions of drug were filtered through a 0.45µ membrane filter and analyzed spectrophotometrically [11].

Partition coefficient and dissociation constant: Partition coefficient was carried out using 1-octanol and water system. Dissociation constants were determined by potentiometrically, spectrophotometrically and photometrically.

Optical rotation: The specific rotation of 2% metoprolol tartrate aqueous solution was determined at 25 °C, using the sodium D line lamp.

Dissolution study of marketed products: *In vitro* release of drugs from IR marketed formulations were used to prove the suitability of the dissolution conditions for the drug in study. The *in vitro* drug release from modified release marketed formulation was used as reference for selection of optimized formulation in case of metoprolol. Six marketed tablets of metoprolol tartrate and hydrochlorothiazide were used for the study. *In vitro* dissolution conditions for marketed products are shown in table 1.

Table 1: *In vitro* dissolution study conditions for marketed products

Parameters	Specifications	
	Conventional DF	**Modified DF**
Dissolution apparatus	Type I apparatus	Type I apparatus
Speed of rotation	50 rpm	50 rpm
Temperature	37 ± 0.5 °C	37 ± 0.5 °C
Time	1 hr	12 hr
Dissolution medium	0.1N HCl	0.1N HCl
Volume of medium	900 ml	900 ml
Sampling time	5, 10, 15, 20, 25 and 30 min	0.5, 1, 2, 4, 6, 8, 10 and 12 hr

Analysis of beeswax and cetyl alcohol: Experiment was performed thrice and results were recorded as (Mean ± S.D.) [12-14]

Preparation of drug pellets: Accurately weighed metoprolol tartrate, sodium bicarbonate and microcrystalline cellulose were sifted through #120 mesh. All ingredients were mixed geometrically in a glass bowl. A 5% w/v PVP K-30 solution was prepared in isopropyl alcohol added to ingredient mixture to form a coherent mass. The coherent mass was passed through axial screw extruder of 16 mesh screen at 100 rpm and the wet pellets were transferred to spheronizer operated at 1000 rpm for 5 min using 1 mm hatch pattern friction plate. Pellets were dried at 60 °C for 24 hr. The dried pellets were sifted to collect 16/20 mesh fractions. The undersize and oversize pellets were rejected. Formulation of metoprolol tartrate pellet is shown in table 2. Similarly, hydrochlorothiazide pellets were prepared using formula shown in table 3 [15].

Table 2: Formulation of pellets of metoprolol tartrate

Sr. No.	Ingredients	Quantity (mg)
1.	Metoprolol tartrate	100
2.	Sodium bicarbonate	20
3.	Microcrystalline cellulose (Avicel PH 101)	30
4.	PVP K-30 solution	Q.S.

Table 3: Formulation of pellets of hydrochlorothiazide

Sr. No.	Ingredients	Quantity (mg)
1	Hydrochlorothiazide	25
2	Microcrystalline cellulose (Avicel PH 101)	10
3	Sodium bicarbonate	5
4	PVP K-30 solution	Q.S.

Hot melt coating of pellets: Metoprolol tartrate pellets of 16/20 mesh fraction were coated in a 12 inch diameter modified coating pan. The coating compositions utilized for present study are shown in table 4. Coating agents were melted with continuous stirring. Metoprolol tartrate pellets were rolled in the coating pan and warmed until a bed temperature of 60 °C was attained. The coating pan was operated at 30 rpm at 30-50% relative humidity. The molten coating mass was loaded uniformly onto the rolling pellets in the form of slow stream. After complete application of the coating mass, the pellets were allowed to roll further for 10 min. During this time the bed temperature was allowed to gradually come down. The pellets were then removed from coating pan and cured at room temperature in the dryer for 24 hr. Similarly the hot melt coating was carried out on hydrochlorothiazide pellets (table 5) [16, 17]. Parameters employed for hot melt coating of metoprolol tartrate/hydrochlorothiazide pellets are given in table 6.

Table 4: Formulation of hot melt coating composition for MPTA pellets

Formulation	Beeswax (mg)	HPMC (mg)
M1	5	--
M2	7	--
M3	10	--
M4	9	1
M5	8	2
M6	7	3

*Coating formula for 100 mg pellets of metoprolol tartrate. Hydroxypropyl methylcellulose was used as release modifier. Dibutyl phthalate (DBP) was used as plasticizer about 10% w/w of coating level.

Table 5: Formulation of hot melt coating composition for HCTZ pellets

Formulation	Cetyl alcohol (mg)	HPMC (mg)
H1	5	--
H2	7	--
H3	10	--
H4	9.5	0.5
H5	9	1
H6	8	2

*Coating formula for 100 mg pellets of hydrochlorothiazide. Hydroxypropyl methylcellulose was used as release modifier. Dibutyl phthalate (DBP) was used as plasticizer about 10% w/w of coating level.

Table 6: Process parameters for preparation of hot-melt MPTA/ HCTZ pellets

Sr. No.	Process parameters	Settings
1	Pellets	200 g
2	Pan speed	30 rpm
3	Pan diameter	12 inch
4	Exhaust air flow rate	100 cfs
5	Room humidity	30-50% RH
6	Processing time	30 min
7	Drying temperature	25 °C
8	Drying time (LOD < 1%)	24 hr

Beeswax and cetyl alcohol are hydrophobic coating agents which restrict the dissolution medium to permeate inside the pellet easily [18]. They were obviously increases floating lag time of pellets with increase in coating level. Therefore an ideal coating material for a floating system should be water permeable in order to initiate the effervescent reaction and the floating process. However, the wet or hydrated coatings should also be impermeable to the generated CO_2 so as to promote and maintain floating. Regarding their mechanical properties, the coatings should be sufficiently flexible in wet state to be able to withstand the pressure of the generated gas and to avoid rupturing. Lipids were used in combination with HPMC which gives a more hydrophilic nature to the film and alters its structure by virtue of pores and channels through which the drug can diffuse easily [19].

Evaluation of pellets

Physicochemical and micromeretic evaluation of pellets: Physicochemical and micromeretic evaluation of pellets were performed.

Drug content: Accurately weighed 500 mg of pellets were grind carefully in the mortar and 50 mg of powdered pellets was transferred carefully to 100 ml volumetric flask and dissolved in 0.1N hydrochloric acid. Sonication was performed using laboratory sonicator for 15 min to extract metoprolol tartrate or hydrochlorothiazide. The final volume was made with 0.1N hydrochloric acid to 100 ml. Drug content was determined using UV- Visible spectrophotometric method.

Floating ability: The floating abilities of the coated effervescent pellets, were determined using 250 ml beaker containing 50 ml 0.1N HCl. Twenty pellets were placed in the medium; the time required to float (floating lag time) and duration for how long they remain float (floating time) were measured by visual observation [20].

***In vitro* dissolution study of metoprolol tartrate and hydrochlorothiazide formulations:** *In vitro* release study of metoprolol tartrate and hydrochlorothiazide formulations was carried out to evaluate the sustained release characteristics imparted by hot melt coating with cetyl alcohol or beeswax formulations. Dissolution studies were performed using USP dissolution apparatus type II. Dissolution conditions used are indicated in table 7. Aliquots of 5 ml were withdrawn at predetermined time intervals and replaced with equivalent amount of fresh dissolution medium. Samples were filtered using 0.45 µ membrane filter. They were analyzed for the determination of % drug release using UV spectrophotometer.

Table 7: *In vitro* dissolution conditions for hot-melt coated pellets

Sr. No.	Parameters	Specifications
	Dissolution apparatus	USP paddle type apparatus
	Paddle speed	100 rpm
	Quantity of pellets	≈ 100 mg of MPTA/25 mg of HCTZ
	Temperature	37 ± 0.5 °C
	Time	12 hr
	Dissolution medium	0.1N hydrochloric acid (pH 1.2)
	Volume of medium	900 ml
	Sampling volume	5 ml
	Sampling volume	1, 2, 4, 6, 8, 10 and 12 hrs

Kinetic data analysis: To understand the exact drug release mechanism from prepared formulations, the dissolution study data were fitted into various mathematical models. From regression coefficients, best fitted models were selected for all the formulations [21, 22].

Stability study: Optimized formulations of MPTA and HCTZ were filled in amber coloured bottles separately and stored at temperature 40±2 °C/75±5% RH for 6 months. Pellets were evaluated for any changes in physicochemical properties and % drug release after six month. Results were compared with data obtained for zero time and room temperature (28±2 °C) and relative humidity (42±2%). The plot of % drug release against time after 6 months of stability study are to be determined [23-25].

Results

Analysis of drugs

Organoleptic characteristics of MPTA and HCTZ: The colour, odour and taste of metoprolol tartrate and hydrochlorothiazide were recorded in table 8.

Table 8: Organoleptic characteristics of MPTA and HCTZ

Sr. No.	Characteristics	Metoprolol tartrate	Hydrochlorothiazide
	Description	Crystalline	Crystalline
	Colour	White	White
	Odour	Odourless	Odourless
	Taste	Tasteless	Slightly bitter

Melting point: The melting points of metoprolol tartrate and hydrochlorothiazide were found in the range of 120-125 °C and 270-275 °C respectively.

Dissociation constant and distribution ratio: Dissociation constant (pKa) of metoprolol tartrate was found to be 9.1 potentiometrically. The dissociation constants (pKa) of hydrochlorothiazide were found to be 8.6, 8.7 and 8.81 potentiometrically, spectrometrically and photometrically respectively. Log KP values for metoprolol tartrate and hydrochlorothiazide were found to be 2.15 and 1.94 respectively.

Optical rotation: Optical rotation of 2% metoprolol tartrate solution prepared in distilled water at 25 °C was found to be +8.5°.

Standard graphs of metoprolol tartrate and hydrochlorothiazide: UV absorption spectrum of metoprolol tartrate in 0.1N HCl and water shows λmax at 221 and 280 nm (shoulder). UV absorption spectrum of hydrochlorothiazide in 0.1N hydrochloric acid and distilled water shows λmax at 270 and 315 nm. Standard graphs of metoprolol tartrate and hydrochlorothiazide were carried out in 0.1N HCl at 221 and 270 nm respectively. Table 9 shows the absorbances of respective concentrations drugs at their respective λmax. Figure 1 and 2 shows that metoprolol tartrate and hydrochlorothiazide were follow Beer's law with regression coefficient (R^2) values 0.999 and 0.999 respectively.

Table 9: Standard curve of MPTA and HCTZ in 0.1N HCl

Metoprolol tartrate		Hydrochlorothiazide	
Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
5	0.157	1	0.107
10	0.312	2	0.215
15	0.473	4	0.432
20	0.628	6	0.644
25	0.789	8	0.859
30	0.936	10	1.054

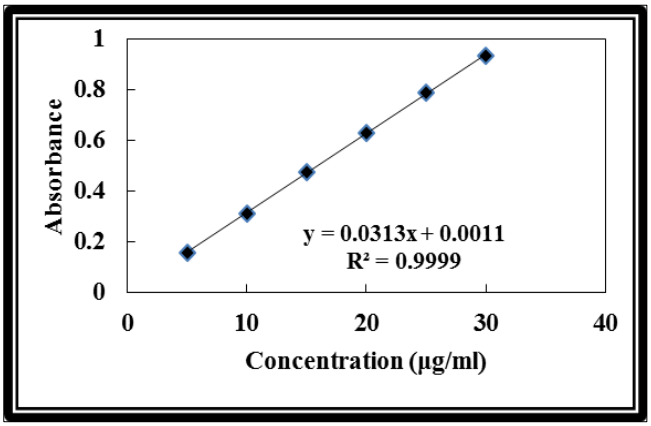

Fig 1: Standard curve of metoprolol tartrate in 0.1N HCl.

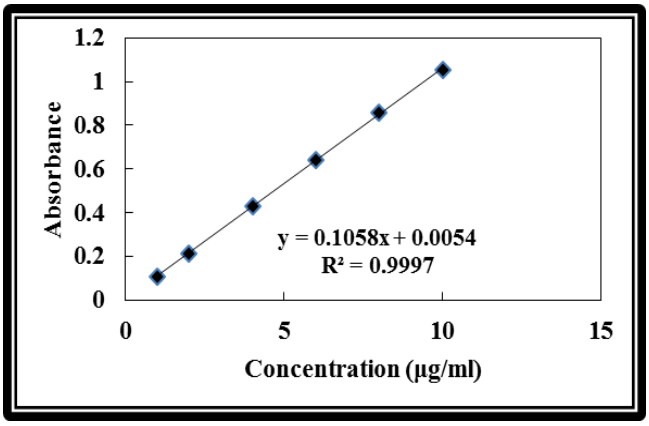

Fig 2: Standard curve of hydrochlorothiazide in 0.1N HCl.

Simultaneous estimation of MPTA and HCTZ using UV spectrophotometry: The multi-component method was developed for the estimation of MPTA and HCTZ in the dosage form. Purity of MPTA sample was found to 99.13%. The purity of HCTZ was found to be 99.66%. The linearity curve response for the drugs was obtained to study Beer's law range. MPTA and HCTZ mixture showed linearity 5-30 µg/ml and 1-12 µg/ml respectively. The average recovery was found to 99.96 ± 0.16 and 100.02 ± 0.03% for MPTA and HCTZ respectively.

Solubility analysis: Metoprolol tartrate was found to be highly soluble in aqueous solvents. The solubility of metoprolol tartrate in non-aqueous solvents was determined (table 11). The solubility of hydrochlorothiazide in various aqueous and nonaqueous solvents was recorded in table 10 and 11 respectively.

Table 10: Solubility of hydrochlorothiazide in aqueous solvents

Solvent	Temperature (°C)	pH of solution	Solubility (mg/ml)
Distilled water	37	7.2	1.08
0.1M hydrochloric acid	25	1.0	0.61
Phosphate buffer	25	7.4	0.62
Simulated gastric fluid	37	1.1	1.08
Simulated intestinal fluid	37	7.5	1.09

Table 11: Solubility of META and HCTZ in non-aqueous solvents

Solvents	Solubility (mg/ml)	
	Metoprolol tartrate	Hydrochlorothiazide
Methanol	> 500	4.02
Chloroform	496	0.14
Acetone	1.1	13.71
Acetonitrile	0.89	2.03
Hexane	0.001	1.27

Analysis of beeswax and cetyl alcohol: Table 12 shows both the coating materials showed the resultant values within the specifications as per USP NF.

Table 12: Analysis of beeswax and cetyl alcohol

Parameters	Beeswax		Cetyl alcohol	
	Limits	Result	Limits	Result
Acid value*	17-24	21.67 ± 1.004	NMT 2	1.28 ± 0.002
Ester value*	72-79	73.49 ± 2.171	NMT 2	0.97 ± 0.008
Hydroxyl value*	--	--	210-232	228 ± 3.128
Iodine value*	8-11	9.85 ± 0.013	NMT 2	1.44 ± 0.001
Peroxide value*	NMT 8	6.33 ± 0.006	--	--
Saponification value*	87-104	94.24 ± 1.622	NMT 2	1.07 ± 0.001
Refractive index (n_D^{79})	1.4834	1.4839	1.4232	1.4211
Melting point (°C)	61-65	60-65	47-53	48-50

*****Indicates result values in (Mean ± S.D.) where samples were analyzed in triplicate.

***In vitro* dissolution study of marketed products:** *In vitro* dissolution study of immediate release dosage forms of metoprolol tartrate and hydrochlorothiazide were carried using 0.1N HCl as dissolution medium. Yashiprolol (Metoprolol tartrate 100 mg) immediate release tablet manufactured by Yashica Pharmaceutical Pvt. Limited, Mumbai, India and Hydrazid (Hydrochlorothiazide 25 mg) immediate release tablet manufactured by Cipla Limited, Mumbai, India shows the release as shown in table 13 and figure 3. Innovator's product used for modified release metoprolol tartrate was Metolar XR-100 manufactured by Cipla Limited, Mumbai, India. TRP for hydrochlorothiazide was constructed and it indicates that the cumulative % drug release at 2, 4, 6, 8, 10 and 12 hours should be 50, 60, 70, 80, 90 and > 90% respectively as shown in table 14 and figure 4.

Table 13: *In vitro* drug release from IR marketed formulations

Time (min)	% Drug release[a]	
	Yashiprolol	Hydrazid
5	87.35 ± 3.87	74.68 ± 3.35
10	101.99 ± 4.78	89.94 ± 3.98
15	101.97 ± 4.66	98.94 ± 4.62
30	101.97 ± 4.59	98.92 ± 4.53
60	101.96 ± 4.47	98.92 ± 4.34

[a] -Values indicates (Mean ± S.D.) when samples were analyzed in triplicate.

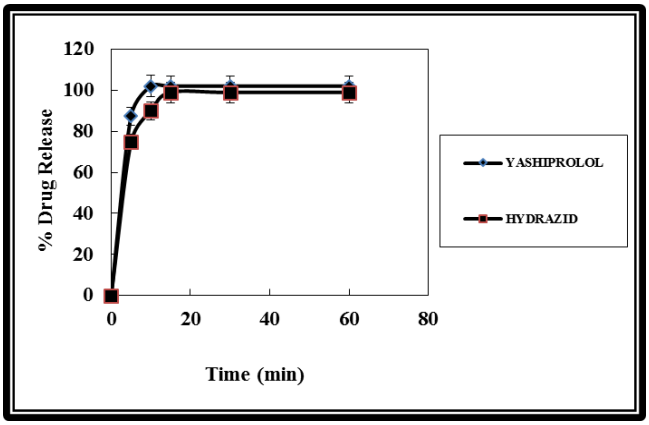

Fig 3: *In vitro* dissolution of IR marketed products of MPTA and HCTZ**.**

Figure 3 shows that both immediate release (IR) marketed products release the drug within 15 min indicates that 0.1N HCl was suitable as dissolution medium.

Table 14: *In vitro* drug release from modified release marketed formulation of MPTA and TRP of HCTZ

Time (hr)	% Drug release	
	Metolar XR-100[b]	TRP (HCTZ)
1	38.54 ± 1.23	--
2	45.11 ± 1.48	50
4	57.89 ± 2.65	60
6	68.56 ± 2.97	70
8	80.33 ± 3.09	80
10	92.71 ± 4.11	90
12	101.05 ± 4.28	> 90

[b] - Values indicate (Mean ± S.D.) when samples were analyzed in triplicate.

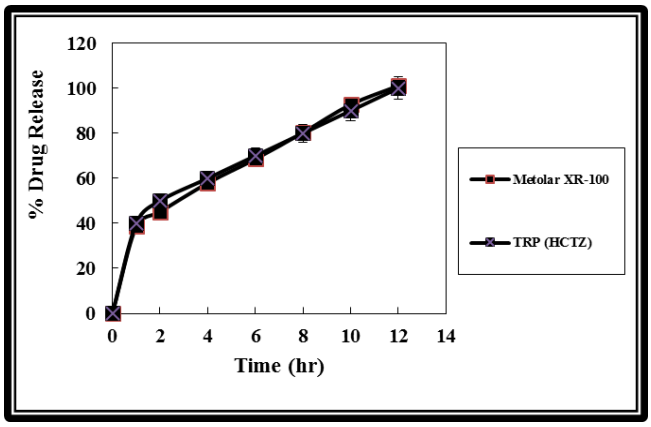

Fig 4: *In vitro* dissolution of modified release marketed formulation (MPTA) and theoretical release profile (HCTZ).

Figure 4 show the % drug release from marketed formulation of metoprolol and theoretical release profile of hydrothiazide. Metolar XR-100 shows initial burst release around 40% in 2 hr and thereafter a controlled release upto 12 hrs.

Evaluation of pellets

Photomicrography: Figure 5 and 6 shows photomicrographs of metoprolol tartrate pellets and hydrochlorothiazide pellets respectively snapped using Intel display camera attached to personnel computer at 30X.

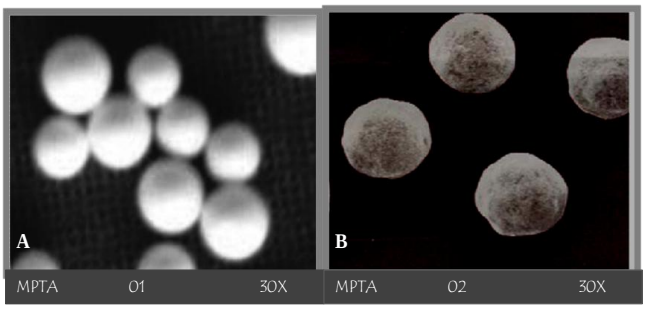

Fig 5: Photomicrograph of (A) uncoated and (B) coated pellets of MPTA**.**

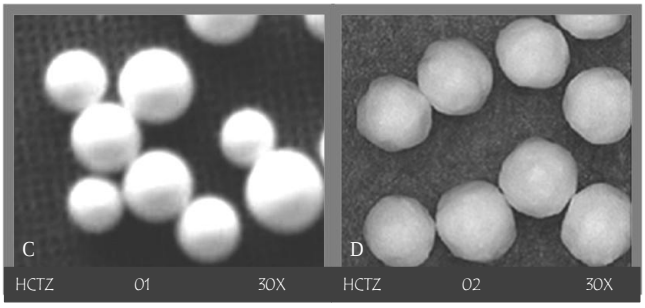

Fig 6: Photomicrograph of (C) uncoated and (D) coated pellets of HCTZ.

Mean pellet size: Mean size of uncoated pellets of metoprolol tartrate and hydrochlorothiazide were found to be 845 and 840 µ respectively. The mean size of coated pellets of metoprolol tartrate and hydrochlorothiazide were ranging from 854 to 926 µ and 860 to 900 µ respectively. The results were shown in table 18 and 19.

Angle of repose: Angle of repose values of uncoated metoprolol tartrate and hydrochlorothiazide pellets were found to be 26.75 and 25.55° respectively. Table 15 shows angle of repose values of all coated pellets of metoprolol tartrate and hydrochlorothiazide, and were varying in the range of 17.27 to 24.15° and 18.18 to 25.85° respectively. This confirms that coated pellets of metoprolol tartrate and hydrochlorothiazide have good to excellent flow property. Uncoated pellets show fair flow property.

Table 15: Angle of repose-flowability of MPTA and HCTZ pellets

Formulation	Angle of repose (°)	Formulation	Angle of repose (°)
M1	24.15	H1	25.85
M2	23.04	H2	22.56
M3	21.42	H3	20.02
M4	20.78	H4	22.62
M5	18.44	H5	19.68
M6	17.27	H6	18.18
M0	26.75	H0	25.55
M5S	18.58	H6S	18.47

Bulk density and tapped density: The bulk and tapped densities of pellet formulations of metoprolol tartrate and hydrochlorothiazide were shown in table 16 and 17. Values obtained were within acceptable range.

Compressibility index (CI): Compressibility index values of metoprolol tartrate and hydrochlorothiazide pellets were shown in table 16 and 17. Compressibility index values of uncoated pellets of metoprolol tartrate and hydrochlorothiazide were found to be 19.072 and 18.789 respectively. Compressibility index of all coated formulations of metoprolol tartrate and hydrochlorothiazide were varying in the range of 9.681 to 14.017 and 9.232 to 12.500 respectively.

Hausner ratio (HR): Hausner ratio of all coated and uncoated pellet formulations were determined shown in table 16 and 17. Hausner ratio values of all coated formulations of metoprolol tartrate and hydrochlorothiazide were found in the range of 1.039 to 1.164 and 1.102 to 1.130 respectively. Hausner ratio of uncoated pellets of metoprolol tartrate and hydrochlorothiazide were found to be 1.237 and 1.228.

Table 16: Micromeretic properties of uncoated and HMC pellets of MPTA

Formulation	Parameter				
	Bulk density*	Tapped density*	Carr index*	Hausner ratio*	Flowability
M1	0.673	0.758	13.376	1.126	Good
M2	0.684	0.761	10.118	1.114	Excellent
M3	0.709	0.785	9.681	1.108	Excellent
M4	0.687	0.799	14.017	1.164	Good
M5	0.749	0.856	12.500	1.143	Good
M6	0.776	0.866	12.500	1.039	Excellent
M0	0.628	0.776	19.072	1.237	Fair
M5S	0.748	0.855	12.510	1.142	Good
* Values indicates mean of triplicate.					

Table 17: Micromeretic properties of uncoated and HMC pellets of HCTZ

Formulations	Parameter				
	Bulk density*	Tapped density*	Carr index*	Hausner ratio*	Flowability
H1	0.668	0.755	11.523	1.130	Good
H2	0.673	0.758	11.213	1.126	Good
H3	0.698	0.769	9.232	1.102	Excellent
H4	0.672	0.768	12.500	1.147	Good
H5	0.694	0.775	10.452	1.117	Good
H6	0.726	0.812	12.500	1.118	Excellent
H0	0.632	0.776	18.798	1.228	Fair
H6S	0.728	0.815	10.674	1.195	Good

* Values indicates mean of triplicate.

Hardness: Hardness of all coated pellets formulations of metoprolol tartrate and hydrochlorothiazide were varying in the range of 2.25 to 2.95 kg/cm^2 and 2.30 to 2.90 kg/cm^2 respectively. Hardness for uncoated pellets of metoprolol tartrate and hydrochlorothiazide were found to be 1.95 and 2.05 kg/cm^2 respectively. Table 18 and 19 shows the results obtained for hardness of all the formulations.

Friability: Friability of coated pellet formulations of metoprolol tartrate and hydrochlorothiazide were varying in the range of 0.120 to 0.233% and 0.140 to 0.228% respectively. Friability of uncoated pellet formulations of metoprolol tartrate and hydrochlorothiazide were found to be 0.423% and 0.385% respectively. Table 18 and 19 shows the results obtained for friability of all the formulations of metoprolol tartrate and hydrochlorothiazide respectively.

Drug content: Drug content in pellets of metoprolol tartrate and hydrochlorothiazide were found in the range of 98.83-100.85% and 98.05-100.65% respectively. The values obtained were within acceptable range (table 18 and 19).

Table 18: Physicochemical evaluation of uncoated and HMC pellets of META

Formulation	Mean pellet size (μ)	Hardness[a] (kg/cm^2)	Friability[a] (%)	Drug content[a] (%)
M1	854	2.25 ± 0.10	0.221 ± 0.002	99.84 ± 1.12
M2	862	2.35 ± 0.15	0.233 ± 0.004	99.79 ± 2.04
M3	873	2.65 ± 0.25	0.214 ± 0.003	100.46 ± 2.23
M4	892	2.80 ± 0.20	0.187 ± 0.005	101.05 ± 1.01
M5	910	2.85 ± 0.05	0.176 ± 0.004	100.11 ± 3.02
M6	926	2.95 ± 0.15	0.120 ± 0.001	98.83 ± 0.17
M0	845	1.95 ± 0.20	0.423 ± 0.006	100.85 ± 2.35
M5S	910	2.90 ± 0.15	0.162 ± 0.003	98.64 ± 2.23

Where, [a] indicates values as (Mean ± S.D.) when sample size is in triplicate, M0-indicates uncoated pellets, M5S indicates formulations M5 stored for stability study as per ICH guidelines for 6 months.

Table 19: Physicochemical evaluation of uncoated and HMC pellets of HCTZ

Formulation	Mean pellet size (μ)	Hardness[a] (kg/cm^2)	Friability[a] (%)	Drug content[a] (%)
H1	860	2.30 ± 0.05	0.215 ± 0.002	99.94 ± 1.22
H2	878	2.45 ± 0.10	0.228 ± 0.004	99.89 ± 3.13
H3	898	2.55 ± 0.15	0.205 ± 0.003	100.09 ± 2.43
H4	870	2.70 ± 0.15	0.195 ± 0.005	98.05 ± 1.37
H5	886	2.85 ± 0.10	0.186 ± 0.004	99.58 ± 2.55
H6	900	2.90 ± 0.05	0.140 ± 0.001	100.65 ± 1.28
H0	840	2.05 ± 0.10	0.385 ± 0.006	100.11 ± 2.19
H6S	905	2.90 ± 0.05	0.142 ± 0.003	98.94 ± 2.23

Where, [a] indicates values as (Mean ± S.D.) when sample size is in triplicate, H0-indicates uncoated pellets, H6S indicates formulations H6 stored for stability study as per ICH guidelines for 6 months.

Floating ability: Floating time and floating lag time for designed pellets were reported in table 20. With increase in concentration of HPMC in the coating composition decrease in the floating lag time was observed. It indicates that HPMC allows rapid wetting of pellets and permits rapid effervescent reaction.

Table 20: Floating ability of hot melt coated pellet formulations

Formulation	Floating parameters		Formulation	Floating parameters	
	Lag time (min)	Floating time (hr)		Lag time (min)	Floating time (hr)
M1	28	9	H1	24	8
M2	42	11	H2	36	10
M3	85	>12	H3	77	>12
M4	8	>12	H4	6	>12
M5	5	>12	H5	4	>12
M6	2	>12	H6	1	>12

***In vitro* dissolution study of metoprolol tartrate pellets:** Figure 7 shows *in vitro* release of metoprolol tartrate from pellets coated with 5 and 7% of beeswax, which can retard the drug release but not more than 6 hr respectively. Wax layer was slightly erode due to movement of paddle as well as vertex formation in dissolution medium. These coating levels were not sufficient to coat overall surface of pellets. Therefore, the faster release of drug than required was observed in case of M1 and M2 formulations. Whereas 10% beeswax containing formulation (M3) release about 64.52% drug in 12 hr.

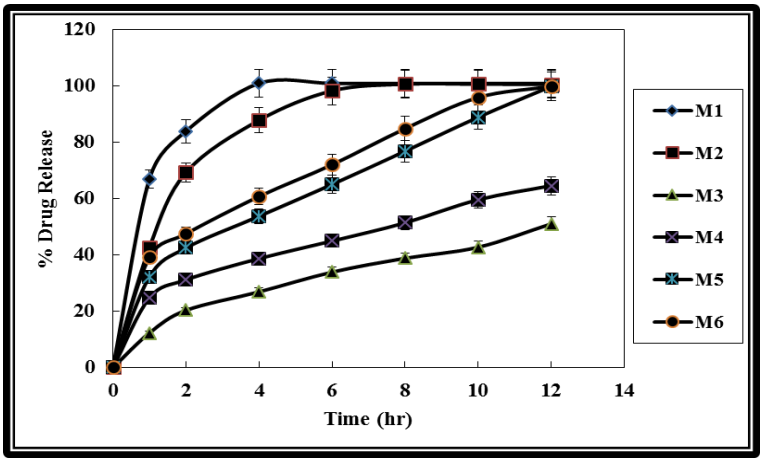

Fig 7: *In vitro* dissolution study of hot melt coated formulations of MPTA**.**

To achieve the target release profile and rapid floating of pellets less than 10 min (desirable) the part of beeswax was replaced by HPMC which was hydrophilic polymer at 10% coating level. Formulation M5 was showing release profile similar to targeted release profile and hence was selected as optimized formulation. In case of M5 formulation the drug release in 2 hr is about 42.42% which was quiet faster may be because of drug release from pellets which were not uniformly coated or drug release before hydration of HPMC polymer shown in figure 7 and table 21. Formulation M5 was showing difference factor (f_1) and similarity factor (f_2) values 8.03 and 61.38 respectively (table 4.29). Hence, M5 was stored for stability testing.

Table 21: *In vitro* drug release from MPTA hot melt coated formulations

Time (hr)	% Drug release[c]					
	M1	M2	M3	M4	M5	M6
1	66.88 ± 3.56	42.56 ± 2.35	12.24 ± 0.62	24.64 ± 1.86	32.12 ± 1.56	39.12 ± 2.11
2	83.84 ± 3.68	69.21 ± 2.88	20.27 ± 0.91	31.12 ± 1.94	42.42 ± 2.78	47.42 ± 2.27
4	100.82 ± 4.39	87.72 ± 3.19	26.84 ± 1.68	38.65 ± 2.14	53.68 ± 2.61	60.68 ± 2.89
6	100.82 ± 4.36	98.18 ± 3.44	33.84 ± 1.48	44.87 ± 2.66	64.96 ± 3.05	71.96 ± 3.22
8	100.81 ± 4.43	100.54 ± 4.06	38.77 ± 2.12	51.35 ± 2.58	76.76 ± 3.58	84.76 ± 4.02
10	100.81 ± 4.41	100.54 ± 4.04	42.68 ± 2.45	59.55 ± 3.18	88.82 ± 4.04	95.82 ± 4.78
12	100.79 ± 4.36	100.48 ± 4.18	50.99 ± 2.89	64.52 ± 3.14	99.76 ± 4.21	99.76 ± 4.67

[c] -Values indicates (Mean± S.D.) when samples were analyzed (n=6).

***In vitro* dissolution study of hydrochlorothiazide pellets:** Figure 8 and table 22 shows *in vitro* release of hydrothiazide from the hot melt coated pellets coated with 5 and 7% of cetyl alcohol which can retard dug release but not more than 6 hr respectively. The wax layer was slightly erode due to movement of paddle as well as dissolution medium (vertex formation) and at these levels of coating agent were insufficient to coat overall surface of the pellets. Therefore faster release of drug was observed from H1 and H2 formulations. Whereas 10% of cetyl alcohol containing formulation (H3) release about 59.41% drug in 12 hr. But the floating lag time was about 90 min which was due to hydrophobicity of cetyl alcohol (undesirable).

Table 22: *In vitro* drug release from HCTZ hot melt coated formulations

Time (hr)	% Drug release[d]					
	H1	H2	H3	H4	H5	H6
1	64.33 ± 3.12	45.63 ± 2.93	21.84 ± 0.65	29.28 ± 1.14	36.47 ± 1.20	39.43 ± 1.84
2	87.54 ± 3.32	66.14 ± 3.02	24.18 ± 1.22	35.34 ± 1.18	45.29 ± 2.43	49.28 ±2.16
4	98.69 ± 4.25	78.72 ± 3.77	30.41 ± 1.47	42.49 ± 2.04	52.59 ± 2.66	59.51 ± 2.72
6	100.89 ± 4.09	92.76 ± 3.12	37.43 ± 1.86	49.08 ± 2.26	60.78 ± 2.89	68.27 ± 3.22
8	100.89 ± 3.98	99.76 ± 4.36	43.26 ± 2.11	55.08 ± 2.49	69.55 ± 3.23	80.54 ± 3.64
10	100.87 ± 4.48	99.74 ± 4.55	49.42 ± 2.74	64.06 ± 2.88	78.64 ± 3.41	91.56 ± 4.33
12	100.87 ± 4.56	99.74 ± 4.48	59.41 ± 3.12	72.46 ± 3.17	86.62 ± 3.82	102.12 ± 4.37

[d] -Values indicates (Mean ± S.D.) when samples were analyzed (n=6).

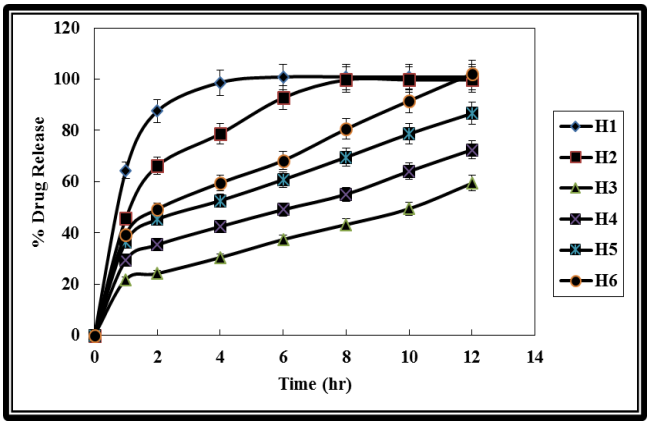

Fig 8: *In vitro* dissolution study of hot melt coated formulations of HCTZ.

To obtain the target release profile and rapid floating of pellet formulation less than 10 min (desirable) the part of cetyl alcohol was replaced by HPMC polymer. In case of H6 formulation the hydrochlorothiazide release in 2 hr was about 49.28% which was quiet faster may be because of drug release from pellets which were not uniformly coated or drug release before hydration of HPMC polymer. Formulation H6 was showing release profile similar to targeted release profile and hence was selected as optimized formulation. Formulation H6 was showing difference factor (f_1) and similarity factor (f_2) values 3.73 and 71.64 respectively (table 26) and used for stability testing.

Molding multi-component system of metoprolol tartrate and hydrochlorothiazide into unit dosage form: Quantity of pellets of metoprolol tartrate equivalent to 100 mg of metoprolol tartrate and pellets of hydrochlorothiazide equivalent to 25 mg were filled into hard gelatin capsule shell of '0' size.

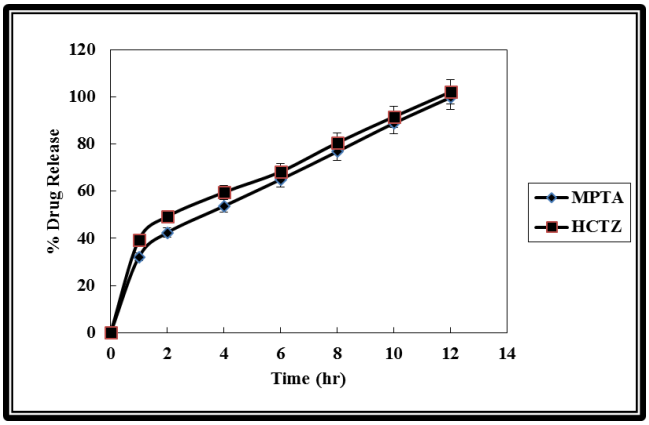

Fig 9: *In vitro* dissolution study of optimized formulation.

Kinetic analysis: Various kinetic models like zero order, first order, Higuchi, Hixon-Crowell and Korsmeyer-Peppas models were applied to the *in vitro* release data of all coated formulations.

Table 23: Kinetic data analysis for MPTA hot melt coated formulations

Formulation	Zero order		First order		Higuchi	Korsmeyer- Peppas		Hixon-Crowell
	K_0	R^2	K	R^2	R^2	R^2	'n'	R^2
M1	5.5831	0.6915	0.2033	0.5528	0.8639[b]	0.4379	0.8731	0.6115
M2	6.8389	0.8177	0.2239	0.6166	0.9431[b]	0.4829	1.0453	0.4829
M3	3.7288	0.9679	0.2208	0.7551	0.9968[b]	0.4271	1.3811	0.8567
M4	4.4797	0.9386	0.2135	0.6830	0.9935[b]	0.4173	1.1741	0.8013
M5	7.1431	0.9617	0.2385	0.6942	0.9963[b]	0.4760	1.1756	0.8352
M6	7.1478	0.9384	0.2331	0.6617	0.9946[b]	0.4715	1.1086	0.8038

Where, [b]- indicates best fitted dissolution model.

Table 24: Kinetic data analysis for HCTZ hot melt coated formulations

Formulation	Zero order		First order		Higuchi	Korsmeyer-	Peppas	Hixon-Crowell
	K_0	R^2	K	R^2	R^2	R^2	'n'	R^2
H1	5.6029	0.6922	0.2035	0.5541	0.8640[c]	0.4401	0.8787	0.6130
H2	6.8035	0.8442	0.2227	0.6185	0.9591[c]	0.4738	1.0272	0.7249
H3	4.0361	0.9549	0.2115	0.7053	0.9878[c]	0.4056	1.2157	0.8229
H4	4.8232	0.9316	0.2137	0.6685	0.9877[c]	0.4201	1.1311	0.7901
H5	5.7771	0.9216	0.2196	0.6532	0.9867[c]	0.4396	1.0852	0.7786
H6	6.9725	0.9388	0.2305	0.6635	0.9913[c]	0.4715	1.1097	0.7999

Where, [c]- indicates best fitted dissolution model

Table 23 and table 24 revealed that both the optimized formulations of metoprolol tartrate (M5) and hydrochlorothiazide (H6) fits in Higuchi model which indicates that the drug release mechanism from dosage form was by diffusion mechanism. But Korsmeyer-Peppas equation showing the value of 'n' between 0.5 -1.0 showing drug release mechanism by non-Fickian diffusion and n > 1 showing drug release mechanism by super case II (erosion is dominated by diffusion).

Stability studies: Stability studies were carried out at 40 °C and 75% RH for 6 month (for accelerated testing) to assess their long-term stability. Figure 10 and table 25 shows no significant change in release pattern after storage for 6 months indicating that the two dissolution profiles were similar with similarity factor (f_2 >50) (table 26). The other parameters like angle of repose, mean pellet size, drug content, hardness, friability and floating ability evaluated were significantly similar with initial values.

Table 25: *In vitro* drug release from stability batches

Time (hr)	% Drug release[e]	
	M5S	H6S
1	30.26 ± 1.43	36.65 ± 1.62
2	40.66 ± 1.87	47.56 ± 1.98
4	52.51 ± 2.07	57.91 ± 2.21
6	66.43 ± 2.45	66.35 ± 3.02
8	74.77 ± 2.69	83.12 ± 3.33
10	84.09 ± 3.28	90.28 ± 3.96
12	97.44 ± 3.88	100.08 ± 4.12

[e] - Values indicate (Mean ± S.D.) when samples were analyzed in triplicate.

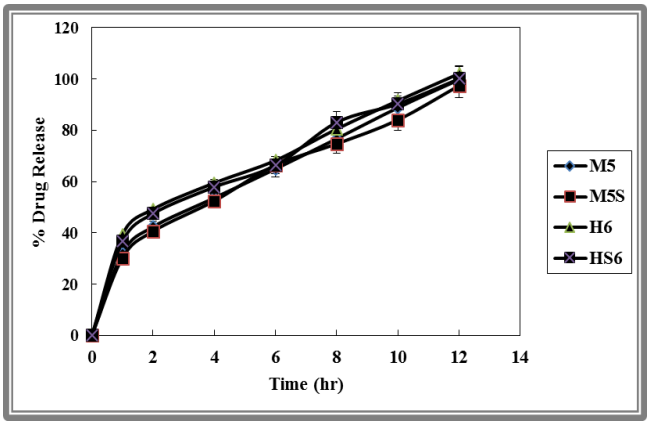

Fig 10: Comparison of *in vitro* dissolution study of optimized formulation with stability batches

Table 26: Difference factor and similarity factor

Sr. No.	Parameters	Difference factor (f_1)	Similarity factor (f_2)
1.	M5 Vs MR	5.78	68.54
2.	H6 Vs TRP	3.56	78.96
3.	M5 Vs M5S	3.14	80.37
4.	H6 Vs H6S	3.08	83.21

Discussion

Modified release multi-component drug delivery system of metoprolol tartrate and hydrochlorothiazide using hot melt coating technique was designed successfully. Pellets of metoprolol tartrate and hydrochlorothiazide with good surface morphology and smooth texture were produced as a result of hot melt coating with beeswax and cetyl alcohol respectively. Photomicrographs of pellets confirmed uniformity of coating of pellets for both the drugs. Coating could be performed very simply within short period. No agglomeration was observed during coating results in excellent percent yield of coated pellets of both the drugs. Beeswax/cetyl alcohol in coating composition facilates free rolling of drug pellets. Coated pellets of both drugs were with good to excellent flow properties which was confirmed from angle of repose, compressibility index and Hausner ratio values. The coated pellet formulations pass the parametric tests like hardness, friability and drug content.

At the given coating levels and coating compositions, the amount of drug release from hot melt coated formulations are shown in table 21 and 22, and illustrated in figure 7 and 8. Drug release from coated pellets was observed to be the function of physicochemical properties of drugs and hot melt coating agents. More specifically, drug release profile was the function of water solubility of drug. Metoprolol tartrate being water soluble drug coated with comparatively higher melting point lipid i.e. beeswax at 10% coating level can retard the release more than 12 hrs. On the other hand, hydrochlorothiazide being very slightly water soluble drug coated with comparatively low melting point lipid i.e. cetyl alcohol at 10% coating level can retard the drug release more than 12 hrs.

Beeswax and cetyl alcohol at 5 and 7% were not sufficient to retard the drug release upto 12 hrs due to erosion of coating and lack of ability of coating overall surface of drug pellets. At 10% coating level beeswax and cetyl alcohol could retard drug release more than 12 hrs for metoprolol tartrate and hydrochlorothiazide formulations respectively. It indicates that the drug release was depends upon the coating levels i.e. as coating level increases the drug release rate from pellets decreases. Since both the drug shows absorption window in stomach and upper part of intestine therefore pellets were formulated in the form of floating pellets. Effervescent pellets coated with only either beeswax or cetyl alcohol shows higher lag time which was not desirable. Therefore beeswax or cetyl alcohol was used in conjugation with HPMC in different ratios where the system could float in few minutes after contact with media and remain floated for prolonged period of time.

Selection of optimized formulation was done on the basis of *in vitro* release profile and floating ability of pellet formulations of both drugs. Formulation M5 and H6 of metoprolol tartrate and hydrochlorothiazide respectively were selected as optimized formulations by comparing their release profiles with marketed modified release formulation of metoprolol and theoretical release profile of hydrochlorothiazide.

Kinetic analysis of release profiles of both drug formulations were fits into Higuchi model confirms drug release mechanism as diffusion. More precisely since Korsmeyer-Peppas equation showing the value of 'n' between 0.5-1.0 showing drug release mechanism by non-Fickian diffusion and n > 1 showing drug release mechanism by super case II (erosion is dominated by diffusion) as shown in table 23 and 24.

Optimized formulations were found to be stable for 6 months when stored at 40 °C and 75% RH for 6 month (for accelerated testing) to assess their long-term stability as per ICH guidelines. Both the drug pellet formulations significantly retain their physicochemical, micromeretic and *in vitro* release characteristics.

Conclusions

Metoprolol tartrate and hydrothiazide pellets were successfully prepared by extrusion- spheronization technique. Both the drug pellets obtained by this means were with spherical shape and relatively smooth surface. Pellets with narrow size distribution were obtained by the present technique. Both the drug pellets coated with wax using hot melt coating technique in a modified coating pan without spray system and demonstrated to be efficient.

Dissolution study of the coated formulations showed that drug release could be controlled by using suitable coating materials like beeswax and cetyl alcohol. The release rate can be regulated by varying coating compositions and coating levels. The hot melt coating can be an eco-friendly, economic, effective and rapid means for preparing modified release multi-component drug delivery systems. The results of present study showed that present hot melt coating technique can constitute an excellent alternative to conventional solvent based coating. Coated pellets of both drugs were with good to excellent flow properties.

The present study has demonstrated that beeswax and cetyl alcohol could be successfully employed as a modified release hot melt coating agent in conjunction with materials such as HPMC as release modifier. It is worthy to mentioning that controlling the release of water soluble as well as poorly water soluble drug could be possible by the present technique by selecting appropriate coating agents . Further *in vivo* study was needed before commercialization of this product.

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