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RESEARCH ARTICLE

QbD-enabled systematic development of gastroretentive multiple-unit microballoons of itopride hydrochloride

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Abstract

The objectives of present studies were to develop the systematically optimized multiple-unit gastroretentive microballoons, i.e. hollow microspheres of itopride hydrochloride (ITH) employing quality by design (QbD)-based approach. Initially, the patient-centric QTPP and CQAs were earmarked, and preliminary studies were conducted to screen the suitable polymer, solvent, solvent ratio, pH and temperature conditions. Microspheres were prepared by non-aqueous solvent evaporation method employing Eudragit S-100. Risk assessment studies carried out by constructing Ishikawa cause-effect fish-bone diagram, and techniques like risk estimation matrix (REM) and failure mode effect analysis (FMEA) facilitated the selection of plausible factors affecting the drug product CQAs, i.e. percent yield, entrapment efficiency (EE) and percent buoyancy. A 3³ Box-Behnken design (BBD) was employed for optimizing CMAs and CPPs selected during factor screening studies employing Taguchi design, i.e. drug-polymer ratio (X_1), stirring temperature (X_2) and stirring speed (X_3). The hollow microspheres, as per BBD, were evaluated for EE, particle size and drug release characteristics. The optimum formulation was embarked upon using numerical desirability function yielding excellent floatation characteristics along with adequate drug release control. Drug-excipient compatibility studies employing FT-IR, DSC and powder XRD revealed absence of significant interaction among the formulation excipients. The SEM studies on the optimized formulation showed hollow and spherical nature of the prepared microspheres. *In vivo* X-ray imaging studies in rabbits confirmed the buoyant nature of the hollow microspheres for 8 h in the upper GI tract. In a nutshell, the current investigations report the successful development of gastroretentive floating microspheres for once-a-day administration of ITH.

Keywords

Formulation by design (FbD), gastroretention, hollow microspheres, hydrodynamically balanced, quality by design (QbD)

History

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Introduction

Since decades, oral intake has been considered as the most preferred route of drug administration owing to its numerous advantages including flexibility in formulation development and improved patient compliance (Gupta et al., 2009; Singh et al., 2012). Alleviating the chronic ailments, invariably calls for the development of oral controlled release (CR) products, especially for highly soluble and permeable BCS class I drugs. The major hiccups encountered during the development of the conventional CR systems is inability to retain and localize the drug delivery system (DDS) within the desired absorptive region of gastrointestinal (GI) tract, invariably

resulting in inadequate extension in therapeutic response and incomplete drug absorption.

Gastroretentive drug delivery systems (GRDDS), in this regard, complement the oral CR formulations by continuously releasing the drug at a controlled rate for a prolonged period through the “drug absorption window” ensuring optimal extent of bioavailability too (Kotreka & Adeyeye, 2011; Pahwa et al., 2012). Among the numerous approaches used to improve the gastric residence time of DDS, the vital ones include, single and multiple-unit floating systems, bioadhesive systems, swelling and expanding systems, raft forming systems and high-density systems (Murphy et al., 2009). With a bulk density less than that of gastric fluids, a floating DDS remains buoyant in the stomach for a prolonged period of time without reducing the gastric emptying rate (Ichikawa et al., 1991). While the system floats on the gastric contents, the drug gets released slowly at the desired rate from the system, resulting in an increased gastroretention time and offers better control over the fluctuations in plasma drug levels (Kawashima et al., 1991). Multiple-unit floating

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systems have added advantages over the single-unit systems not only owing to their efficient drug release characteristics for extended periods of time, but also for reduced inter-subject and/or intra-subject variability in GI absorption (Pawar et al., 2010). Of late, the hollow microspheres, popularly termed as microballoons, have been increasingly employed for improvement in residence time of drug in the GI tract as well as its oral bioavailability (Sato et al., 2004a; Awasthi et al., 2012). With size less than 200 μm , these low-density non-effervescent microballoons have demonstrated sufficient buoyancy to retain drug in the gastric contents for prolonged periods of time (Sato et al., 2004b,c).

Itopride Hydrochloride (ITH) is a prokinetic agent used for the treatment of functional dyspepsia and other GI disorders like gastric fullness, anorexia, upper abdominal pain, chronic gastritis and non-ulcer dyspepsia. It exhibits shorter half-life (i.e. <6 h) and narrow absorption window in the stomach and upper part of the small intestine, leading to incomplete drug absorption and faster drug elimination, eventually to sub-optimal plasma drug levels (Gupta et al., 2004). Owing to the inability of conventional CR formulations to accomplish desired drug release profile within the absorption window, it was planned to develop the GR microballoons of ITH for sustained release profile of drug delivery with improved therapeutic efficacy.

Development of GR systems involves a number of formulation and process variables like, type of polymer, concentration of polymer, selection of apt method of preparation, concentration of crosslinking agent and stirring speed (Singh et al., 2005a; Vora et al., 2013). Optimizing the formulation composition and the manufacturing process(es) of such DDS using traditional one-factor-at-a-time (OFAT) is a Herculean task, resulting in just workable solutions with expenditure of a great deal of time, money and effort (Singh et al., 2005b, 2013). Systematic optimization of DDS employing Quality by Design (QbD)-based approach of Formulation by Design (FbD), in this regard, has been lately practiced to provide comprehensive understanding of the formulation system by identifying plausible interaction(s) among the product and/or process-related factors to produce “the best” possible formulation under a given set of conditions with minimal experimentation and utilization of resources (Singh et al., 2011). FbD methodology involves defining the quality target product profile (QTPP), and critical quality attributes (CQAs), identification of critical material attributes (CMAs) and critical process parameters (CPPs) by screening and risk assessment, data analysis using design of experiments (DoE), modelization and optimum search through response surface methodology (RSM) to embark upon the design space and postulation of control strategy for continuous improvement (Lionberger et al., 2008). The FbD paradigm has yielded higher fruition in developing the optimized GRDDS of diverse kinds from our laboratories, including diltiazem (Singh & Ahuja, 2002), atenolol (Singh et al., 2006), hydralazine (Singh et al., 2009), tramadol (Singh et al., 2010), lamivudine (Singh et al., 2012) and rivastigmine (Kapil et al., 2012, 2013).

Attempts were, therefore, made to prepare the FbD-optimized GR floating microballoons of ITH using Eudragit

S-100 as the release controlling polymer, and their subsequent *in vitro* evaluation for entrapment efficiency (EE), particle size, buoyancy and drug release characteristics, and *in vivo* evaluation of GR performance in rabbits through X-ray imaging.

Materials and methods

Materials

ITH was provided *ex-gratis* by M/s Cadila Pharmaceuticals Ltd, Jammu, India. Eudragit S-100 was received as a gift sample from M/s Evonik, Mumbai, India. High-density liquid paraffin (HLP) and petroleum ether were purchased from M/s Merck, Mumbai, India. All other chemicals, solvents and reagents used were of analytical grade and were used as obtained.

Methods

Defining the QTPP and CQAs

As the first step towards QbD-based product development for GR microballoons of ITH, the patient-centric QTPP was defined encompassing the summary of quality characteristics of the drug product to achieve prolonged release profile of drug delivery. In order to meet the QTPP, various quality attributes (QAs) were identified *viz.* EE (indicative of drug loading in the hollow microspheres), particle size (imperative for discerning the drug release and absorption potential through GI tract), percent drug release in 18 h (i.e. Q_{18h}) and time required for 60% drug release (i.e. $T_{60\%}$) (marker of drug release from microballoons). Various elements of QTPP for development of GR floating microballoons of ITH have been summarized in Table 1, while Table 2 enlists the respective justification(s) of selecting each CQA.

Initial risk assessment studies

The risk assessment studies were carried out to identify the CMAs and/or CPPs for GR microballoons plausibly affecting the CQAs of drug product. Ishikawa fish-bone diagram was constructed to establish the potential cause-effect relationship among the product and process variables employing Minitab 17 software (M/s Minitab Inc., Philadelphia, PA). Prioritization studies were carried out for selecting the CMAs/CPPs with high risk by constructing the Risk Estimation Matrix (REM) for qualitative analysis of risk by assigning low, medium and high-risk(s) levels to the each material attribute (MA) and/or process parameter (PP) (Fahmy et al., 2012; Vora et al., 2013). Further, the Failure Mode and Effect Analysis (FMEA) was carried out for quantitative estimation of risk(s) and to detect the plausibility of failure modes associated with drug product, if any. Rank order scores, ranging between 1 and 10 each, were assigned to the CMAs for severity, detectability and occurrence of risk(s), to calculate the risk priority number (RPN) as per the formula given in Equation (1) (Fahmy et al., 2012). The factors associated with high RPN were finally subjected to factor screening studies employing Taguchi design.

$$\text{RPN} = \text{Severity}(S) \times \text{Occurrence}(O) \times \text{Detectability}(D).$$

(1)

Table 1. Quality target product profile (QTPP) for GR hollow microballoons of ITH.

QTPP elements	Target	Justification
Dosage form type	Gastroretentive system	Helps in maintaining the therapeutic effect of drug for prolonged periods of time by retaining the formulation in GIT for extended time periods
Drug delivery type	Microballoons	Selection of GR floating microballoons help in enhancing the residence time of drug formulation in stomach and upper GIT leading to complete absorption of drug within its absorption window
Route of administration	Oral	Recommended route for delivery of ITH is oral and the available marketed formulations (i.e. tablets) are also meant for oral intake only
Dosage strength	150 mg	It is the unit dose of ITH which needs to be incorporated for once-a-daily administration
Packaging	Hard gelatine capsules	The microballoons can easily be delivered by filling in hard gelatin capsules with improved patient compliance, portability and manufacturing ease
Stability	At least 24 months at room temperature	To maintain therapeutic potential of the drug during storage period

GI, gastrointestinal; GR, gastroretentive; ITH, itopride hydrochloride.

Table 2. Critical quality attributes (CQAs) for GR hollow microballoons of ITH and their justifications.

Quality attributes of the drug product	Target	Is this a CQA?	Justification
Physical attributes			
Color	Acceptable to patients	No	Color, odor and appearance were not considered as critical, as these are not directly linked to patient efficacy and safety.
Odor	No unpleasant order		
Appearance	Acceptable to patients		
Drug content	100%	No	Drug content is a vital parameter for any pharmaceutical dosage form for attaining maximal plasma concentration of the drug. Unlike tablets, microballoons are not the unit dose formulations, thus it was regarded as moderately critical.
Percent buoyancy	100%	No	Higher value of percent buoyancy is required for longer residence time of the drug formulation in the gastric region. As the developed GR microballoons were hollow in nature having inherent ability to completely float upto 24 h, hence it was taken up as less critical.
Particle size	Low	Yes	As the microballoons are administered through oral route, the particle size was thought to exert significant influence in attaining prolonged gastric retention of the drug formulation, and thus its therapeutic performance. Hence, it was considered as critical.
Entrapment efficiency	100%	Yes	Higher values of entrapment efficiency is vital for accomplishing maximal drug release regulation from the dosage form and hence the therapeutic concentration of the drug. Thus, it was considered as critical.
Time required for 60% drug release ($T_{60\%}$)	~8 h	Yes	This parameter is an indicator of sustained release profile of drug release from the prepared microballoon formulations, thus was taken up as highly critical.
Cumulative amount of drug release in 18 h (Q_{18h})	~75.0%	Yes	

Preparation of GR microballoons

The microballoons of ITH were prepared by non-aqueous solvent evaporation method (Phutane et al., 2010). Briefly, the drug (20–100 mg) was dissolved in aqueous methanolic solution (1:4) and the polymer, i.e. Eudragit S-100 (0.1 g) was dissolved in acetone (10 mL) to form a clear solution. Both the phases were mixed with each other under stirring condition. Finally, the mixture containing the blend of drug and polymer were poured in 50 mL of HLP, maintained at 28 °C with continuous stirring at 1000 rpm for 2 h to allow complete evaporation of solvents. The microballoons formed were collected by filtration, washed with petroleum ether (40–60 °C), and subsequently dried overnight in oven at 40 °C. Each formulation was prepared in triplicate. The hollow microspheres, thus obtained, were packed in a glass vial and stored in desiccator. During all these trials,

the composition of the mixture of organic solvent (i.e. acetone) to aqueous methanol was kept as constant, in the ratio of 20:1 v/v.

Factor screening studies

The seven-factor eight-run Taguchi design was used for factor screening studies to identify the effect of each MAs and/or PPs on the CQAs of GR hollow microspheres of ITH. Table 3 illustrates the Taguchi design matrix selected for the preparation of GR microballoons along with their respective low and high levels. A total of eight trial formulations were thus prepared as per the screening design, and evaluated for percent yield, EE and percent buoyancy, respectively. Model was fitted using linear polynomial equations, and coefficients for each factors was evaluated corresponding to the CQAs of drug product by obviating the interaction term(s). The Pareto

Table 3. Formulation and process variables with their respective high and low levels investigated employing Taguchi design.

Runs	Drug: polymer ratio	Stirring temp.	Stirring speed	Vol of HLP	Vol of acetone	Vol of aq: MeOH	Stirrer type
1	+1	+1	−1	+1	−1	+1	+1
2	−1	+1	+1	−1	+1	+1	+1
3	−1	−1	−1	−1	−1	−1	+1
4	+1	−1	+1	−1	−1	+1	−1
5	+1	+1	−1	−1	+1	−1	−1
6	−1	+1	+1	+1	−1	−1	−1
7	+1	−1	+1	+1	+1	−1	+1
8	−1	−1	−1	+1	+1	+1	−1

Levels of the factors studied	Levels	
	Low (−1)	High(+1)
Drug–polymer ratio (mg)	1:1	1:5
Stirring temperature (°C)	25	35
Stirring speed (rpm)	600	1000
Volume of HLP (mL)	40	60
Volume of acetone (mL)	06	12
Volume of aqueous-MeOH (mL)	0.2	0.6
Stirrer type	Mechanical stirrer	Magnetic stirrer

Table 4. Formulation composition of GR hollow microballoons prepared as per BBD.

Code	Trial	Factor 1	Factor 2	Factor 3
TA	1	0.00	0.00	0.00
TB	2	0.00	1.00	1.00
TC	3	0.00	−1.00	1.00
TD	4	1.00	0.00	−1.00
TE	5	1.00	−1.00	0.00
TF	6	−1.00	−1.00	0.00
TG	7	1.00	0.00	1.00
TH	8	0.00	0.00	0.00
TI	9	−1.00	0.00	1.00
TJ	10	0.00	1.00	−1.00
TK	11	0.00	0.00	0.00
TL	12	0.00	0.00	0.00
TM	13	0.00	−1.00	−1.00
TN	14	−1.00	0.00	−1.00
TO	15	0.00	0.00	0.00
TP	16	−1.00	1.00	0.00
TQ	17	1.00	1.00	0.00

Translation of coded factors into physical units

Factors	Coded levels		
	−1	0	1
Drug: Polymer ratio	1:3	1:3.5	1:4
Stirring temperature (°C)	25	30	35
Stirring speed (rpm)	600	800	1000

charts were used for quantitatively identifying the effect of each MAs/PPs on the selected CQAs for screening.

Optimization of GR microballoons using experimental design

Systematic optimization of GR microballoons was accomplished employing Box-Behnken Design (BBD) using the highly influential CMA/CPPs selected using the factor screening and risk assessment studies. Table 4 illustrates the design matrix as per BBD containing a total of 17 different formulations prepared employing drug–polymer ratio (X_1), temperature (X_2) and stirring speed (X_3) as the CMA/CPPs at three different levels, i.e. low (−1), intermediate (0) and high

(+1) levels, including quintuplicate studies at the center point (0,0,0) formulations. All the prepared formulations were evaluated for various CQAs viz. EE, particle size, Q_{18h} and $T_{60\%}$, respectively.

Characterization of the GR microballoon formulations

Percent yield. Percent yield was calculated from the weight of dried microballoons recovered from each of the formulations and the sum of initial dry weight of starting materials as stated in Equation (2).

$$\text{Percent yield} = \frac{\text{Actual weight of microspheres}}{\text{Total weight of drug and excipients}} \times 100. \quad (2)$$

Entrapment efficiency. An accurately weighed amount of microballoons, equivalent to 10 mg of drug, was dissolved in 5.0 mL of methanol and the volume was made upto 100 mL with 0.1 N HCl. The solution was macerated to facilitate complete extraction of the drug by evaporating methanol. The solution was filtered through Whatman filter paper, diluted suitably and analyzed spectrophotometrically at 258 nm using UV–Visible Spectrophotometer 3000⁺ (M/s Labindia Instruments Pvt. Ltd., Mumbai, India) (Gupta et al., 2010). Entrapment efficiency (%) was calculated as per the formula mentioned in Equation (3).

$$\text{Entrapment efficiency (\%)} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug}} \times 100. \quad (3)$$

In vitro buoyancy studies. The floating characteristics of the hollow microspheres were evaluated by *in vitro* buoyancy studies in simulated gastric fluid (SGF, pH 1.2) without pepsin. An accurately weighed amount of microballoons were filled in size 0 hard gelatin capsules. These capsules were transferred to the dissolution medium (SGF pH 1.2, 900 mL)

taken in USP Type II dissolution apparatus kept at $37 \pm 0.5^\circ\text{C}$ and 50 rpm. At specified periodic intervals during 24 h dissolution run, the floating and sedimented portions of the microballoons were collected, dried and weighed separately to calculate the percent buoyancy using Equation (4).

$$\text{Percent buoyancy} = \frac{\text{Weight of hollow microspheres}}{\text{Total weight of microspheres}} \times 100. \quad (4)$$

In vitro drug release studies. *In vitro* drug release studies on the GR microballoons were carried out in triplicate using the USP Type II apparatus in 900 mL of SGF (pH 1.2) maintained at $37 \pm 0.5^\circ\text{C}$ and 50 ± 2.0 rpm under sink conditions. Accurately weighed amount of microballoons containing dose equivalent to 100 mg of ITH were filled in size 0 hard gelatin capsules and placed under dissolution conditions. Aliquot of samples (5 mL) were withdrawn periodically at suitable time intervals and replenished with an equivalent volume of plain dissolution medium. Samples were analyzed spectrophotometrically at 258 nm employing a UV-Vis spectrophotometer 3000⁺ (M/s Labindia Instruments Pvt. Ltd., Mumbai, India). The raw data obtained from *in vitro* drug release studies were analyzed using ZOREL software. The software has the in-built provisions for applying the correction factor for volume and drug losses during sampling (Equation (5)), and calculating the values of amount of drug dissolved, percent release, rate of drug release and log fraction released at varied times (Singh et al., 1997; Singh & Singh, 1998).

$$C_i = A_i \frac{V_s}{V_t} \times \sum_{i=1}^{n-1} A_i \left[\frac{V_t}{V_s - V_t} \right] \quad (5)$$

where, C_i = corrected absorbance; A_i = absorbance of i th reading; V_s = sample volume; V_t = total volume of dissolution medium. The values of percent of drug released in 18 h (i.e. Q_{18h}) were computed from the drug release profile and the time taken to release 60% of drug (i.e. $T_{60\%}$) for all the formulations was computed using Stineman interpolation option of Minitab 17 software (M/s Minitab Inc., Philadelphia, PA). Drug release data were fitted into Korsmeyer model for non-swellable compressed matrices, as described in Equation (6) (Korsmeyer et al., 1983).

$$\frac{M_t}{M_\infty} = Kt^n \quad (6)$$

where, M_t is amount of drug released at time “ t ”, M_∞ is amount of drug released at an infinite time, K is the kinetic constant and n is the Fickian diffusion coefficient.

Particle size analysis. Accurately weighed quantities (0.1 g) of microballoons were dispersed in 0.1 N HCl containing 0.02 %w/v SLS with stirring and ultrasonication. Average particle size and particle size distribution of microballoons were determined using Mastersizer 2000 (M/s Malvern Instruments Ltd., Worcestershire, UK).

Evaluation of micromeritic properties

The microballoons were characterized for various micromeritic parameters *viz.* angle of repose, bulk and tapped

density, Carr's Index (CI) and Hausner's Ratio (H). The angle of repose was measured by funnel method and calculated by using the Equation (7).

$$\tan \theta = \frac{2h}{d} \quad \text{or} \quad \theta = \tan^{-1} \frac{2h}{d} \quad (7)$$

where, h and d are the height and diameter of the heap of microballoons.

For determining the bulk and tapped density, accurately weighed amount of microballoons were transferred into a 10-mL measuring cylinder, and the volume occupied was noted before and after tapping, with the help of a tapped density apparatus (M/s Electrolab, Mumbai, India). Bulk density was calculated as a ratio of mass of microballoons to bulk volume, whereas tapped density was calculated as a ratio of mass of microballoons to tapped volume for which the cylinder was tapped gently until there was no change in volume.

CI was calculated using the following formula stated in Equation (8):

$$\text{Carr's Index} = 1 - \frac{\rho_B}{\rho_T} \times 100. \quad (8)$$

H was calculated as a ratio of bulk density to tapped density.

$$\text{Hausner's ratio} = \frac{\rho_T}{\rho_B} \quad (9)$$

where, ρ_T and ρ_B represent the values of tapped density and bulk density, respectively.

Optimization data analysis and validation of QbD

The QbD optimization data analysis was carried out after evaluating prepared microsphere formulations for various CQAs like, EE, particle size, Q_{18h} and $T_{60\%}$. Subsequently, mathematical modelling was carried out employing second-order quadratic model to explore the plausibility of significant interaction(s) among the studied CMAs. Only the polynomial coefficients corresponding to each model term, which were found to be significant as per the Student's t -test was considered during framing the polynomial equation(s). One-way ANOVA was carried out by analyzing the model parameters like model p value, coefficient of correlation (r^2) and lack of fit. Response surface analysis was carried out with the help of 3D-plots for thorough understanding of the effect of each CMA and/or CPP on the CQAs. Search for optimum formulation was carried out using numerical optimization procedure of desirability function by “trading-off” of various CQAs as per the selected acceptance criteria, i.e. maximization of EE, Q_{18h} and $T_{60\%}$, and minimization of particle size.

Validation of the QbD methodology was carried out by selecting six confirmatory check-point formulations from the knowledge space region. The validation formulations were evaluated, and the observed and predicted values for each CQA were critically compared. The linear correlation plots were constructed between the observed and predicted responses, forcing the line through the origin. Further, the percent prediction error was also calculated with respect to the observed responses, and residual plots were constructed between the observed responses and percent bias.

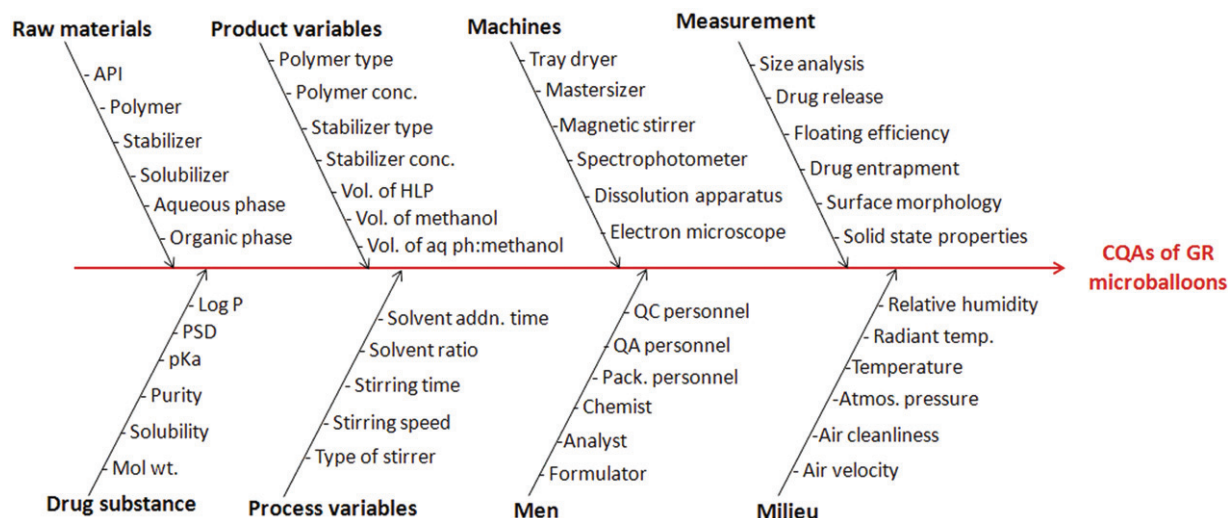


Figure 1. Ishikawa fish-bone diagram depicting the cause-and-effect relationship among the formulation and process variable for formulation of GR hollow microballoons of ITH.

Drug release comparison with marketed brand

Drug release profile of the optimized microballoons was compared with the marketed brand, Ganaton® (M/s Abbott Ltd., Mumbai, India) of ITH, as per the previously described method.

Scanning electron microscopy (SEM)

The surface morphology of optimized hollow microspheres was examined by SEM JSM 6100 (M/s JEOL, Tokyo, Japan). The dried microballoons were subjected to gold coating after mounting them on brass stubs using carbon paste and kept in a desiccator for one week. The microballoons were scanned under acceleration voltage of 10 kv and the images depicting the surface topology were obtained.

Drug-excipient compatibility studies

Fourier transform infrared (FT-IR) spectroscopy. The FT-IR spectroscopy was performed to characterize the possible interactions between the drug and excipients, if any. The FT-IR spectra of drug and polymer mixture were recorded in KBr disc over the range 4000–400 cm^{-1} using an FTIR spectrophotometer (M/s Perkin Elmer, Waltham, MA).

Differential scanning calorimetry (DSC) studies. The DSC studies were carried out to investigate the thermodynamic compatibility between physical mixture of drug and polymer selected for preparation of microballoons based on their melting temperature and glass transition temperature. The drug–polymer physical mixture was prepared by simple mixing with the help of mortar and pestle. Approximately 3–5 mg of each sample was transferred in Aluminium pan and heated at a rate of 10 $^{\circ}\text{C}.\text{min}^{-1}$ up to 300 $^{\circ}\text{C}$ under nitrogen environment at a flow rate 20 $\text{mL}.\text{min}^{-1}$. Thermal data analyses of DSC thermograms were conducted using the Q Series Thermal Advantage DSC software (M/s TA Instruments, New Castle, DE).

Powder X-ray diffraction (P-XRD) studies. The P-XRD studies were carried out for solid-state characterization of the drug,

polymers and optimized microballoons. The diffraction pattern of samples were recorded by X-ray diffractometer, Philips PW 17291 (M/s Philips Instruments, DA Best, The Netherlands) using Ni-filtered, Cu kV radiation, at a voltage of 40 kV. The XRD spectra, thus obtained, were compared for solid-state characterization of the drug in the prepared microsphere formulations.

In vivo imaging studies. *In vivo* studies for evaluation of GR potential of the microballoons was performed using X-ray imaging technique as per the protocol approved by the Institutional Ethical Committee, Maharishi Markandeshwar University, Mullana, Ambala, India. Unisex rabbits (weighing 2–2.5 kg) were housed under standard laboratory conditions at $25 \pm 2^{\circ}\text{C}$ and $55 \pm 5\%$ RH with access to standard diet and tap water *ad libitum*. Prior to initiation of the studies, the animals were kept overnight under fasting condition in order to avoid difficulties during imaging. The X-ray images of empty stomach were taken prior to conduct of experiments. Barium meal (2 mL) was administered to animals to identify the location of gastric tissue. Animals were administered with GR microballoons containing barium sulphate filled in size 4 hard gelatin capsules along with sufficient amount of water. After oral dosing, the animals were placed in the upright position for imaging to locate both control and microballoons formulations in tract using X-ray machine (M/s Siemens 300 MA with fluoroscopy, München, Germany) at different time intervals like 0.5, 1, 1.5, 3, 6 and 8 h, respectively.

Results and discussion

Risk assessment studies

Figure 1 depicts the Ishikawa fish-bone diagram for GR microballoons of ITH portraying the cause–effect relationship among the factors affecting the drug product CQAs. Table 5 illustrates the REM carried out for qualitative analysis of risk associated with each MA and/or PP. The REM suggested that factors, i.e. drug–polymer ratio, volume of aqueous phase, stirrer type, stirring speed and stirring temperature were found to be high risk, while the factors like type of

Table 5. REM for initial risk assessment of GR hollow microballoons of ITH.

CQAs	Risk Estimation Matrix									
	Drug-polymer ratio	Polymer type	Vol. of acetone	Vol. of aq. phase: methanol	Vol. of HLP	Stirrer type	Stirring speed	Stabilizer concentration	pH	Temp.
Size	High	Med	Med	High	Low	High	High	Med	Low	High
Yield	High	Med	Med	Med	Low	High	High	Low	Med	High
EE	High	Med	Med	Med	Low	Med	High	Med	Low	High
Q _{18h}	High	Med	Low	Low	Low	Med	Med	Low	Low	Low
T _{60%}	High	Med	Low	Low	Low	Med	Med	Low	Low	Low

EE, entrapment efficiency; Q_{18h}, cumulative amount of drug release in 18 h; T_{60%}, time required for 60% drug release; HLP, high-density liquid paraffin.

High risk Medium risk Low risk

Table 6. Summary of FMEA analysis illustrating RPN scores for various formulation and process variables affecting the CQAs.

Sl. no.	Failure modes	Severity (S)	Occurrence (O)	Detection (D)	RPN (S O D)	Consequences on CQAs
1	Drug-polymer ratio	8	6	6	288	Yield, Size, EE, Q ₁₈ , T ₆₀
2	Polymer type	5	4	2	40	Q ₁₈ , T ₆₀
3	Vol. of acetone	6	6	5	180	Yield, Size, EE
4	Vol. of aq. phase	5	5	5	125	Yield, Size, EE
5	Vol. of MeOH	7	6	5	210	Yield, Size, EE
6	Volume of HLP	7	4	5	140	EE, Q ₁₈ , T ₆₀
7	Stirrer type	6	5	5	150	Size, Q ₁₈ , T ₆₀
8	Stirring speed	7	7	4	196	Yield, Size
9	Temperature	6	5	4	120	Size, EE
10	Stabilizer conc.	4	3	2	24	Yield, EE
11	pH	3	2	2	12	Yield, Size, EE

EE, entrapment efficiency; Q_{18h}, cumulative amount of drug release in 18 h; T_{60%}, time required for 60% drug release.

polymer, volume of methanol, HLP, stabilizer concentration were associated with medium risk. Based on the REM analysis, extensive risk assessment was carried out by assigning ordinal scores to each MA and PP. Table 6 enlist the details of MAs and PPs employed during FMEA and their calculated RPN scores, which quantitatively describe their effect and plausible consequences on drug product CQAs. A cut-off value of RPN (i.e. 100 or above) was selected for discriminating the high-risk factors against the low-risk factors. High RPN scores (i.e. above 100) were observed for drug-polymer ratio, volume of acetone, volume of aqueous phase: methanol, volume of liquid paraffin, stirrer type, stirring speed and stirring temperature, respectively.

Factor screening studies

Factor screening studies were further carried out on the input variables short-listed using REM and FMEA analysis to finally embark upon the CMAs employing Taguchi design. The first-order polynomial equations along with their corresponding polynomial coefficients, generated for each of the quality attributes employed during the factor screening studies, indicated the absence of any interaction(s) among the factors. Further, the influence of each factor on the response variables was also evaluated with the help of Pareto charts. Significant influence of all the three factors like drug-polymer ratio, stirring speed and stirring temperature was observed on the percentage yield of the floating

microballoons of ITH, as these values were found to be higher in magnitude than the *t*-value limit of the Pareto chart (Figure 2A). The Pareto charts depicted in Figure 2(B) corroborate considerable impact of drug-polymer ratio and stirring speed on EE, while Figure 2(C) illustrates very significant effect of drug-polymer ratio on percent buoyancy as the values crossed the Bonferroni limit. In a nutshell, the factors, i.e. drug-polymer ratio, stirring speed and stirring temperature were selected as the CMAs, while other four variables considered during screening studies were fixed at a constant value owing to their insignificant influence on the CQAs for microballoon formulations.

Characterization of GR floating microballoons

Percent yield

The formulation composition of the microballoons prepared as per the experimental design exhibited yield ranging widely between 48% and 95%. This clearly vouches that the choice of formulation composition has very significant impact on entrapment of the drug in the polymer matrix for formation of microballoons.

Entrapment efficiency

All the microballoons prepared as per the experimental design showed good entrapment, ranging widely between 73% and 91%, indicating superior drug loading in the microballoons for attaining desired therapeutic action. The largest value of

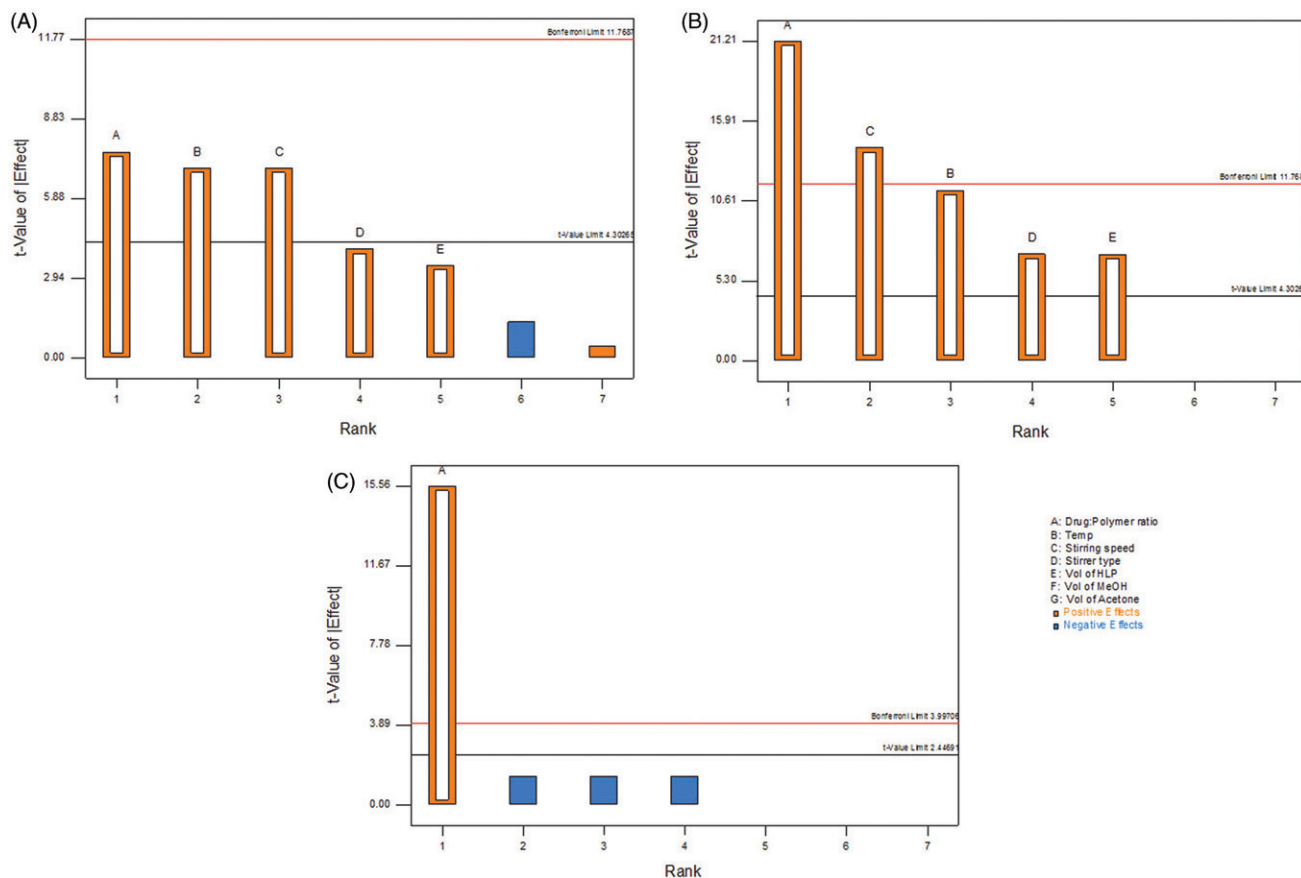


Figure 2. Pareto charts for screening of influential factors as per Taguchi design using chosen critical quality attributes (CQAs), (A) percent yield, (B) entrapment efficiency, (C) floating time.

EE, however, was observed at the intermediate levels of the polymer.

In vitro buoyancy studies

In vitro buoyancy studies revealed that 51% to 85% of the hollow microsphere formulations remained under floating condition for 24-h time period. All the prepared microballoons showed instantaneous onset of floating behavior *in vitro* with zero lag time attributable to the hollow nature of the microspheres leading to buoyant systems. This would help in achieving higher retention time of the microballoons in stomach for prolonged duration of time.

In vitro drug release studies

The drug release profile of the formulations prepared as per the experimental design showed a noticeable initial burst release in the first 2 h, followed by nearly sustained release profile of drug release upto 24 h. The initial burst release effect may be ascribed to the release of drug on the surface of the microballoons, followed by diffusional drug release from the inner core (Obeidat & Price, 2006; Chawla et al., 2012; Singh et al., 2012). The values of Q_{18h} for the prepared hollow microspheres ranged quite widely between 60.2 and 98.7%, portraying a somewhat linear decreasing trend with increase in the concentration of the polymer, i.e. Eudragit S-100. High magnitude of $T_{60\%}$, ranging between 8.4 and 11 h, vouched sustained drug release profile of the formulations. Further, the values of release rate exponent (n),

ranging between 0.120 and 0.311, ratified a Fickian or quasi-Fickian mechanism of drug release (Korsmeyer et al., 1983). Albeit Eudragit S-100 is known to be a non-swellable polymer tending only to dissolve at pH above 7, the value of “ n ” showed increasing trend with increase in the concentration of the polymer, plausibly attributed to its diffusional contribution (Lee et al., 2000; Jain et al., 2006; Ahuja et al., 2007).

Response surface analyses

Table 7 enlists the values of the coefficients of second-order quadratic polynomial equations, as per Equation (10), for each CQA along with ANOVA parameters like model p value, r^2 and predicted error sum of squares (PRESS). The coefficients of the polynomial equation generated using multiple linear regression analysis (MLRA) for all the CQAs, i.e. EE, particle size, Q_{18h} and $T_{60\%}$ along with high values of r^2 ranging between 0.9421 and 0.9968 ($p < 0.005$ in each case), and low values of PRESS confirmed goodness of fit of the data.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_3^2 + \beta_7 X_1 X_2 + \beta_8 X_2 X_3 + \beta_9 X_1 X_3 \dots \quad (10)$$

Figure 3 depicts the 3D-response surface plots for EE, particle size, Q_{18h} and $T_{60\%}$. The response surface analyses provide improved understanding of the interaction(s) between the CMAs and their impact on CQAs of the GR microballoons of ITH.

As portrayed in Figure 3(A), a twisted response surface plot was observed for the CQA, i.e. EE, where a rise in the levels of polymer showed initial decrease followed by an increasing trend at low levels of stirring temperature. On the other hand, increase in the levels of temperature showed an improving trend followed by a plateau phase. The identical relationship was also observed for response surface plots between polymer concentration versus stirring speed and temperature versus stirring speed, respectively (data not shown). Maximum EE was observed at the low levels of

Table 7. Coefficient of model terms and statistical parameters obtained for second order quadratic equations for the studied CQAs.

Coefficient code	Polynomial coefficients for CQAs			
	Entrapment efficiency	Particle size	Q _{18h}	T _{60%}
β_0	+87.18	448.16	+69.4	+8.86
β_1	-0.725	196.54	-16.7125	+5.16625
β_2	+3.8	-2.03	-1.5875	+0.1525
β_3	+3.6	-20.94	+1.625	-0.13875
β_4	-0.15	-4.58	+0.175	-0.03
β_5	+1.2	-11.95	-0.75	-0.0475
β_6	+1.25	-11.58	+1.5	-0.375
β_7	+4.685	-93.83	+10.0125	-3.25875
β_8	-5.015	1.65	-0.2375	+0.04375
β_9	-2.915	4.17	+0.8375	-0.02875
Model <i>p</i> value	<i>p</i> < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.001
<i>R</i> ²	0.9831	0.9974	0.9805	0.9961
PRESS	53.41	96.47	28.19	64.03

PRESS, predicted error sum of squares.

polymer and the higher levels of stirring temperature and stirring speed. The presence of curved lines in response surface confirmed the presence of interaction(s) among the CMAs.

Figure 3(B) portrays the 3D-response surface plot for the particle size, where increase in the levels of CMA, i.e. polymer concentration, showed an ascending trend at all the levels. The effect of temperature on particle size, on the other hand, was found to be negligible. Likewise, almost a linearly increasing trend was observed with stirring temperature and stirring speed (data not shown). Smallest particle size was observed at low level of polymer and low temperature.

The 3D-response surface plot depicted in Figure 3(C) reveals that the amount of polymer has linearly descending influence on Q_{18h}. However, the effect of stirring temperature was found to be almost negligible. Likewise, an analogous trend was also observed with polymer concentration and stirring speed (data not shown). Figure 3(D) portrays the 3D-response surface plot for T_{60%}, where a sharp rise in the values of T_{60%} was observed upon increasing the amount of polymer, ostensibly owing to the sustained release profile of drug release from the microballoons. However, a miniscule influence was observed with stirring temperature and stirring speed on T_{60%} (data not shown).

Particle size analysis

Particle size analysis of the microballoons prepared as per the experimental design was found to range between 240 and 1150 μ m, ratifying the micro-structure of the prepared

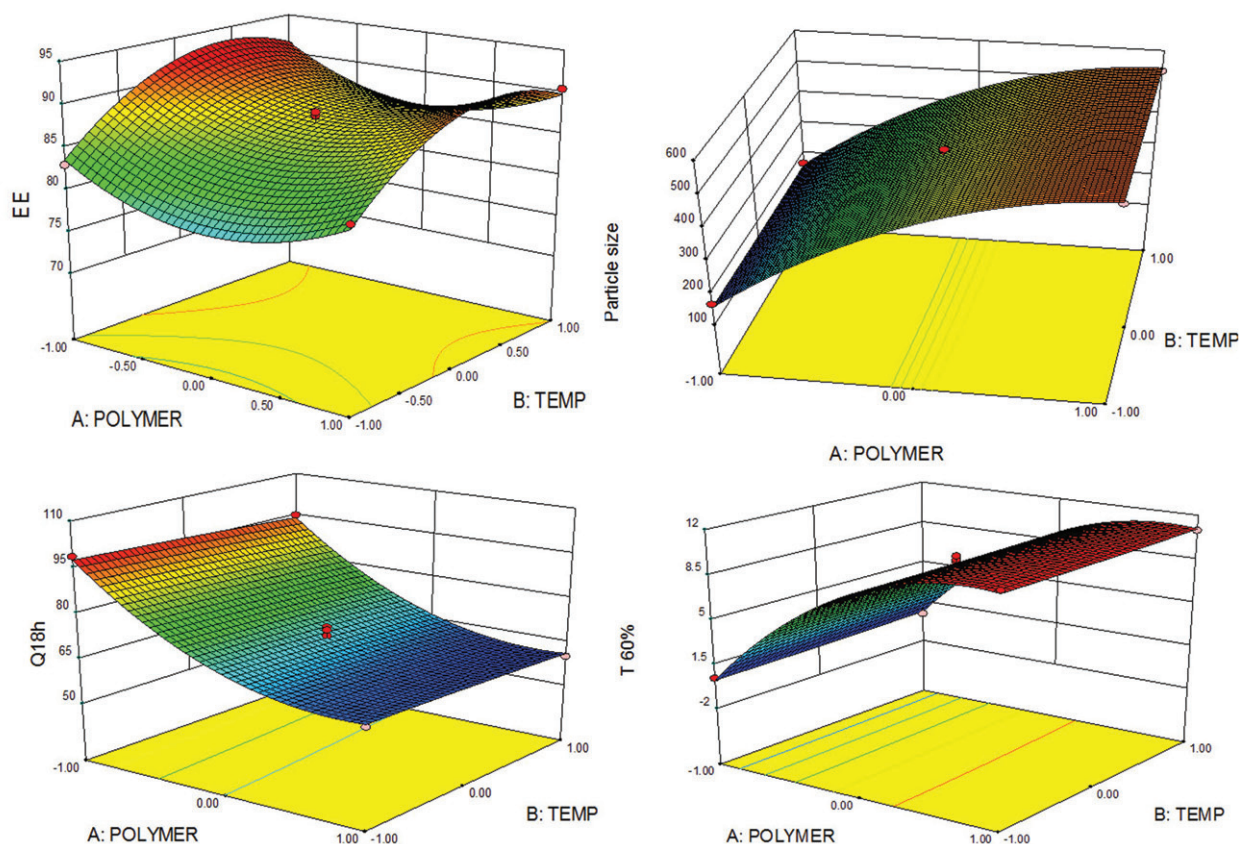


Figure 3. Response surface plots showing the influence of CMAs and CPPs, i.e. amount of polymer, stirring temperature and stirring speed on CQAs, (A) EE, (B) particle size, (C) T_{60%} and (D) Q_{18h} of GR microballoons of ITH.

formulations. The variation in mean particle size was ascribed to the variation in drug–polymer ratio along with plausible influence of stirring speed during preparation of microballoons. Increase in the concentration of polymer tended to cause significant augmentation in the particle size, while the factors like stirring speed and stirring temperature reduced the particle size of microballoons noticeably.

Evaluation of micromeritic properties

Evaluation of the micromeritic properties revealing CI between 10.3 and 20%, Hausner's ratio less than 1.25, and

angle of repose (θ) less than 25° , confirm favorable flow characteristics of the prepared microspheres.

Search for optimum formulation and validation of QbD methodology

Selection of the optimized formulation was embarked upon using numerical optimization by desirability function while “trading off” various CQAs to attain the desired goals, i.e. minimization of particle size, and maximization of EE, Q_{18h} and $T_{60\%}$ to obtain desirability close to 1. The optimized formulation corresponded to drug–polymer ratio of 1:3.31,

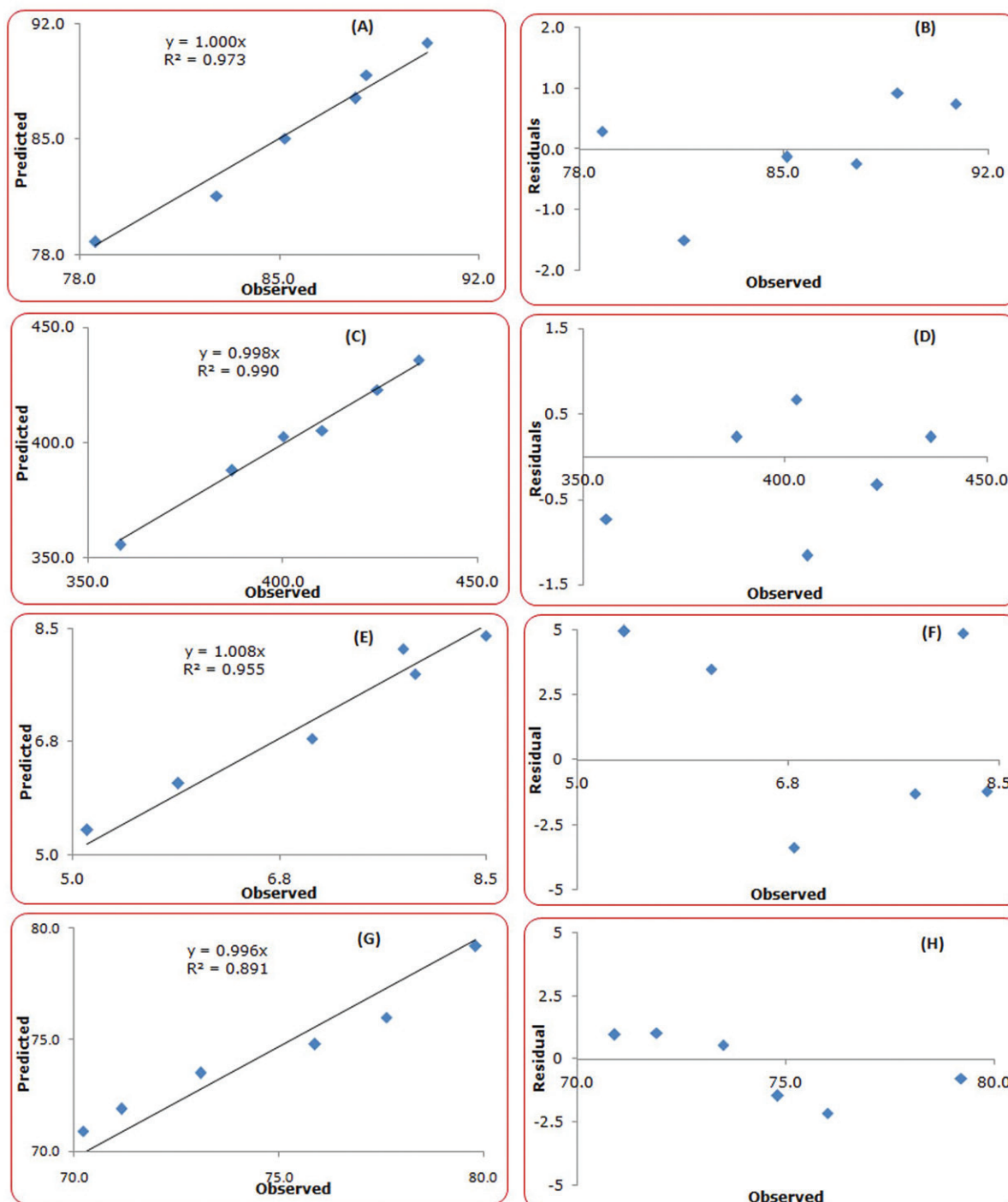


Figure 4. Linear correlation plots and residual plots between the observed and predicted values of various CQAs, (A–B) entrapment efficiency, (C–D) particle size, (E–F) $T_{60\%}$ and (G–H) Q_{18h} .

stirring temperature of 28 °C and stirring speed of 1000 rpm, exhibiting EE of 85%, particle size of 400 μm , percent buoyancy of 87%, $Q_{18\text{h}}$ as 75.3% and $T_{60\%} \sim 8\text{h}$, respectively.

Validation of the QbD methodology was accomplished by preparing six check-point formulations and comparing their

observed responses with those predicted ones. The prediction error (i.e. bias) for the CQAs varied between -3.95 and 5.22% with overall mean \pm SD as $-0.98\% \pm 0.22$. Figure 4 illustrates the linear correlation plots drawn between the predicted and observed responses demonstrating high values

Figure 5. Dissolution profiles of optimized GR microballoon formulation of ITH and marketed tablet (Ganton). The inset depicts mean rate of drug release versus mid-point of time intervals from optimized microballoons vis-à-vis the marketed tablet.

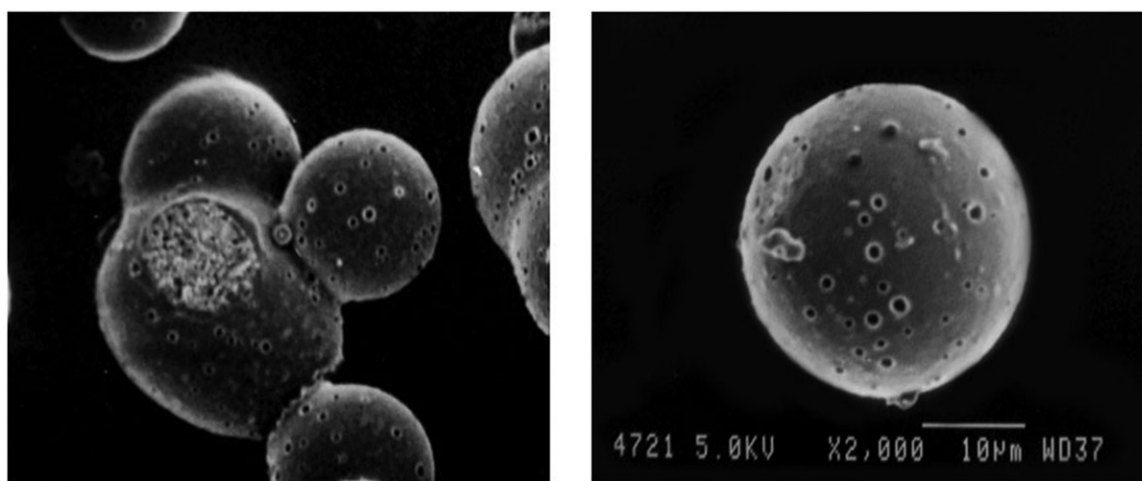
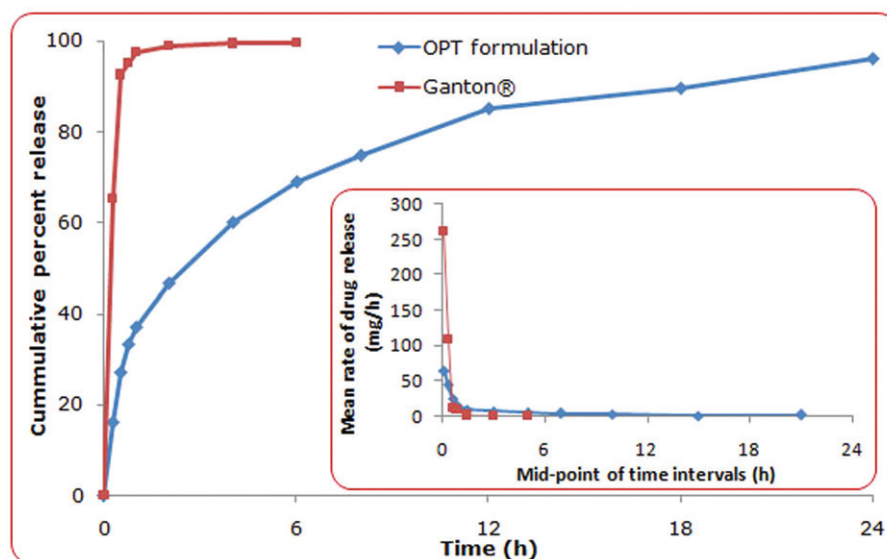


Figure 6. Scanning electron microscopy images of the optimized GR hollow microspheres.

Figure 7. FTIR spectra of pure drug, Eudragit S-100, physical mixture of drug and polymer, and optimized microballoon formulation.

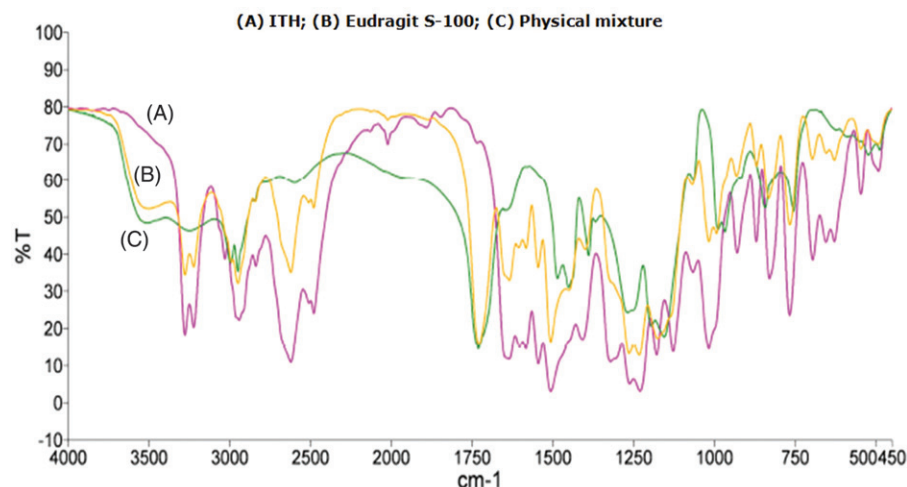


Figure 8. DSC thermograms of pure drug, Eudragit S-100, physical mixture of drug and polymer, and optimized GR microballoons.

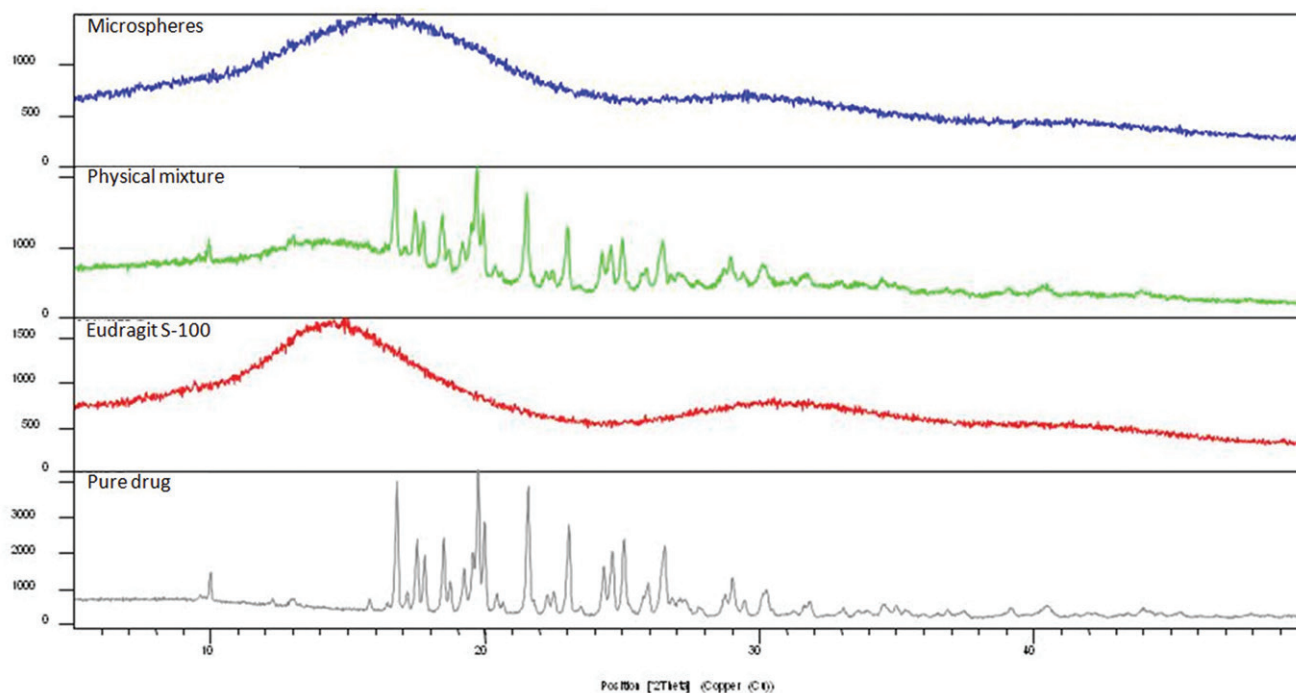
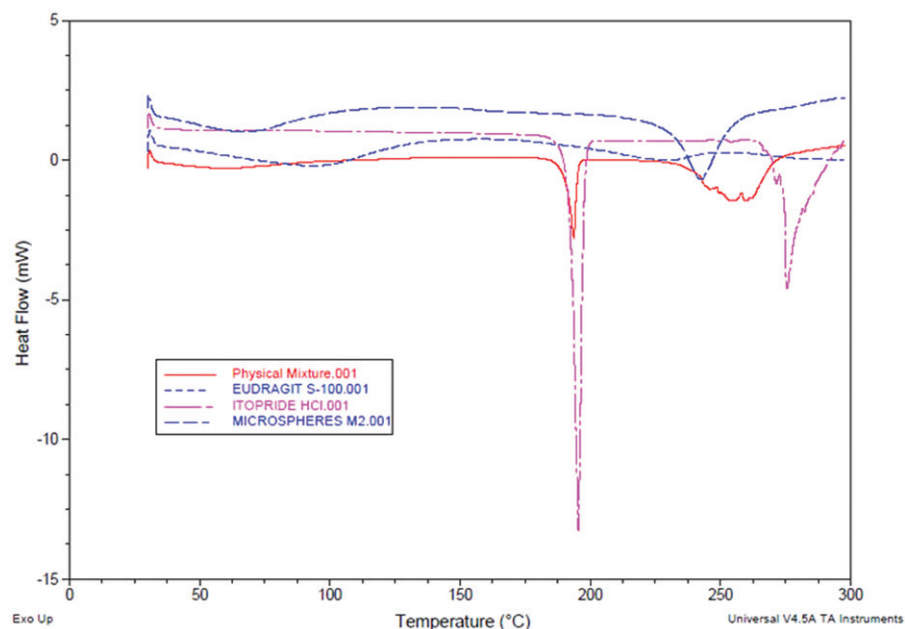


Figure 9. PXRD patterns of pure drug, Eudragit S-100, physical mixture of drug and polymer, and optimized GR microballoons.

of “r”, ranging between 0.886 and 0.932, thus ratifying the excellent goodness of fit ($p < 0.05$ in each case). The corresponding residual plots were also quite regulated with a relatively narrow, uniform and random scatter around zero axes, indicating high degree of prognostic ability of the QbD approach.

Drug release comparison with marketed brand

Figure 5 portrays the comparative drug release profile for ITH from the conventional marketed tablet (Ganaton) and the optimum formulation of GR hollow microspheres.

Strikingly different drug release profiles were observed for both the formulations. The marketed tablet showed almost complete drug release in less than 1 h owing to its immediate release nature, while the microballoons showed complete drug release in 24 h ratifying its extended release nature. The value of diffusional release exponent (n) of 0.458 for optimized microballoon formulation indicated non-Fickian, i.e. anomalous behavior, while a value of 0.136 for the marketed formulation construed distinct diffusion based mechanism (Korsmeyer et al., 1983; Singh & Singh, 1998; Costa & Lobo, 2001). The inset depicts the mean rate of drug release versus mid-point of time intervals for optimized

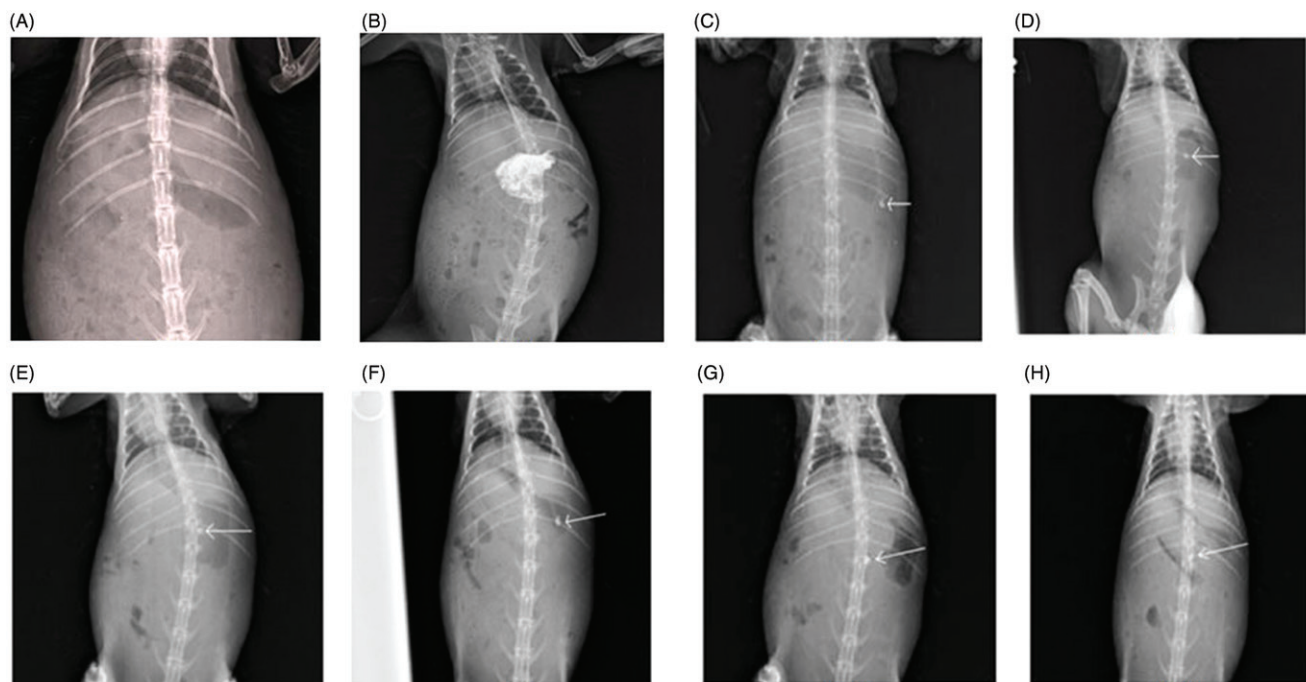


Figure 10. X-ray images of the rabbit stomach, (A) without microballoons, (B) with barium meal, (C) after 30 min administration of microballoons, (D) after 2 h administration of microballoons, (E) after at 3 h administration of microballoons, (F) after 4 h administration of microballoons, (G) after 6 h administration of microballoons, (H) after at 8 h administration of microballoons.

hollow microspheres vis-à-vis the marketed formulation, which corroborates markedly superior sustained release characteristics from the hollow microspheres.

Scanning electron microscopy

Figure 6 portrays the SEM image of optimized microballoons, which were found to be hollow and spherical in shape with presence of pores on the surface. Also, the surface of microballoons was smooth in appearance, indicating complete entrapment of drug within their hollow cavity.

Drug-excipient compatibility studies

FTIR spectroscopy. The FT-IR spectra of pure drug, physical mixture of drug-with polymers and optimized microballoons are shown in Figure 7, showing lack of any significant shift in the wave number (cm^{-1}) or functional groups of the drug in physical mixture with polymer and in the microballoons vis-à-vis the pure drug. This corroborates absence of any physiochemical interaction(s) and incompatibility between the drug and polymer.

Differential scanning calorimetry studies. The DSC thermograms of pure drug, physical mixture of drug with Eudragit S-100 and prepared microballoons are shown in Figure 8. DSC thermogram of ITH showed endothermic peak at 195.49°C indicating its melting point in the range of $191\text{--}195^\circ\text{C}$. Comparison of the DSC thermograms of pure drug with physical mixture and microballoons ratified the absence of any physiochemical incompatibility between the drug and polymer. However, the characteristic shift in peak of drug in microballoons at around 242.66°C corroborates change in physical nature of the drug in microballoons from the erstwhile crystalline to amorphous state. This could be further

explained by XRD analysis data wherein no crystalline peak of ITH was observed.

Powder X-ray diffraction (P-XRD) studies. Figure 9 depicts the XRD patterns of pure drug, physical mixture and microballoons. X-ray diffractogram of pure drug exhibited crystalline characteristics with diffraction angle (2θ) and d spacing at 16.78° , 19.74° , 21.57° , 23.05° and 5.28° , 4.49° , 4.12° , 3.86° , respectively. Similarly, the X-ray diffractogram of physical mixture of drug and polymer also showed identical diffraction patterns (2θ) and d spacing at 16.74° , 19.69° , 21.5° , 23° and 5.29° , 4.51° , 4.13° , 3.86° , respectively. However, X-ray diffractograms of the microballoons showed diffused spectra without any characteristic peak(s) of the drug, ostensibly owing to the complete encapsulation of drug within the microballoons.

In vivo imaging studies. Figure 10(A) and (B) refer to the X-ray images of empty stomach of rabbit before and after administration of Barium meal, respectively, whereas Figure 10(C)–(H) reveal that the QbD optimized microballoons were retained in the gastric region of the rabbit indicating prolonged residence time of the microballoons even after 8 h of administration, ostensibly owing to their floating characteristics for extended period of time.

Conclusions

The present studies successfully vouch the use of rational QbD approach in the systematic development of optimized GR systems employing simple, effectual and cost-effectual floating microballoons for improving gastric residence time and site-specific drug delivery of ITH. Implementation of QbD-based strategies helped in selecting apt formulation

and/or process variables for formulation of multiple-unit floating hollow microspheres, and subsequently arriving at the optimum delivery system with desired floatational and drug release characteristics. Thus, the *in vitro* and *in vivo* studies ratify the successful CR and GR propensities of the proposed optimized formulation for once-in-a day administration, leading eventually to effective, safe and patient-compliant outcomes. Further pharmacokinetic and/or pharmacodynamic studies would be useful to corroborate its extended duration of action and enhancement in bioavailable fraction. The promising results and the technical know-how from the current work can also be extrapolated for accomplishing the GR characteristics of other BCS class I drugs too.

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Declaration of interest

The authors declare no conflict(s) of interest in this work. The research work has been carried by funding of University Grant Commission, New Delhi, India.

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