Formulation and *In-vitro* Characterization of floating microcapsules as gastroretentive drug delivery system containing Itopride hydrochloride by W/O/O multiple emulsion solvent diffusion technique

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KEYWORDS

Gastroretentive, microcapsules, Double emulsion method, Itopride hydrochloride

ABSTRACT

Itopride hydrochloride loaded floating microcapsules were prepared by W/O/O multiple emulsion solvent diffusion method using ethyl cellulose and eudragit RSPO 100 as drug release rate controlling polymers. Drug containing aqueous phase was emulsified in ACN: DCM primary organic phase. This W/O primary emulsion was further emulsified in continuous phase (light liquid paraffin) containing emulsifying agent (span 80).Formulated microcapsules were harvested by filtration and subsequent washing with petroleum ether. Further microcapsules were evaluated for flow properties, %product yield, particle size, %EE, buoyancy, *in-vitro* drug release, SEM, FTIR and DSC analysis**.**Floating microcapsules were prepared with varying proportions of EC and eudragit RSPO 100. Microcapsules containing drug: EC: Eudragit RSPO 100 (Formulation F5) in proportion of 1:2:1shows desired properties. All formulations show good to excellent flow properties. F5 formulation shows 91.41± 2.84% production yield, mean particle size was29.39± 5.45µm, %buoyancy 88.27±1.75%, EE 98.53±0.349%. Cumulative % drug release from microcapsules of F5 formulation was 98.99± 1.90% in 24hours and following Korsmeyer–Peppas kinetic model for drug release with R^2 value 0.9805. SEM analysis revealed formation of spherical microcapsules with rough surface indicates encapsulation of drug within polymer coat. FTIR and DSC analysis shows no interaction between drug and polymers used in formulation.Formulated multiple unit floating gastroretentive microcapsules of Itopride hydrochloride have potential to delivered drug in upper part of GIT for extended period of time, thereby reducing dosing frequency, enhance bioavailability and improved patient compliance..

INTRODUCTION

Development of multiple unit floating drug delivery systems have gained more popularity due to their physical characteristics such as uniform particle size, free flowing, uniform distribution throughout GI tract, more absorption, reduce risk of dose dumping, drug release at constant controlled rate, increase gastrointestinal transit time and reduced local drug irritation(Adebisi &Convey, 2011; Kesharvani, 2020). Gastroretentive floating microcapsules are low density systems remained buoyant on gastric fluid in stomach for extended period. Microcapsules

containing gel forming polymers forms gel barrier on contact with gastric fluid and release drug slowly in upper part of GIT.(Ichikawa, Watanabe & Miyake, 1991).

Several methods developed for preparation of microcapsules are spray drying, phase separation and solvent evaporation technique etc. Phase separation techniques have some disadvantages such as aggregation and presence of residual solvent in resulting microcapsules, whereas drug substance exposed to heat in spray drying technique. (Ruiz et al., 1989; Takada, 1994).

Hydrophobic drugs are easily and successfully encapsulated by emulsification solvent evaporation technique. But microcapsules of hydrophilic drugs are not prepared by O/W single emulsion solvent evaporation method due to low entrapment and rapid diffusion of drug into external phase. So double emulsion solvent evaporation technique is employed for preparation of microcapsules to reduce partitioning of drug into continuous phase. (Ashjari, Khoee & Mahdavian, 2012). Microcapsules of hydrophobic drugs prepared by W/O/O double emulsion solvent evaporation technique have more encapsulation efficiency than W/O/W emulsification method, because hydrophilic drugs cannot diffuse into external phase (oil phase). (Aydongan, Comoglu, Pehlivanoglu, Dogan, Comoglu & Dogan, 2015).

Itopride is a benzamide derivative used in treatment of gastroesophageal reflux disease. It is novel prokinetic agent which stimulates gastrointestinal motility by increasing acetyl choline synthesis. ITH act as dopamine D_2 receptor blocker and acetyl cholinesterase inhibitor. Itopride hydrochloride used in treatment of gastrointestinal symptoms caused due to decreased motility of GIT, abdominal pain, heartburn, anorexia, feeling of gastric fullness, non-ulcer dyspepsia, chronic gastritis, nausea and vomiting. (Kim, Kim, Choi, Shon, Kim & Seo, 2005).Itopride hydrochloride is a water soluble drug; get absorbed in upper part of GIT. Therapeutic dose of ITH is 50mg (t.i.d), systemic bioavailability is 60% and elimination half-life ($t_{1/2}$) is 6hours. However it shows narrow absorption window in the stomach. Therefore it is essential to develop gastroretentive drug delivery system, which release drug slowly in upper part of GIT, thereby reduce dosing frequency and increase bioavailability. (Patel, Patel & Patel, 2020).

In present research work Itopride hydrochloride floating microcapsules were prepared by W/O/O double emulsion solvent diffusion method. Microcapsules were formulated using different proportion of ethyl cellulose and eudragit RSPO 100 as release controlling polymers. Acetonitrile and dichloromethane as primary oil phase and light liquid paraffin as secondary continuous phase was used in formation of multiple emulsion. Temperature and speed of agitation was optimized from trial batches.

MATERIALS AND METHODS

Materials

Itopride hydrochloride(ITH) (D.K. Pharma Chem. Ltd. Thane), Ethyl cellulose, Acetonitrile (Research Lab Fine Chem Industries, Mumbai) Eudragit RSPO-100(Evonik, Mumbai), Dichloromethane, Span-80, Light liquid paraffin (Loba Chemie Pvt.Ltd, Mumbai).

Methods

Method of preparation of Itopride hydrochloride Microcapsules

Gastro retentive floating microcapsules of Itopride hydrochloride (ITH) were formulated by w/o/o double emulsion solvent diffusion method (Composition shown in Table 1). Drug was dissolved in water to form internal water phase. Water phase was emulsified in acetonitrile and Dichloromethane (O_1) as co-solvent solution containing ethyl cellulose and/or eudragit RSPO 100 in different ratios to form primary emulsion by stirring on magnetic stirrer. Formed primary emulsion was added slowly to light liquid paraffin second continuous phase (O_2) containing

span 80. It was stirred at 1000rpm using mechanical stirrer for 2h until complete evaporation of organic solvent. Formed microcapsules were collected by decantation and filtration using whatman filter paper no. 41. Finally, microcapsules washed thoroughly with petroleum ether (40-60℃). Then the microcapsules were collected and dried in oven at 30℃ overnight (Kharab., 2021;Jelvehgari., 2010). Formulated microcapsules were stored in glass vial for further evaluation.

Table 1: Composition of floating Microcapsule of Itopride Hydrochloride(ITH).

Sr. No.	Ingredients	F1	F2	F3	F4	F5	F6
1	ITH: Ethyl cellulose	1:1	-	1:3	1:5	-	-
2	ITH: Eudragit RSPO 100	--	1:1	-	-	-	-
3	ITH:EC: Eudragit RSPO 100	--	-	-	-	1:2:1	1:3:2
4	Acetonitrile (mL)	25	25	25	25	25	25
5	Dichloromethane (mL)	10	10	10	10	10	10
6	Span 80 (mL)	1	1	1	1	1	1
7	Light liquid paraffin (mL)	100	100	100	100	100	100

Preliminary studies

For the preparation of microcapsules various formulations and process variables were studied. Process and formulation variables screened by keeping one variable changing and other were constant. Prepared formulations were evaluated for physical appearance using binocular microscope.

Effect of drug polymer ratio

Based on literature survey microcapsules were prepared by using eudragit RSPO 100 and ethyl cellulose in varying ratios with drug. Formulations with different proportions of (ITH: Ethyl cellulose: Eudragit RSPO100) drug-polymer ratio (1:1, 1:3, 1:5, 1:2:1, 1:3:2) and polymer-polymer ratio were prepared to determine effect of drug-polymer and polymer-polymer ratio on morphology of microcapsules. For screening of other variables use of combinations of ethyl cellulose and eudragit RSPO 100 were selected.

Effect of temperature and speed of agitation

To study the effect of temperature and stirring speed various batches of ITH loaded microcapsules were prepared by varying the temperature (30℃, 40℃ and 50℃) and speed of agitation (500rpm, 1000rpm and 1500rpm) using combination of polymers.

Evaluation of Itopride Hydrochloride Floating Microcapsules

Micromeritic properties

All Formulations of Itopride Hydrochloride microcapsules were evaluated for micromeritic parameters such as angle of repose, bulk density, tap density, Carr's Index (CI), and Hausner's Ratio etc.(Galatage et al., 2019;Kapoor, 2012).

Angle of repose

Angle of repose is the maximum angle possible between the surface of a pile of the microcapsules and the horizontal plane. Lower the angle of repose better will be the flow property of microcapsules. Angle of repose was measured by fixed funnel method.

Angle of repose was calculated using following formula,

$$\tan \theta = h/r \qquad (1)$$

Where,

h = height of the pile of microcapsules and

r = radius of the base of the pile

Bulk density

Bulk density (BD) was calculated by accurately weighing microcapsules and shifted into 10ml measuring cylinder. Measure volume occupied by microcapsules at zero tapings.

BD = Weight of microcapsule / Volume of microcapsules without tapings (2)

Tap density:

Tap density (TD) was calculated as ratio of weight of microcapsules to tap volume until no change in volume using Bulk density apparatus (Pathak Electrical work Mumbai).

TD = Weight of microcapsules / Tap volume of microcapsules (3)

Carr's Index (% Compressibility)

Carr's Index (CI) was calculated using following formula,

$$CI = (TD - BD / TD) \times 100 \quad (4)$$

Hauser's ratio

Hausner's Ratio was calculated by following formula,

$$\text{Hausner's Ratio} = TD / BD \quad (5)$$

Particle size Analysis (Galatage et al., 2019;Kapoor, 2012)

Optical microscopy was used to determine particle size of floating ITH microcapsules. The optical microscope was fitted with an ocular micrometer and a stage micrometer. The eyepiece micrometer was calibrated with stage micrometer. The sample was placed on slide and mounted on the mechanical stage. The shape and size of microcapsules were observed and measure visually using optical microscope (Besto). The mean particle size and standard deviation was calculated by measuring 100 floating microcapsules.

% Production Yield (Kumar et al., 2021; Jyoti, 2018; Galatage,2019)

Product yield of each formulation was calculated by dividing the weight of floating microcapsules recovered from each batch in relation to sum of initial weight of drug and excipients used in the preparation of microcapsules. Percent product yield was determined using following formula:

$$\%\text{Product Yield} = (\text{Weight of Floating Microcapsules} / \text{Total dry weight of Starting material (Drug + polymer)} \times 100 \quad (6)$$

% Encapsulation Efficiency (Bansal et al.,2016;Gupta.,2010;Shah, 2012)

Itopride hydrochloride loaded microcapsules theoretically equivalent to 10mg of drug was weighed and crushed in mortar pestle. Crushed microcapsules were dispersed in 5mL methanol and then volume was made up to 100mLusing 0.1N HCl. The dispersion was sonicated 5min for complete extraction of drug. The solution was macerated for 24h. The solution was then filtered using whatman filter paper, diluted suitably and analysed spectrometrically at 258nm using UV Visible Spectrophotometer.

Percent Encapsulation Efficiency (EE) was calculated using following formula

$$\%EE = (\text{Practical Drug Content} / \text{Theoretical Drug Content}) \times 100 \quad (7)$$

***In- Vitro* Buoyancy (Floating ability)** (Lee et al, 1999; Kohli, 2015; Pandit, 2013)

Floating ability of microcapsules was performed by spreading of 50mg of microcapsules over the surface of dispersion medium kept in USP type II (paddle) dissolution test apparatus at speed of 50rpm at 37°C ± 0.5 for 24h. After 24h, the floating and sedimented portion of microcapsules were filtered, dried and weighed separately.

Percent *In-Vitro* Buoyancy was calculated using following formula:

$$\%\ \textit{In-Vitro}\ \text{Buoyancy} = (W_f / W_f + W_{nf}) \times 100 \quad (8)$$

Where,

W_f = Weight of floating Microcapsules

W_{nf} = Weight of non-floating microcapsules

***In-Vitro* Drug Release** (Dandag et.al,2013;Bose,2013;Mamatha,2018)

In-vitro drug release characteristics of Itopride hydrochloride floating microcapsules were carried out by employing USP type II (LABINDIA) dissolution test apparatus in triplicate. Drug release studies were performed using 900mL of 0.1N HCl solution (pH 1.2) as dissolution medium maintained at 37±0.5°C and 75 rpm under sink condition. Accurately weighed amount of microcapsules equivalent to 150mg of Itopride hydrochloride were filled in zero sized hard gelatine empty capsule shell and placed under dissolution conditions. 5mL aliquot of samples

were withdrawn at predetermined time intervals for 24h and replenished with 5mL of dissolution medium to maintain sink condition. Above withdrawn 5mL samples was filtered through whatman filter paper. After suitable dilution samples were analysed spectrophotometrically at 258nm.

Drug release kinetics (Abboju & Bhujugundla,2018)

In-vitro drug release data was fitted to different kinetic models to explain release kinetics. zero order, first order, Korsmeyer-Peppas, Higuchi and Hixson Crowell are different linear and nonlinear kinetic models which describe release of drug from pharmaceutical dosage form. The model with highest correlation coefficient (R^2) was considered to be the best fitting one.

Scanning Electron Microscopy (Jung et al.,2000; Kharab,2021)

The morphology and surface characteristics of the microcapsules were determined by using scanning electron microscopy imaging analysis (FEI Nova Nano SEM450). The sample was mounted onto an aluminium stub and sputter coated with gold particles in an argon atmosphere by sputter coater.

Fourier Transform Infrared Spectroscopy (FTIR) (Patel et al.,2020;Shah,2012)

FTIR study gives the information about the compatibility between drug and Polymer. FTIR (Bruker ALPHA II) spectroscopic analysis was performed for Itopride hydrochloride, physical mixture of drug-polymer and Itopride hydrochloride loaded microcapsules (F5). All the discs were prepared by punching the sample and KBr powder mixture (1:1) at 20psi for 10min using KBr press. The prepared disc was placed in sample holder and scanned at the transmission mode in the region of 4000-400cm^{-1}.

Differential Scanning Colorimetry (DSC) (Bansal, Beeg, Asthana, Garg, & Asthana ,2016)

DSC thermogram were used to characterize physical state of drug and to detect any physic-chemical change or incompatibility between drug and polymers selected for preparation of microcapsules depending upon their melting temperature and glass transition temperature. Drug, physical mixture of drug-polymer and Itopride Hydrochloride loaded microcapsules selected as sample to perform DSC (Shimadzu DSC-60) analysis. Approximately 3-5mg of each sample was transferred in Aluminium pan and heated at a rate of 10^0C/min up to 300°C in nitrogen atmosphere at a 20mL/min flow rate.

Results and Discussion

Gastroretentive multiple unit microcapsules were prepared by $W/O_1/O_2$ double emulsion solvent diffusion method. Internal aqueous phase containing Itopride Hydrochloride was emulsified in acetonitrile/dichloromethane co-solvent solution containing polymers (ethyl cellulose, eudragit RSPO 100) in different ratio to prepare primary emulsion (W/O_1). Light Liquid paraffin was selected as continuous phase, because Itopride hydrochloride, ethyl cellulose and eudragit RSPO100 were hardly soluble in light liquid paraffin. This primary emulsion was then added slowly to light liquid paraffin contain Span 80 as surfactant to form $W/O_1/O_2$ emulsion. Some amount of time was required to form stable emulsion to allow complete diffusion and evaporation of organic solvent and solidification of polymer. Formed microcapsules were washed with petroleum ether (40-60°C) complete removal of light liquid paraffin. Acetonitrile was selected since it was unique solvent among other organic solvent (such as acetone, alcohol) which is polar, water miscible and oil miscible. But formation of primary emulsion cannot be assured due to water miscibility of acetonitrile, as the aqueous Itopride hydrochloride was added to the polymer solution the polymer will precipitate. Therefore, to avoid this co-solvent system was used for preparation of polymer solution. Co-solvent system includes acetonitrile (ACN)/ dichloromethane (DCM) a non- polar solvent. Due to oil miscibility of DCM, solvent removal also occurs by extraction in the continuous phase. After mixing primary emulsion in the continuous phase DCM undergoes rapid diffusion in the oil phase and polymer solution became viscous. This prevents migration of aqueous drug phase

thereby increasing encapsulation efficiency. DCM play important role in increasing partitioning of ACN to external oil phase and reduce solidification time.

Preliminary Studies

All batches of microcapsules were prepared by varying proportion of drug-polymers ratio (ITH: Ethyl cellulose: Eudragit RSPO100) that is 1:1, 1:3, 1:5, 1:2:1, 1:3:2. To study effect of temperature formulations were prepared at different temperature. It was concluded; at 30℃ (room temperature) uniform spherical shape microcapsules were formed. At high temperature shape of microcapsules were not uniform and spherical. Effect of stirring speed on formation of microcapsules was studied. It was observed that 1000rpm was optimum speed for getting uniform and spherical microcapsules (Figure 1).

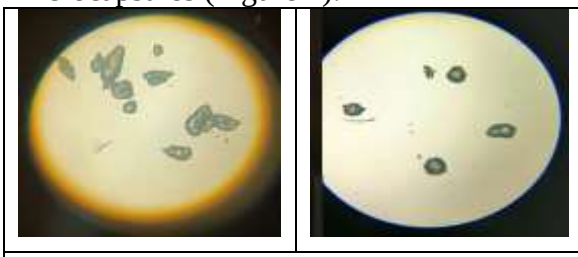


Figure 1. Microscopic images of Itopride hydrochloride microcapsules at different temperature and stirring speed.

Itopride hydrochloride loaded floating microcapsules were for evaluated for flow properties, %production yield, particle size, % buoyancy, % encapsulation efficiency, *in-vitro* drug release and kinetic model fitting for drug release. Topographical information was obtained by performing scanning electron microscopy (SEM) for all microcapsule batches. IR and DSC studies were carried out to get information regarding drug-polymer interaction.

Characterization of microcapsules

Flow properties of Microcapsules

Angle of Repose

The values of angle of repose for all formulations were found in the range of 20.30±0.333°to 24.09±0.679°(Table 2). The flow of microcapsules was found less than 25°.This indicates that the flow of microcapsules was excellent.

Bulk Density

Bulk density of Itopride Hydrochloride loaded microcapsules was found in between 0.288 ±0.005 to 0.364 ±0.008 (Table 2.). Bulk density of all formulations lower than the density of gastric content (1.007gm/cc). Thus gastroretentive floating microcapsules were remained buoyant in stomach for a prolonged period of time.

Tapped Density

All formulations of Itopride Hydrochloride show tapped density in between 0.324±0.01 to 0.419± 0.008 (Table 2).

Compressibility (Carr's Index)

Compressibility was indirectly related to the relative to flow rate, cohesiveness, particle size, shape and moisture content. It was the method to predict flow property of microcapsules. % compressibility for all microcapsules found in the range of 10.84 ± 1.49 to 14.18 ± 0.284 (Table 2). This indicates that the flow of microcapsules shows good to excellent flow character.

Hausner's Ratio

Hausner's ratio for all formulations of Itopride Hydrochloride microcapsules was found less than 1.16 (Table 2) indicates that the flow of microcapsules was good.

Table 2 : Flow Properties of all batches (F1 to F6) of ITH floating microcapsule

Formulations	Angle of Repose(º)	Bulk Density (g/cc)	Tapped Density (gm/cc)	Carr's Index (%)	Hausner's Ratio
F1	24.09 ± 0.679	0.3062 ±0.005	0.351 ± 0.011	12.64 ± 1.895	1.14 ± 0.025
F2	22.20 ± 0.415	0.288 ± 0.005	0.324 ± 0.01	10.84 ± 1.49	1.12 ± 0.017
F3	23.46 ± 0.405	0.294 ± 0.003	0.333 ± 0.007	11.68 ± 1.11	1.13 ± 0.015
F4	23.87 ± 0.702	0.309 ±0. 007	0.359 ± 0.008	14.18 ± 0.284	1.16 ± 0.005
F5	23.35 ± 0.70	0.364 ± 0.008	0.419 ± 0.008	13.12 ± 1.35	1.15 ± 0.015
F6	20.30 ± 0.333	0.305 ± 0.007	0.343 ± 0.013	10.86 ±1.32	1.12 ± 0.015

Evaluation of floating Microcapsules

Results of all batches of Itopride Hydrochloride floating microcapsules were evaluated for production yield (%), particle size (µm), buoyancy (%) and encapsulation efficiency (%). (Table 3).

% production Yield

Percent production yield of gastroretentive floating microcapsules ranging widely between 77.93 ± 2.84% to 93.10 ± 1.57% (Table 3). F6 formulation shows maximum production yield (93.10%) while F1 shows minimum production yield (77.93%). This clearly indicate that at lower concentration of polymer the production yield was less as the concentration increases production yield also increases.

Particle Size

The average particle size of all six formulations was in the range of 13.05 ± 3.6µm to 68.93± 5.642µm (Table 3). When the concentration of ethyl cellulose was low (F1), particle size was found to be low 13.05µm and at high concentration particle size was found to be (F4) 47.70µm. At high concentration of both ethyl cellulose and eudragit RSPO100 the particle size was found to be maximum (F6) that was 68.93±5.642µm. It was concluded that the proportion of polymers increases, particle size also increases. This may be due to increased viscosity of organic phase produced by high concentration of ethyl cellulose and eudragit RSPO 100 which results in larger sized emulsion droplets and consequently larger size of microcapsules.

% Buoyancy

In-vitro buoyancy study was carried out to determine floating ability of microcapsules. *In-vitro* buoyancy study revealed that all floating microcapsule formulations show good floating ability ranged from 65.77 ± 1.053% to 95.91 ± 1.32% (Table 3). All formulations remained floated for 24h.All formulations shows instantaneous onset of floating behaviour with zero lag time. This would help in achieving higher retention time of microcapsules in stomach.

Encapsulation Efficiency (%EE)

Encapsulation Efficiency (%) was a key indicator of loading efficiency of drug for development of microcapsules. % encapsulation efficiency for all six formulations was found in the range of 80.3 ± 0.950%to 97.91 ± 0.669% (Table 3). Among all these formulations F6 formulation shows maximum encapsulation efficiency which was 97.91%. EE increased with increased in the ratio of polymer and ratio of eudragit RSPO100 indicating an improvement in the loading efficiency of Itopride Hydrochloride.

Table 3: Evaluation of all batches (F1 to F6) of Itopride Hydrochloride floating microcapsules.

Formulations	Production yield (%)	Particle size (µm)	Buoyancy (%)	Encapsulation Efficiency (%)
F1	77.93 ± 2.84	13.05 ± 3.6	65.77 ± 1.053	80.3 ± 0.950
F2	78.77± 2.01	16.29 ± 0.71	66.8 ± 2.12	89.02 ± 0.557
F3	83.63 ±1.87	20.21 ± 2.63	74.13 ± 0.987	85.87 ± 0.9734
F4	88.07 ± 2.57	47.70 ± 20.88	91.27 ± 0.771	94.08 ± 0.359
F5	91.41 ± 2.84	29.39 ± 5.450	88.27 ± 1.75	98.53 ± 0.349
F6	93.10 ± 1.57	68.93 ± 5.642	95.91 ± 1.32	97.91 ± 0.669

***In-vitro* Drug Release Study**

In-vitro dissolution study was carried out for all batches of Itopride hydrochlroride floating microcapsules in 0.1N HCl dissolution medium at 37±0.5℃ and 75rpm. Cumulative % drug release for F-1 to F-6 was obtained by plotting %cumulative drug release versus time (h) shown in Figure 2 and Table 4. % CDR for F-1 was found to be 99.23% in 16h, due to less amount of polymer. However formulation F-2 and F-3 shows 98.43% and 97.58% drug release respectively in 18h. Prolongation of drug release to 18h due to use of combination of ethyl cellulose and eudragit RSPO 100. Eudragit RSPO 100 able to retard drug release for longer duration compare to ethyl cellulose. % Cumulative drug release for F-4, F-5 and F- 6 was found to be 95.17%, 98.99% and 90.9% respectively for 24h. Increase in proportion of polymers causes decrease in drug release for longer duration. F-5 formulation shows optimum drug release in 24h, so combinbination of polymes shows better results.

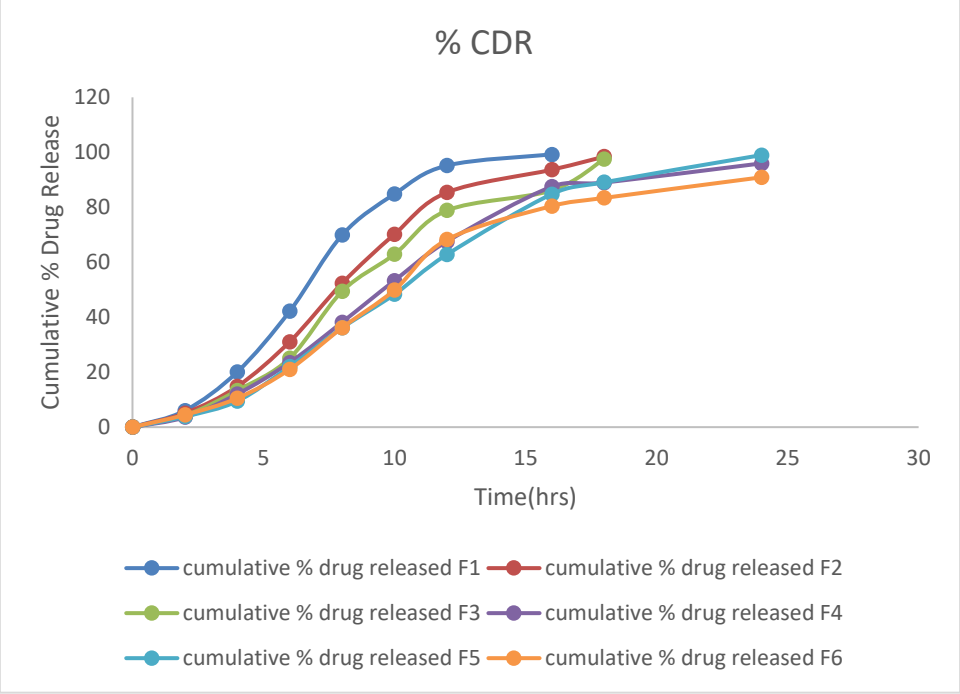


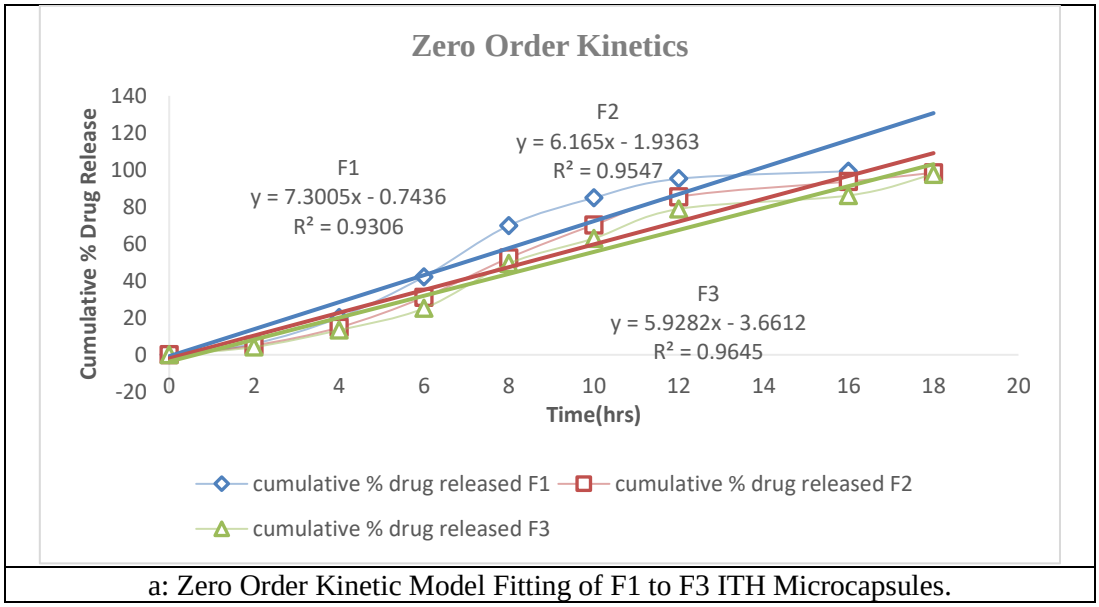
Figure 2. %CDR profile of Itopride hydrochloride microcapsules F1 to F6 Formulations

Table 4: % CDR of Itopride hydrochloride microcapsules for F1 to F6 Formulations.

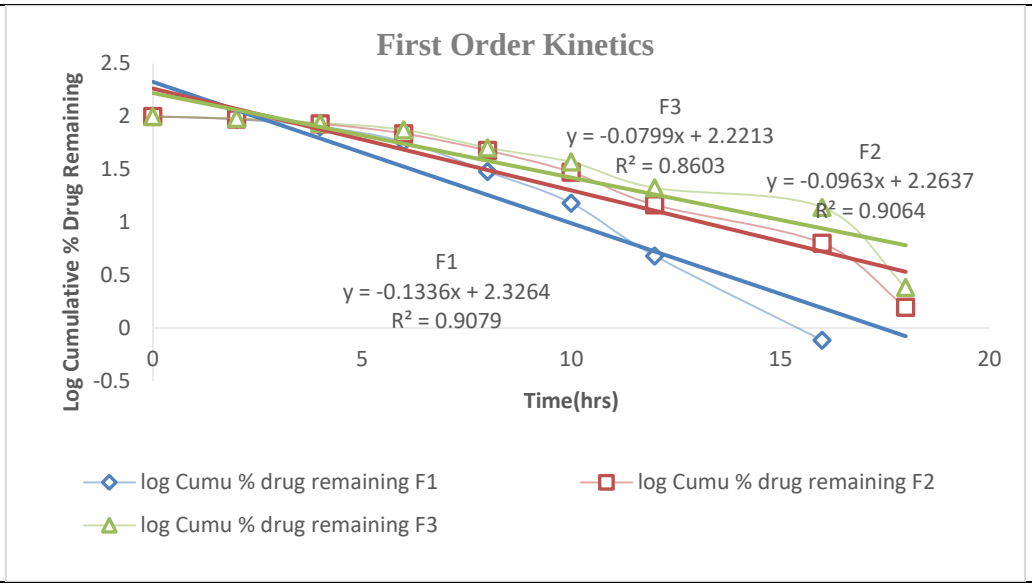
Time (h)	cumulative % drug released F1	cumulative % drug released F2	cumulative % drug released F3	cumulative % drug released F4	cumulative % drug released F5	cumulative % drug released F6
0	0	0	0	0	0	0
2	6.02±2.10	5.1±0.77	4.22±2.21	3.61±2.09	3.86±0.88	4.48±1.85
4	20.11±0.57	14.82±1.65	13.36±1.13	12.04±1.42	9.47±1.18	10.52±2.50
6	42.19±2.16	31.09±2.12	25.1±2.60	23.51±1.89	22.05±1.06	21.04±1.74
8	69.9± 1.87	52.37±1.42	49.41±1.19	38.18±1.50	36.13±3.01	36.18±1.92
10	84.84±1.17	70.22±1.86	62.93±1.12	53.34±0.75	48.34±1.45	49.91±0.99
12	95.19±0.84	85.41±1.11	78.85±0.67	67.55±0.99	62.81±2.04	68.25±1.31
16	99.23±1.90	93.67±2.15	86.14±1.01	87.49±2.35	84.78±1.62	80.45±1.05
18	--	98.43±1.08	97.58±0.58	88.97±1.25	89.11±1.18	83.41±1.77
24	--	--	--	95.97±1.53	98.99±1.90	90.9±1.90

***In-vitro* drug release kinetic models**

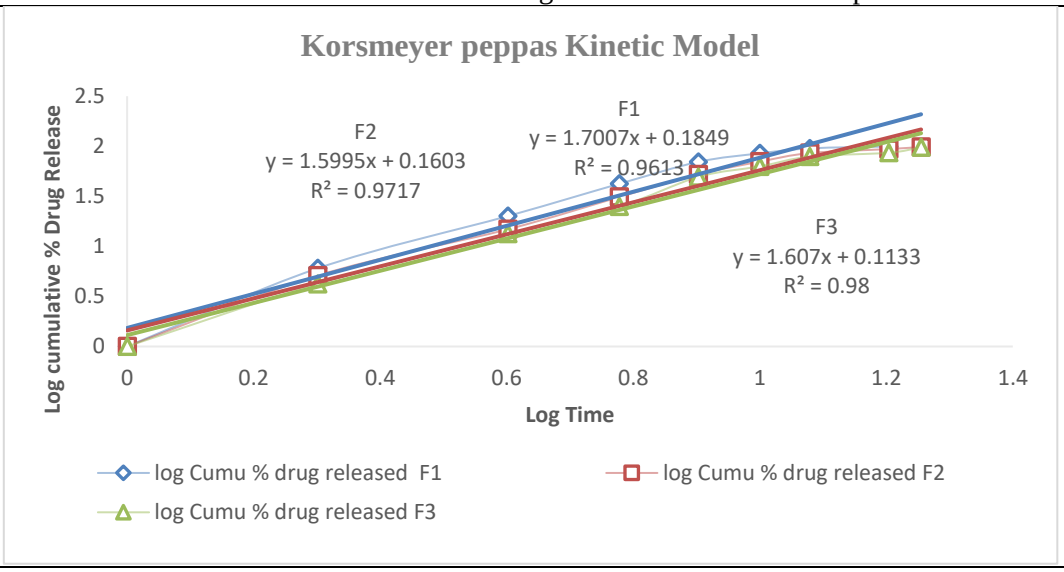
Data obtained from *in-vitro* dissolution study was fitted to different drug release mathematical kinetic models such as zero order, first order, Korsmeyer-Peppas, Higuchi and Hixson Crowell model. Kinetic model fitting was carried out to understand mechanism of drug release through dosage form. The results of model fitting were shows in Figure 3, 4 and Table 5. The drug release from formulations F-2 to F-6 floating microcapsules follow Korsmeyer-Peppas kinetic model with R^2 value close to one. This model obtained by plotting log cumulative % drug release versus log time. Only F-1 formulation follow Hixson Crowell kinetic diffusion model with R^2= 0.9656.



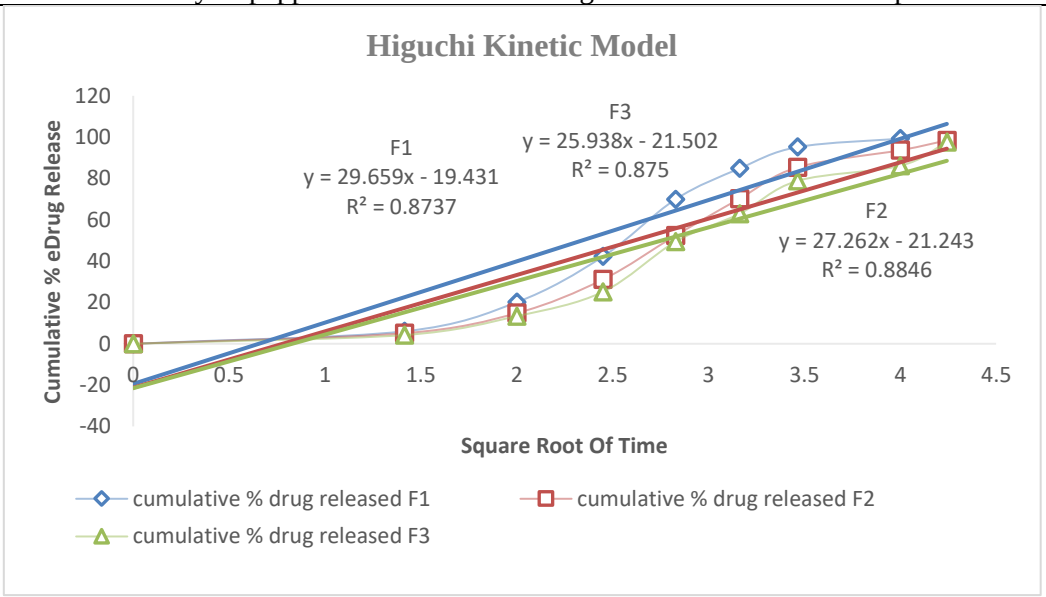
a: Zero Order Kinetic Model Fitting of F1 to F3 ITH Microcapsules.



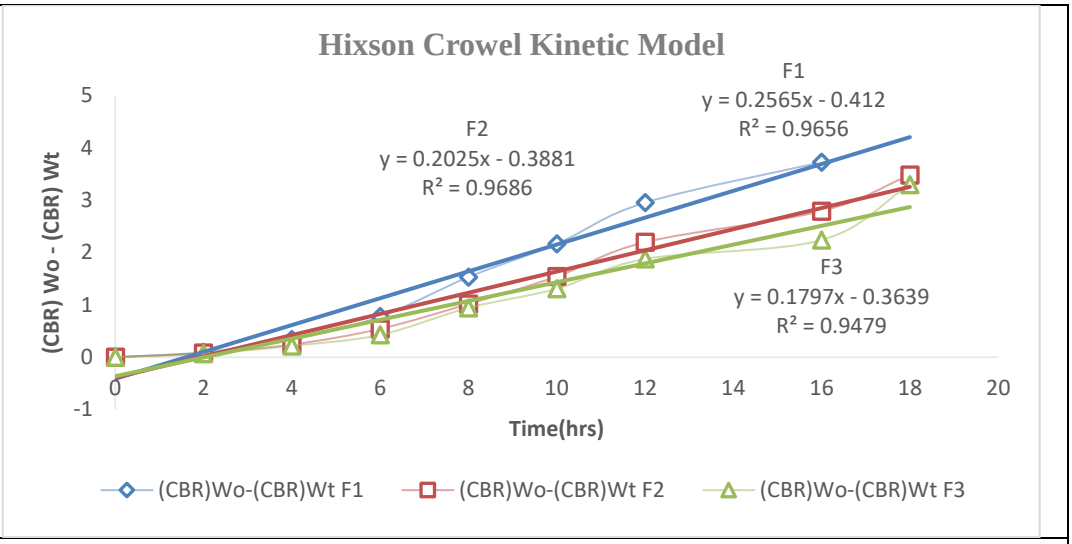
b: First Order Kinetic Model Fitting of F1 to F3 ITH Microcapsules.



c: Korsmeyer- peppas Kinetic Model Fitting of F1 to F3 ITH Microcapsules.

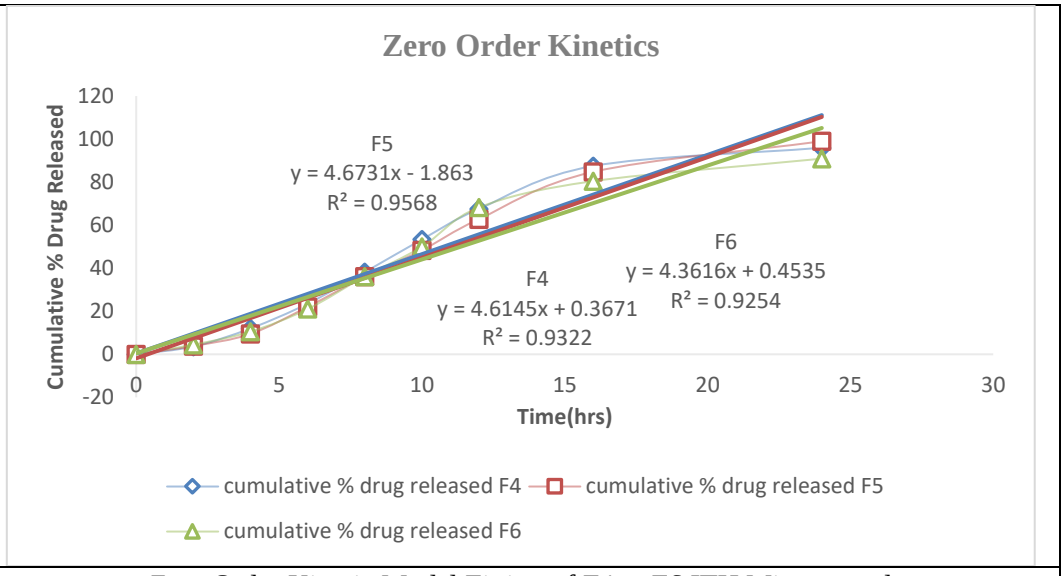


d: Higuchi Kinetic Model Fitting of F1 to F3 ITH Microcapsules.

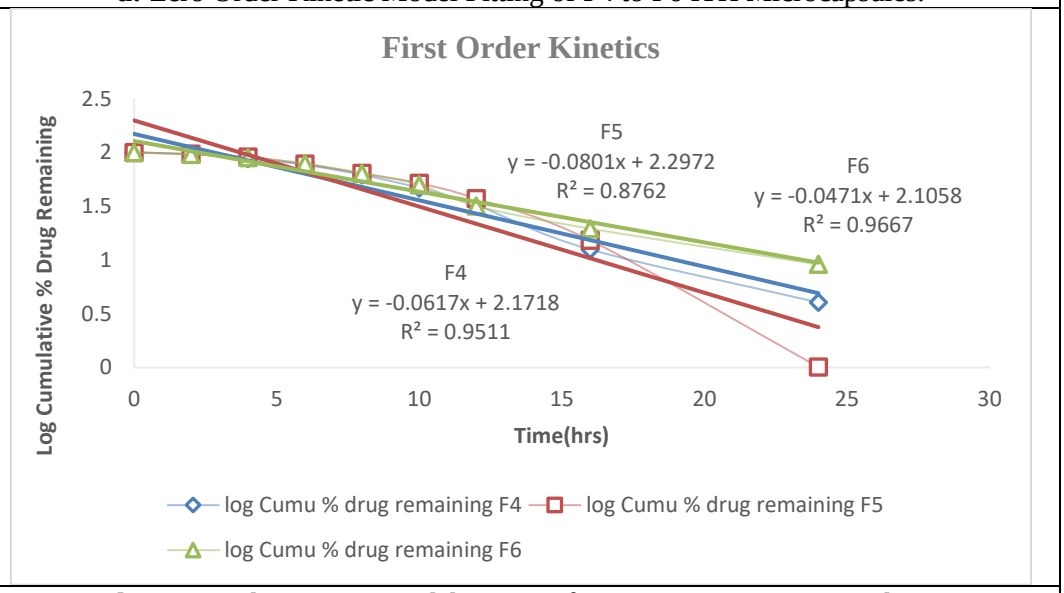


e: Hixson Crowell Kinetic Model Fitting of F1 to F3 ITH Microcapsules.

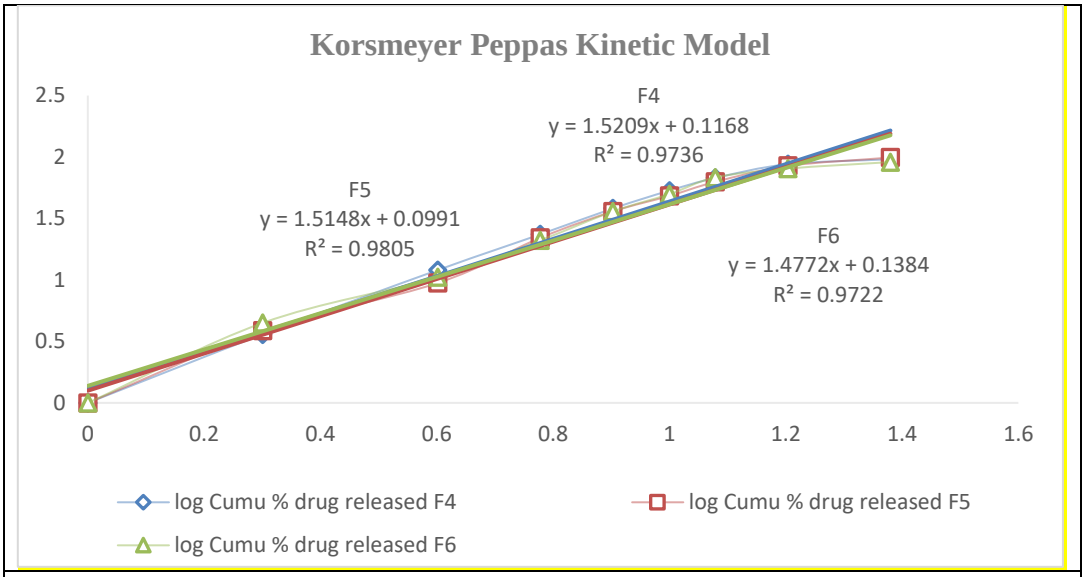
Figure 3. Different kinetic model fitting of Itopride hydrochloride loaded microcapsules for F1to F3.



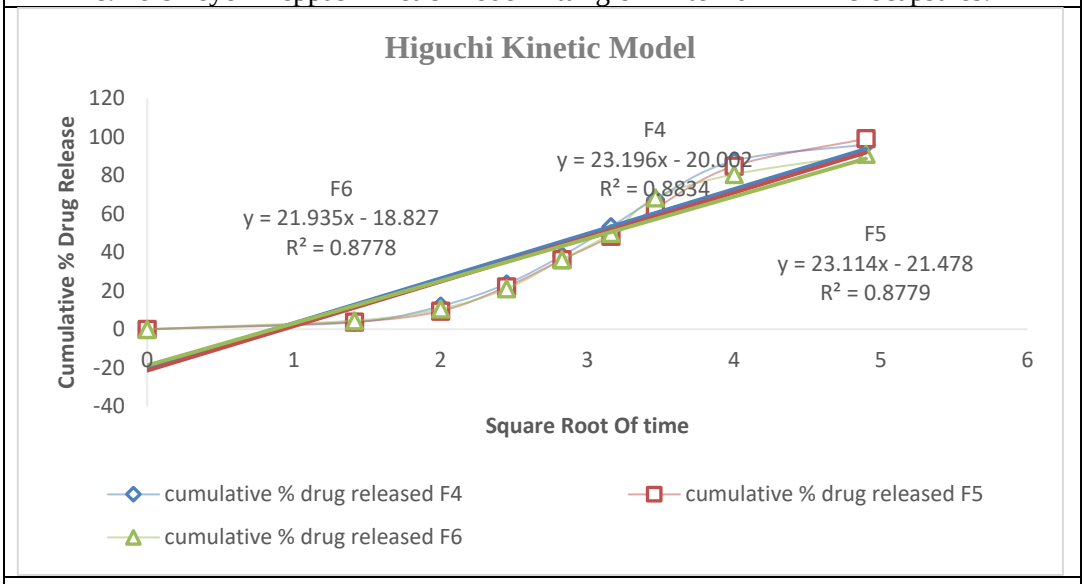
a: Zero Order Kinetic Model Fitting of F4 to F6 ITH Microcapsules.



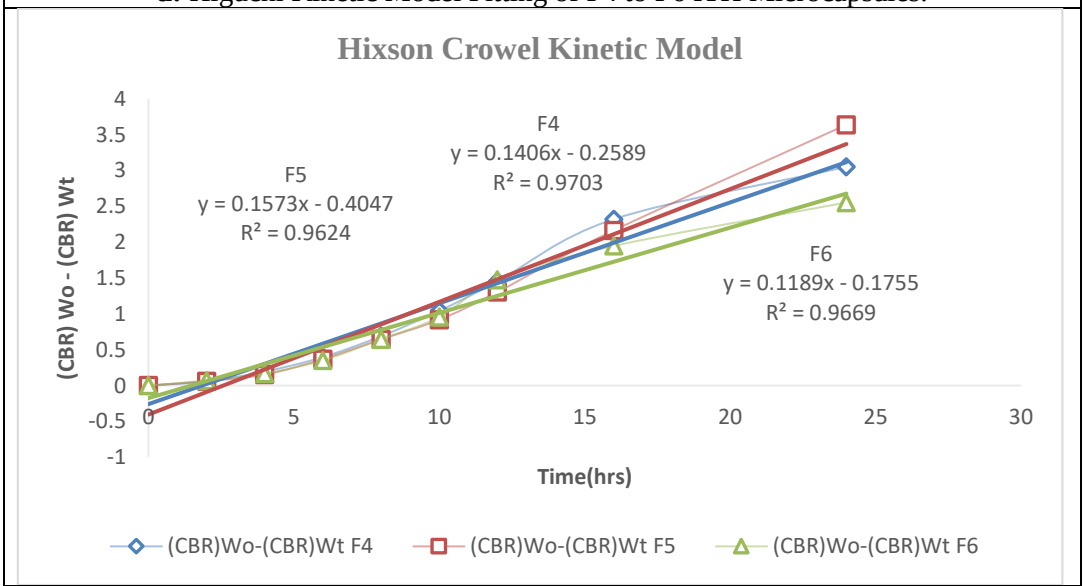
b: First Order Kinetic Model Fitting of F4 to F6 ITH Microcapsules.



c:Korsmeyer- Peppas Kinetic Model Fitting of F4 to F6 ITH Microcapsules.



d: Higuchi Kinetic Model Fitting of F4 to F6 ITH Microcapsules.



e:Hixson Crowell Kinetic Model Fitting of F4 to F6 ITH Microcapsules.

Figure 4. Different kinetic model fitting of Itopride hydrochloride loaded microcapsules for F4to F6.

Table 5: Different kinetic model fitting of Itopride hydrochloride loaded microcapsules for F1 to F6.

Formulation	Zero order (R^2)	First order (R^2)	Korsmeyer – Peppas (R^2)	Higuchi (R^2)	Hixson Crowell (R^2)
F-1	0.9306	0.9079	0.9613	0.8737	0.9656
F-2	0.9547	0.9064	0.9717	0.8846	0.9686
F-3	0.9645	0.8603	0.98	0.875	0.9479
F-4	0.9322	0.9511	0.9736	0.8834	0.9703
F-5	0.9568	0.8762	0.9805	0.8779	0.9624
F-6	0.9254	0.9667	0.9722	0.8778	0.9669

Scanning Electron Microscopy (SEM)

Morphology of Itopride Hydrochloride and microcapsules of all formulations were analysed by SEM. SEM images of Itopride Hydrochloride revealed existence of needle like crystals with broad particle size distribution (Figure 5. a). The structural and surface morphology of all formulations (F1-F6) showed the prepared microcapsules were spherical and nearly spherical in shape and had rough surface (Figure 5.b-g). If the W/O primary emulsion was stable until solidification of polymer, the inside of microcapsules would be filled with W/O emulsion droplets and polymer would solidify around emulsion droplets. When the stability of W/O was poor then coalescence of emulsion droplet occurred, prior to the solidification of polymers leads to the formation of pores on the surface of microcapsules (Figure 5. h and i).

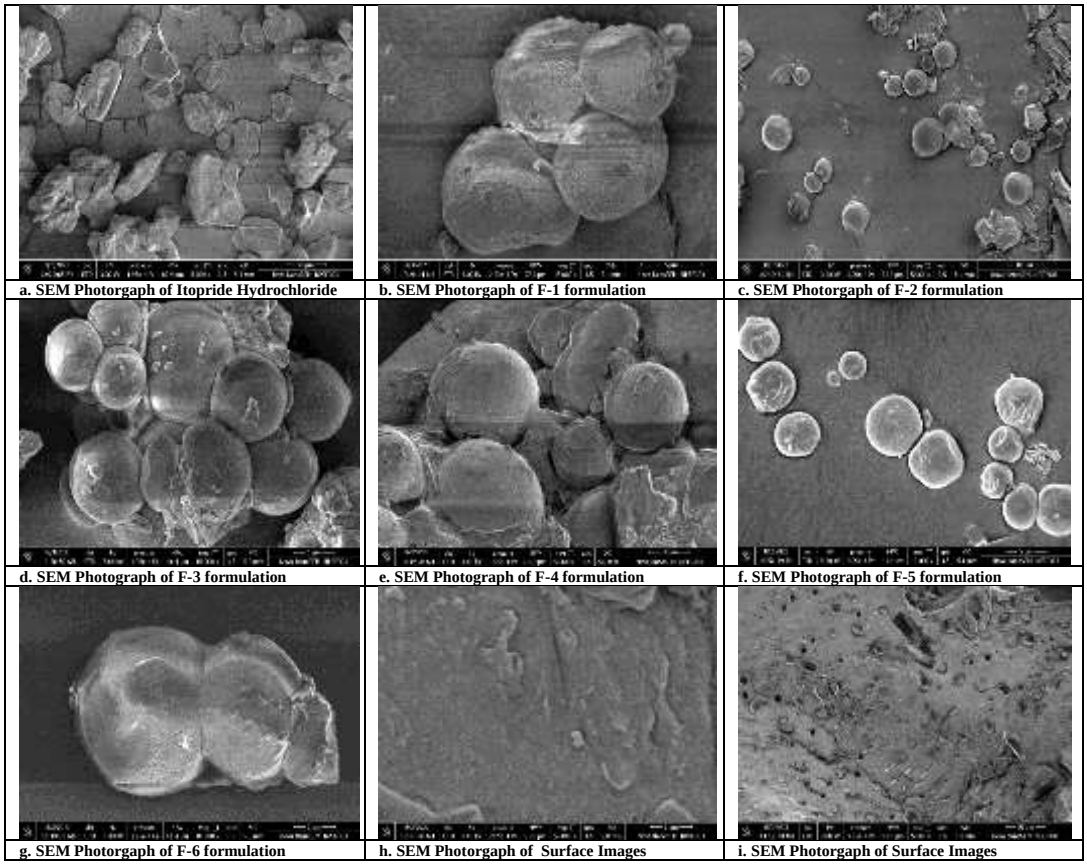


Figure 5.SEM Photograph of Itopride hydrochloride, Formulation F-1 to F-6 and surface images.

FTIR Spectroscopy

FTIR Spectra of Itopride Hydrochloride, physical mixture of drug and polymers and Itopride Hydrochloride loaded microcapsules of F5 formulation were recorded. FTIR spectrum of Itopride Hydrochloride showed prominent peaks such as, C-N (amine) at 1003.59 cm^{-1}, N-H

stretching (amide) at 3277.12 cm^{-1}, C=O (amide) at 1637.98cm^{-1}, C=C stretching (alkene aromatic) at 1503.12 cm^{-1} and C-O-C asymmetrical ether stretching (alkyl) at 1225.05cm^{-1} (Figure 6 and Table 6).

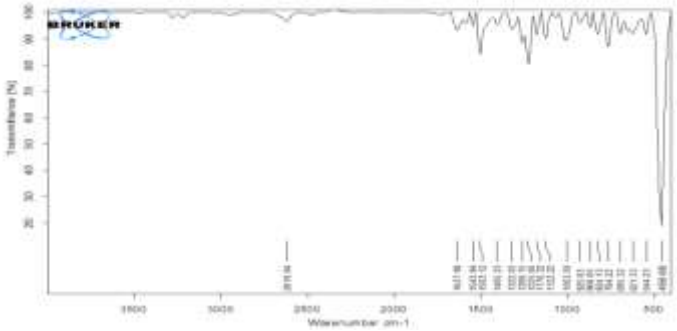


Figure 6. FTIR Spectra of Itopride Hydrochloride.

Table 6: Interpretation of FTIR spectra of Itopride hydrochloride, physical mixture and F 5 formulation.

Bond	Types of Compound	Frequency Range (cm^{-1})	Observed Peak (cm^{-1}) Drug	Observed Peak (cm^{-1}) Physical Mixture	Observed Peak (cm^{-1}) F 5 Formulation
C-N	Amine	1350-1000	1003.59	1121.08	1056.37
N-H	Amide (Stretching)	3500-3100	3277.12	3277.21	--
C=O	Amide	1680-1630	1637.98	1637.77	1638.12
C-H	Alkane (Stretching)	3000-2850	-	2971.67	2973.80
C=C	Alkene (Aromatic Stretching)	1600-1500	1503.12	1502.77	1545.68
C-O-C	Asymmetrical ether stretching	1300-1050	1225.05	1226.10	1231.44

FTIR spectra of physical mixture and microcapsules of F5 batch showed some of characteristic peaks of Itopride Hydrochloride such as C-N(amine), C=O(amide), C-H (alkane), C=C (aromatic alkenes) and C-O-C (asymmetric ether stretching) in the range1350-1000, 1680-1630, 3000-2850,1600-1500 and 1300-1050 respectively (as shown in Figure 7 and 8, Table 6). Hence it confirms that there was no interaction occurred between drug and components of the formulations. FTIR spectroscopic studies revealed that Itopride Hydrochloride was compatible with formulation ingredients (ethyl cellulose, eudragit RSPO 100).

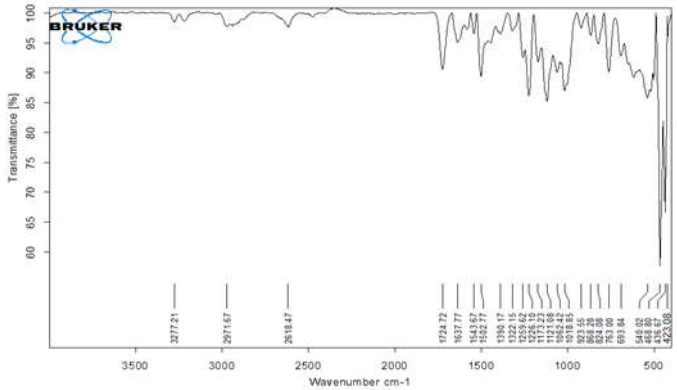


Figure 7. FTIR Spectra of Physical mixture.

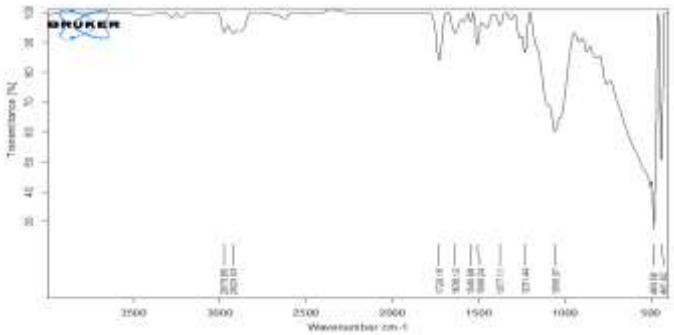


Figure 8. FTIR Spectra of F-5 formulation.

Differential Scanning Colorimetry (DSC)

DSC thermogram of pure Itopride hydrochloride, physical mixture and F-5 formulation is shown in Figure 9,10,11. DSC thermogram of ITH shows sharp endothermic peak at 193.95^{0}C, however physical mixture and F-5 formulation shows endothermic peak at 193.97^{0}C and 171.36^{0}C respectively. Slight shift and reduction in intensity of peak may be due to dispersion of drug in polymer mixture and encapsulation of drug in polymer coat. This does not indicate any chemical change or interaction between drug and polymers.

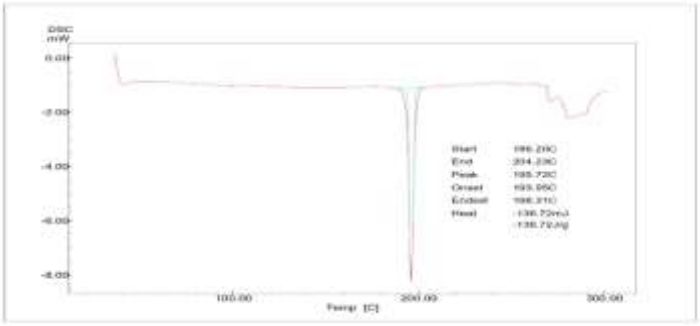


Figure 9. DSC thermogram of Itopride hydrochloride.

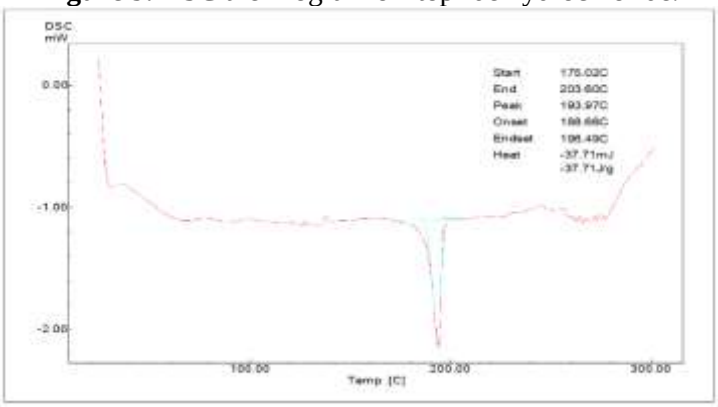


Figure 10. DSC thermogram of physical mixture.

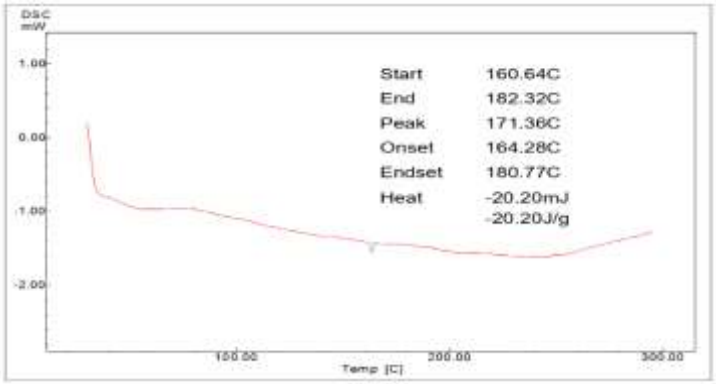


Figure 11. DSC thermogram of F 5.

CONCLUSION

Itopride hydrochloride was successfully loaded in microcapsules by W/O/O multiple emulsion solvent diffusion method. Optimized formulation shows excellent floating behaviour, entrapment efficiency and desired drug release pattern. Formulation remained floated in gastric fluid for longer duration. Ethyl cellulose and eudragit RSPO 100 polymers able to encapsulate drug within polymer coat. Polymer coat controls drug release for extended period of time.

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