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RESEARCH ARTICLE

Statistical optimization of controlled release microspheres containing cetirizine hydrochloride as a model for water soluble drugs

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Abstract

The purpose was to improve the encapsulation efficiency of cetirizine hydrochloride (CTZ) microspheres as a model for water soluble drugs and control its release by applying response surface methodology. A 3³ Box–Behnken design was used to determine the effect of drug/polymer ratio (X_1), surfactant concentration (X_2) and stirring speed (X_3), on the mean particle size (Y_1), percentage encapsulation efficiency (Y_2) and cumulative percent drug released for 12 h (Y_3). Emulsion solvent evaporation (ESE) technique was applied utilizing Eudragit RS100 as coating polymer and span 80 as surfactant. All formulations were evaluated for micromeritic properties and morphologically characterized by scanning electron microscopy (SEM). The relative bioavailability of the optimized microspheres was compared with CTZ marketed product after oral administration on healthy human volunteers using a double blind, randomized, cross-over design. The results revealed that the mean particle sizes of the microspheres ranged from 62 to 348 μm and the efficiency of entrapment ranged from 36.3% to 70.1%. The optimized CTZ microspheres exhibited a slow and controlled release over 12 h. The pharmacokinetic data of optimized CTZ microspheres showed prolonged t_{max} , decreased C_{max} and $\text{AUC}_{0-\infty}$ value of 3309 ± 211 ng h/ml indicating improved relative bioavailability by 169.4% compared with marketed tablets.

Keywords

Box–Behnken, cetirizine HCl, Eudragit RS100, emulsion solvent evaporation, microspheres, response surface methodology

History

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Introduction

Several challenges face the encapsulation of freely water soluble drugs within microspheres to control and sustain their release and actions. These challenges include high drug loading, escaping or degradation during the preparation, and difficulty to attain a predictable release from the microspheres. To achieve these goals, optimization of the formulation and process variables has been made to the solvent evaporation process^{1,2}.

Controlling the release of the drug can be attained through microencapsulation process^{3,4}, it also protects the sensitive drug against light and heat so enhances its stability⁵, modifies its solubility and absorption⁶ and reduces drug side effects on gastrointestinal mucosa^{7,8}. ESE is one of the best methods developed for formulation of microspheres due to its ease of preparation without effect on the activity of the encapsulated drug. This technique is a simple way for obtaining microspheres of different drugs using a wide variety of polymers^{9–11}. The process involves dissolving the coating materials in a volatile

solvent which is to be dispersed in an immiscible liquid vehicle phase with continuous stirring until the formation of an emulsion with a definite particle size. Subsequent evaporation of the volatile liquid is achieved either under reduced pressure or at atmospheric temperature to give solid polymeric microparticles^{9,12}.

Eudragit® RS 100 is widely used as coating materials as it is an inexpensive, stable, biocompatible and easily fabricated polymer. Eudragit® RS 100 is referred to as ammoniomethacrylate copolymers, with 5% functional quaternary ammonium groups, with a resultant pH independent permeability¹³. Also, it is a water-insoluble so is used as a coat material for sustained release formulation¹⁴.

Several factors that influence the release rate of drug from microspheres prepared by the ESE include; molecular weight of the polymer and viscosity of its solutions^{15,16}, ratio of drug to wall former and concentration of emulsifier and pore inducer¹⁷, addition of a non-solvent prior to microencapsulation¹⁸, type of emulsifier, co-solvent and drug/polymer ratio¹⁹, the addition of additives such as PEG 1000²⁰ and the rate of increasing temperature of water bath during microsphere preparation²¹.

Optimization is a technique that utilizes the available resources to obtain the best possible results. The process of try and error that was extensively used is now replaced by the optimization technique that finds wide range of application in chemistry and pharmaceutical industry^{22,23}. Optimization techniques are

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employed for the design and development of controlled-release dosage forms^{24–27}. Such studies represent the application of appropriate experimental designs, generation of polynomial relationships and optimum research methods using applicable software.

CTZ is a highly selective antagonist of the H₁-receptor not associated with sedation used in the treatment of allergic rhinitis and chronic urticaria. It is a water soluble drug which is rapidly absorbed. The drug has C_{max} reached 1 h after oral administration. CTZ undergoes a low degree of first-pass metabolism in the liver; metabolized to limited extent by oxidative dealkylation to a metabolite with negligible antihistaminic activity. It is approximately 93% bound to plasma proteins, and has a plasma elimination half life of approximately 8–9 h which does not change with multiple dosing. Its pharmacokinetic is dose-independent and plasma levels are proportional to the dose administered over the clinically studied range of 5–20 mg^{28–30}.

In this study, ESE technique was used to prepare controlled-release microspheres of a highly selective antagonist of the H₁-receptor, CTZ, as model for water soluble drugs. The influence of several factors on particle size, entrapment efficiency and dissolution properties of the resulting microspheres were investigated using Box–Behnken experimental design. This design is useful for developing, improving and optimizing the processes. Statistical design reduces the number of experiments and time required for optimization of formulation, thus proving to be far more efficient and cost effective than conventional methods of product development.

Materials and methods

Materials

CTZ HCl was kindly supplied as a gift from Deef Pharmaceutical Industries Co. (Alqassim, KSA). Eudragit RS 100 was kindly supplied by Evonik Industries AG-Werk Rohm (Darmstadt, Germany). Dichloromethane was purchased from Prolabo (France); *n*-Hexane 95% from Honil Limited (London, UK); Acetonitrile HPLC grade from Merck (Darmstadt, Germany); Potassium dihydrogen orthophosphate from BDH chemicals Ltd (London, UK); Light Liquid Paraffin from WINLAB (UK); and Sorbitan monooleate, NF, Span 80 from Sigma Chemical Co. (Steinheim, Germany). All materials used were obtained and used without any further purification.

Methods

Box–Behnken experimental design

A 3³ Box–Behnken experimental design was used in this study using statistical package Statgraphics® plus software, version 4 (Manugistics Inc., Rockville, MD). The factors studied were: the drug to polymer ratio (*X*₁), the surfactant concentration (*X*₂) and the stirring speed (*X*₃). Preliminary studies also provided a setting of the levels for each formulation variable. The selected responses were the mean particle size of the microspheres (*Y*₁), the entrapment efficiency (*Y*₂) and the cumulative percent drug released after 12 h (*Y*₃). The factors level and responses of the design are presented in Table 1. The design is appropriate to study the quadratic response surfaces and to construct the second-order polynomial models. A total of 15 experimental runs, shown in Table 2, are required.

Preparation of microspheres

All CTZ microspheres formulae depicted in Table 2 were prepared by the ESE^{12,31,32}. Briefly, the drug was dissolved in the polymeric, Eudragit RS100, solution previously dissolved in

Table 1. Independent and dependent variables and their levels for Box–Behnken design.

Factors	Levels			Response	Constraints		
	–1	0	+1		Minimum	Maximum	Goal
<i>X</i> ₁	1:5	1:4	1:3	<i>Y</i> ₁	309	551	Minimize
<i>X</i> ₂	2	3	4	<i>Y</i> ₂	36.28	70.63	Maximize
<i>X</i> ₃	300	400	500	<i>Y</i> ₃	77.94	101.15	Minimize

Table 2. Experimental runs and their observed responses.

Run	Factors (coded values)			Responses		
	<i>X</i> ₁	<i>X</i> ₂	<i>X</i> ₃	<i>Y</i> ₁	<i>Y</i> ₂	<i>Y</i> ₃
1	0	+1	+1	486	64.92	89.32
2	+1	–1	0	387	65.82	77.94
3	–1	0	–1	401	36.28	100.2
4	0	–1	+1	462	70.63	92.56
5	0	0	0	465	59.75	91.12
6	–1	–1	0	358	39.26	98.91
7	+1	0	–1	497	69.49	80.19
8	–1	+1	0	309	48.48	99.36
9	0	0	0	491	60.41	90.73
10	0	+1	–1	476	60.06	88.39
11	–1	0	+1	376	43.25	101.2
12	+1	0	+1	469	66.15	82.75
13	+1	+1	0	551	64.17	85.23
14	0	–1	–1	464	60.89	90.52
15	0	0	0	469	61.52	90.65

dichloromethane at drug to polymer ratios 1:3, 1:4 or 1:5 forming the internal phase. The resultant dispersion was added drop wise to liquid paraffin (external phase) containing 2, 3 or 4% Span 80 and stirred at 300, 400 or 500 rpm (Steady stir Digital, 855 Fisher Scientific, Waltham, MA). The stirring was continued to evaporate the solvent at room temperature. Microspheres were filtered off after decantation of liquid paraffin, washed with *n*-hexane and then dried at room temperature for 24 h.

Characterization of the prepared microspheres

Determination of the entrapment efficiency

Weighed amounts of each microsphere formulation (50 mg) was dissolved in methanol, sonicated for 10 min and the drug content was determined using HPLC (Agilent 1200 series, equipped with a UV diode array detector, and an automatic sampling system, Germany) at 229 nm. Entrapment efficiency of microspheres was calculated as follows:

$$\text{Entrapment efficiency} = \frac{\text{Actual drug entrapped}}{\text{Theoretical drug content}} \times 100 \quad (1)$$

Micromeritic properties evaluation

Particle size analysis. The distribution of particle size was evaluated by sieve analysis on an electric shaker at high amplitude for 10 min. The weight of microspheres retained on each individual sieve was recorded and the microspheres were collected and used for further characterization. The mean particle size of microspheres was calculated using Equation (2).

$$\text{Mean particle size} = \frac{\sum \left(\text{Mean particle size of the fraction} \times \text{Weight fraction} \right)}{\sum \text{Weight fraction}} \quad (2)$$

The linear graph obtained by plotting logarithm of the particle size against the cumulative percent frequency on the probability scale was used to calculate the geometric mean diameter (d_g) in μm . The slope of the obtained line is known as the geometric standard deviation (σ_g) which can be calculated by Equation (3)³³.

$$\sigma_g = \frac{d_{50}}{d_{16}} = \frac{d_{84}}{d_{50}} \quad (3)$$

Flow properties

Bulk density. The bulk density of the microspheres was calculated by dividing the weight of 50 g powder by the volume (in cm^3) of the powder in a 100-ml graduated cylinder³³. The tapped density was determined dividing the weight (in g) by the volume (in cm^3) obtained after tapping of the powder within the graduated cylinder until constant volume. The bulk, and tapped densities were measured to evaluate the packability of microspheres. Hausner ratio and Carr's index can be estimated from Equation (4).

$$C.I. = 100 \times \frac{d_T - d_B}{d_T} \quad (4)$$

Angle of repose. The angle of repose of drug-loaded microspheres was calculated using the fixed-base cone method according to the formula ($\tan \theta = h/r$), where h = cone height, r = radius of circular base formed³³.

Surface morphology of microspheres. The surface morphology of the microspheres was examined by scanning electron microscope (Philips XL30, Netherlands) operated at 4–25 kV on samples gold-sputtered for 120 s at 10 mA, under argon at low pressure.

In vitro dissolution study

USP Dissolution Tester Apparatus II (ERWEKA DT 700, Germany) at $37 \pm 0.5^\circ\text{C}$ was used for dissolution test. The paddles were rotated at a speed of 50 rpm. Capsules filled with amount of microspheres equivalent to 10 mg of CTZ were placed in the cylinder with 900 ml distilled water. Sample aliquots were withdrawn and replaced with fresh dissolution media at time intervals of 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 h. The drug content in the filtered samples was analyzed using HPLC (Agilent 1200 series equipped with UV, diode array detector, and an automatic sampling system, Germany) using reversed-phase column $25 \text{ cm} \times 4.6 \text{ mm}$ (i.d.) C18, $5 \mu\text{m}$ – Intersil® ODS-3 (GL Sciences Inc., Japan). The mobile phase consisted of a mixture of (28:72) acetonitrile/0.05 M potassium dihydrogen orthophosphate (pH 3.5), and the flow rate was 1 ml/min. CTZ content was determined at 229 nm after injecting a volume of $10 \mu\text{l}$. CTZ was detected at a retention time of 3.5 min. Assay performance was evaluated through determination of specificity, recovery, linearity, the limit of quantification (LOQ), the limit of detection (LOD), precision, accuracy as reported in the International Conference on Harmonization guidelines. The release experiments were done in triplicate.

Mathematical modeling of CTZ release

The release profiles of CTZ from microspheres can be modeled mathematically to obtain information concerning the release mechanism. The release data obtained were fitted to different kinetic models: zero-order³⁴, first-order³⁵, Higuchi³⁶ and Korsmeyer–Peppas models^{37,38} which is often used to describe the drug release behavior from the microspheres.

In vivo study on human volunteers

In vivo study was carried out to compare the pharmacokinetics of CTZ from microspheres formulation (treatment A) and the conventional Zyrtec® tablet (GlaxoSmithKline, Egypt) treatment B. A single dose of CTZ (10 mg) was given to the volunteers using a double blind, randomized, cross-over design in which fasting subjects were asked to take a single oral dose of prepared microspheres equivalent to 10 mg CTZ or 10 mg of the reference product with 250 ml of tap water in each period of the study. The washout period in this study was 1 week.

Subject population

Six healthy Egyptian male volunteers aged between 20 and 40 years (median weight: $73 \pm 8.5 \text{ kg}$ and median height: $174 \pm 6.7 \text{ cm}$) were chosen. Health status of the volunteers was confirmed by complete medical history, physical examination and laboratory analysis. No concurrent medication was allowed during the course of the study, and each subject read, understood and signed an informed written consent.

Sample collection

The protocol of the study was conducted according to Helsinki agreement protocol and according to the requirements of the ethical committee of the faculty of Medicine, Beni Suef University, Egypt that reviewed and approved the protocol from the Ethical point of view on its expedited meeting on the 17 December 2013. The drug was administered orally after fasting overnight with 250 ml of tap water in each period of the study and washout period of 1 week. Blood samples (5 ml) were collected at different time intervals: 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 18 and 24 h. The blood samples were then centrifuged at 3000 rpm and clear plasma was then collected and stored at -20°C till assayed.

Chromatographic conditions

Determination of CTZ in plasma was adopted by a modified HPLC method as reported³⁹. The HPLC apparatus consisted of Isocratic pump LC-10 AS and a UV/VIS detector SPD-10 A connected to a C-R6A Integrator (Shimadzu, Kyoto, Japan). The analytical column was Ponapak C18 HPLC column, $4.6 \times 250 \text{ I.D. mm}$, particle size 125 A (Waters Associates, Ireland). The mobile phase consisted of methanol: water in a ratio of 60:40 (v/v), containing 0.5% triethylamine and adjust to pH 3 using 0.1 N HCl. The components of the mobile phase were mixed filtered through Millipore filter ($0.45 \mu\text{m}$ nylon membrane). The mobile phase flow rate was 1.2 ml min^{-1} and the detection wavelength was 229 nm.

Plasma analysis

Plasma sample was mixed with acetonitrile and the internal standard (Erythromycin). The mixture was vortexed and then centrifuged at 3000 rpm. The upper layer was separated and transferred to another tube then filtered through $0.45 \mu\text{m}$ Millipore® filter for analysis with HPLC. $20 \mu\text{l}$ of each sample was injected to the column for analysis. Spectrophotometric detection at 229 nm was interpreted in the form of the reported peak areas. The recovery (20–500 ng/ml) varied between 97.16 and 99.86%.

Pharmacokinetic analysis

Pharmacokinetic parameters from plasma data following the oral administration of the two formulae were estimated using, WinNonlin®, version 1.5 (Scientific Consulting, Inc., Cary, NC). Two-way ANOVA followed by Sidak's multi-comparison

test using GraphPad Prism 6 (GraphPad software, Inc., San Diego, CA) has been used to verify the differences in drug bioavailability between two investigated groups. The level of statistical significance was chosen as $p \leq 0.05$. $C_{\max}$ (ng/ml) and $t_{\max}$ (h) were the observed maximum drug concentration and the time needed to reach this concentration, respectively. The area under the curve, $AUC_{(0-24)}$ (ng h/ml) was calculated from which the relative bioavailability ($AUC_{\text{test}}/AUC_{\text{standard}} \times 100$) was determined.

Results

The efficiency of the prepared microspheres to entrap the drug was ranged from 36.28 (F_3) to 70.06% (F_{10}) as displayed in Table 2. The particle size analysis of the prepared microspheres is presented in Table 3. The arithmetic mean diameter ranged from 309 to 551 μm in runs 8 and 13, respectively. The size distribution can be specified by two values; the geometric mean diameter (d_g) and the geometric standard deviation (σ_g). From the frequency distribution data, d_g of the prepared microspheres was ranged from 410 in run 8 to 692 μm in run 13 and σ_g were ranged from 0.653 and 0.894. The prepared microspheres exhibited good flow properties in most of the prepared batches as shown in Table 3. Hausner ratio was ranged from 1.12 in run 2 to 1.54 in run 13; and Carr's index was ranged from 10.6 in run 2 to 35.2 in run 13. Moreover, most of the formulations have acceptable angle of repose that lies in the range of 29.5–35°.

Figures 3 and 4 illustrate the release profiles of the formulations designed by Box–Behnken design. The *in vitro* release profile of formulations 3, 6, 8 and 11 is characterized by a high burst release (>40% after 0.5 h) and complete release of the drug after 12 h. On the other hand, formulae 2, 7 and 12 showed controlled release patterns of drug indicating the appropriate choice of the range of the formulation variables.

The model that best fits the release data was evaluated by the correlation coefficient (r) except in Peppas model which

evaluated by n value. The calculated (r) values were 0.985, 0.993 and 0.978 for the *in vitro* release of CTZ from formulae 2, 7 and 12, respectively, indicating that CTZ release was followed the diffusion mechanism. Also, kinetic treatment results revealed that the n values for the same formulae were 0.462, 0.469 and 0.488, respectively, that confirmed Fickian diffusion mechanism for the release of CTZ from the prepared microspheres.

Estimation of quantitative effects of the factors

Analysis of variance and lack of fit parameters for testing the model according to the data of the responses (Y_1 – Y_3) are shown in Table 4. According to ANOVA, p value of 0.05 or less is often considered evidence of a regression effect. p Values of 0.007, 0.0051 and 0.0031 indicated a significant effect of the independent factors on the responses Y_1 , Y_2 and Y_3 , respectively. In the lack of fit statistics, the F-ratio is 2.509, 26.64 and 119.26 for Y_1 , Y_2 and Y_3 , respectively, and the p values are 0.298, 0.054 and 0.062 for the same responses. The p value is larger than the significance level $\alpha = 0.05$, which is sufficient evidence at the $\alpha = 0.05$ level to conclude that there is no lack of linear fit and the model fits the observed data.

Results in Table 5 show factor effects of the Box–Behnken model and associated p values for all three responses resulted from ANOVA. According to these results, X_1 has a significant antagonistic effect on Y_1 and Y_2 with p values of 0.0008 and 0.0002, respectively, while X_1 has a significant synergistic effect on Y_3 with p value of 0.0001. In addition, Y_1 was significantly affected by the antagonistic effect of the quadratic term of X_1^2 and the interaction effect of X_1X_2 with p values of 0.0051 and 0.005, respectively. While, the quadratic term of X_1^2 affected Y_2 antagonistically with p value 0.007. Whereas, the observed results revealed that X_2 and X_3 had no significant effect on Y_1 , Y_2 and Y_3 .

A 3^3 Box–Behnken design provides a polynomial equation to obtain the levels of X_1 , X_2 and X_3 to get optimum values of Y_1 , Y_2

Table 3. Micromeretic properties of CTZ microspheres.

Run	d_{av}	d_g	σ_g	Hausner ratio	Carr's index	Angle of repose
1	486	550	0.793	1.42	29.5	32.7
2	387	445	0.795	1.12	10.6	30.4
3	401	470	0.810	1.37	26.8	33.7
4	462	620	0.653	1.38	27.7	34.9
5	465	515	0.805	1.28	22.0	30.4
6	358	440	0.822	1.19	16.2	29.5
7	497	515	0.780	1.43	30.2	38.6
8	309	410	0.746	1.40	28.8	41.2
9	491	580	0.817	1.39	28.2	36.4
10	476	692	0.894	1.50	35.3	41.3
11	376	410	0.759	1.47	31.9	35.9
12	569	580	0.739	1.18	17.1	30.5
13	551	665	0.821	1.54	35.2	34.4
14	446	550	0.776	1.29	22.7	32.4
15	469	560	0.757	1.22	17.9	31.3

Table 5. Estimated effects and associated p Values for all three responses.

Factor	Response					
	Y_1		Y_2		Y_3	
	Estimated effect	p Value	Estimated effect	p Value	Estimated effect	p Value
X_1	−115.0	0.0008 ^a	−24.59	0.0002 ^a	18.38	0.0001 ^a
X_2	37.75	0.0618	0.2575	0.9238	0.59	0.712
X_3	−11.25	0.5068	4.5575	0.1352	1.62	0.334
X_1^2	−110.0	0.0051 ^a	−16.46	0.0072 ^a	0.40	0.864
$X_1 X_2$	−106.5	0.0050 ^a	5.435	0.1936	−3.42	0.172
$X_1 X_3$	1.5	0.9489	5.155	0.2138	−0.81	0.723
X_2^2	−37.5	0.1665	4.205	0.3152	−1.35	0.572
$X_2 X_3$	6.0	0.7983	−2.44	0.5302	−0.56	0.806
X_3^2	31.5	0.2321	2.925	0.4727	0.08	0.974

^aSignificant effect of factors on individual responses is shown with asterisk.

Table 4. Analysis of variance and lack of fit parameters of testing the model in portions.

Response model	ANOVA for responses Y_1 – Y_3					Lack of fit		
	DF	Sum of squares	Mean square	F ratio	p Value	F ratio	p Value	R^2
Y_1	9	56399.98	6266.66	11.869	0.0070 ^a	2.509	0.298	0.9906
Y_2	9	1604.443	178.271	13.599	0.0051 ^a	26.64	0.054	0.9990
Y_3	9	689.3207	76.5912	16.834	0.0031 ^a	119.26	0.062	0.9998

DF: degree of freedom.

^aSignificant effect on the response.

and Y_3 , at constrained conditions. A new formula was prepared according to the predicted X_1 , X_2 and X_3 levels to measure the obtained responses and compared with the calculated values. Moreover, the design proposed two checkpoint formulation batches to be replicated and the observed responses compared to the predicted values. The observed, predicted values and the residuals for the optimized and checkpoint formulations of CTZ microspheres were displayed in Table 6.

The mean plasma concentration–time curve following the administration of the optimized formula of CTZ microspheres and

Table 6. Predicted, observed values and the residuals for the optimized and checkpoint formulations of CTZ microspheres.

Factors	Level	Responses	Predicted	Observed	Residuals
Optimized formulation					
X_1	1:5	Y_1	388	375	13
X_2	2	Y_2	69.2	73.3	−4.1
X_3	389	Y_3	79.0	81.5	−2.5
Checkpoint 1					
X_1	1:4.88	Y_1	436	451	−15
X_2	2.5	Y_2	48.0	50.2	−2.2
X_3	350	Y_3	96.0	94.3	1.7
Checkpoint 2					
X_1	1:3.55	Y_1	513	520	−7
X_2	3.5	Y_2	64.9	62.1	2.8
X_3	450	Y_3	87.9	90.5	−2.6

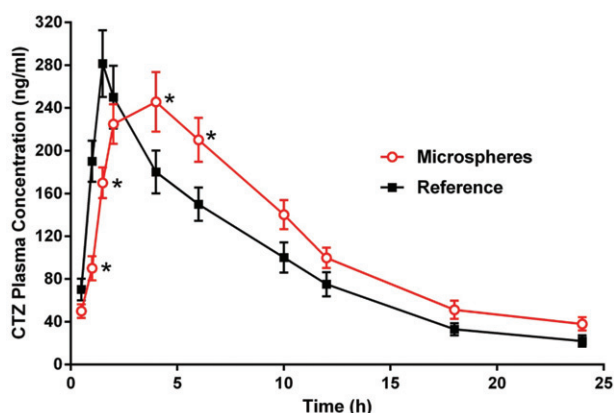


Figure 1. Plasma concentration of CTZ following the oral administration of single dose 10 mg of Zyrtec® tablets and the optimized formula of CTZ microsphere equivalent to 10 mg to human healthy volunteers. Data represent the mean values of $n = 6 \pm$ S.D. *Significant difference at $p < 0.001$.

Zyrtec tablets are illustrated in Figure 1. The pharmacokinetic study (Table 7) revealed increase in the bioavailability of CTZ microsphere by more than 1.69-fold relative to the marketed oral tablet. In addition, results revealed increase in the MRT in case of microsphere (12.58 h) compared with the reference tablet (7.46 h).

Discussion

To develop controlled release microspheres that entrap a freely water soluble drug like CTZ with optimum sphere size suitable to flow freely combined with maximum entrapment efficiency, the polymer to drug ratio, surfactant concentration and the stirring speed are important parameters affecting the drug release pattern. A 3^3 Box–Behnken experimental design was carried out to optimize and evaluate the selected variables X_1 , X_2 and X_3 main effects, interaction effects and quadratic effects at different levels as shown in Table 1. Considerable variation between particle size, entrapment efficiency and the amount of drug released after 12 h was obtained based on different combinations of variables and variable levels.

The results revealed acceptable entrapment efficiency for CTZ in the prepared microspheres, regardless its water solubility and narrow size distribution about the mean for all formulations³³. According to USP 29-NF24, there are six formulae have Hausner ratio less than 1.3 and Carr's index less than 25 which indicate passable to good flow properties of the prepared microspheres. The lower the angle of repose, the lower the fictional forces existing within the particulate mass that indicates good flowability of the microspheres and suggests that the microspheres can be easily handled during processing and filling into a capsule⁴⁰. The surface of the microspheres was quite spherical and smooth as seen in the photomicrograph (Figure 2) taken from SEM pictures for the optimized formula of dried microspheres.

The results of drug release from the prepared microspheres displayed that the small amount of polymer used in the formulations 3, 6, 8 and 11 is not enough to reduce dose dumping of highly soluble drug. However, it is obvious that increasing the ratio of polymer in the microspheres; as in case of formulations 2, 7 and 12; resulted in a remarkable decrease in the drug release rate.

Pareto charts (Figure 5) are used to demonstrate the effect (in decreasing order of importance) of the independent variables and their interactions on the dependant variables. The chart includes a vertical reference line at the critical p value of 0.05. An effect that exceeds the vertical line is considered to be statistically significant^{41,42}. The fact that the bars for the variable X_1 extend after the reference line and the relatively larger coefficient for these terms in the regression equations for Y_1 , Y_2 and Y_3 indicate that drug to polymer ratio is significant in increasing both the microsphere size and entrapment efficiency as well as controlling the release

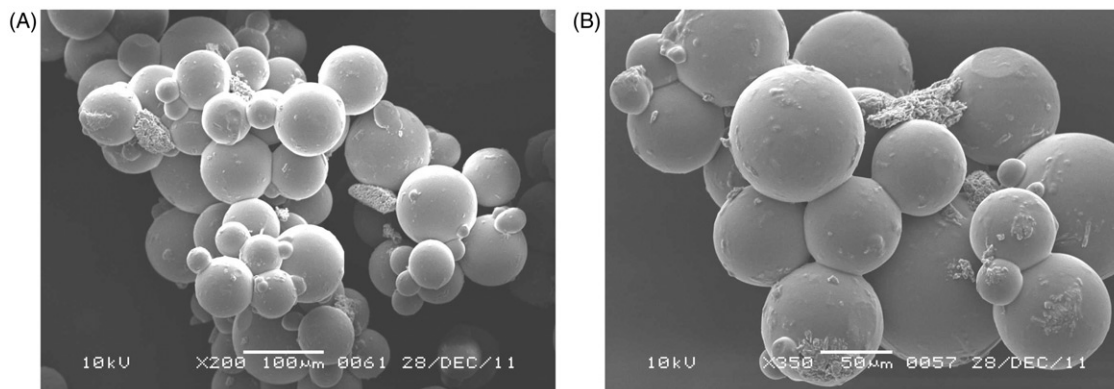


Figure 2. Scanning electron photomicrographs of the optimized CTZ microspheres at (A) 200× magnification and (B) 350× magnification.

of CTZ from the microspheres throughout the dissolution time. The positive coefficients of independent variable X_1 indicate that they have a retardant effect on CTZ release through the increase in thickness of coating layer. The positive coefficient of the independent variable X_1 indicates that it has a controlling effect on CTZ release. It could be assumed that the variation in drug to polymer ratio is closely related to the change in the porosity of microspheres. Thus, microspheres prepared at higher drug to polymer ratio show faster initial drug release due to rapid liquid penetration⁴³. While, X_2 and X_3 have no significant effect on all responses, this finding displayed from their bars which does not reach to the reference line in the Pareto charts of Y_1 , Y_2 and Y_3 .

An inverse relationship was found between X_1 and Y_1 . The increase of X_1 from 1:5 to 1:3 decreases Y_1 from 387 in run 2 to 358 in run 6 and from 497 in run 7 to 401 in run 3. This could be explained that higher polymer concentration produced a more viscous dispersion which reduces the shearing action, resulting in larger droplets during the emulsification and consequently larger microspheres were produced after evaporation¹². It also could be suggested that the higher polymer concentration led to higher frequency of collision, resulting in fusion of the droplets producing larger microspheres⁹.

X_1 has the main effect on determining the entrapment efficiency of the formulations. There was a direct relationship between X_1 and Y_2 . As the ratio of polymer increased from 1:3 to 1:5, Y_2 will increased from 36.28 in run 3 to 69.49% in run 7; from 48.48 in run 8 to 64.17% in run 13, and from 39.26 in run 6 to 65.82% in run 2. It is obvious that the increase of X_1 markedly increase Y_2 which can be attributed to the presence of polymer at higher concentration, results in solidification of microspheres at a faster rate. This reduces the leaching of drug to the external phase; consequently, the encapsulation efficiency is increased as reported in⁴⁴. On the other hand, there is a slight or no effect in Y_2 by increasing X_2 and X_3 .

In all runs, with the increase in X_1 , there was a decrease in the amount of drug released. This could be attributed to the greater binding of the drug with the polymer. Larger amounts of drug adhered tightly to the polymer matrix, as the concentration of polymer increases that result in a decrease in the rate of drug release from the microspheres. The Y_3 response ranged from 77.94% in run 2 to 101.15% in run 11 as indicated in Figures 3 and 4. It was found that X_1 has the main effect on determining the release of CTZ from the prepared microspheres. There was an inverse relationship between X_1 and Y_3 . As the ratio of X_1 increased from 1:5 to 1:3, Y_3 decrease from 100.2 in run 3 to 80.19 in run 7, from 98.91 in run 6 to 77.94 in run 2 and from 99.36 in run 8 to 85.23 in run 13. It is clear from the Pareto charts and main effect plots (Figure 5) that X_1 has the main effect on

determining the release of CTZ from the microspheres and there is no significant effect of both X_2 and X_3 on Y_3 .

3D response surface Figure 6 (left side) and contour plots for the estimated responses Figure 6 (right side) were constructed according to the model polynomial functions to assess the change of the response surface. Since the model has more than two factors, one factor was held constant for each diagram, therefore, a total of 9 response surface plots was produced, 3 for each response. From which three were selected to represent the main effect of factors on the responses. Also, contour plots gave an idea about the exact percent of X_1 , X_2 and X_3 at which the responses becomes at desired level.

After creating the polynomial equations [Equations (5)–(7)] relating the factors and responses, the process was optimized. Optimization was achieved to obtain the levels of X_1 – X_3 which maximize Y_2 and minimize both Y_1 and Y_3 . To validate these values, two checkpoints and optimal formulations were prepared according to the predicted levels of X_1 , X_2 and X_3 (Table 6).

The obtained responses obtained from the new formulations that prepared according to the predicted levels of X_1 , X_2 and X_3 , were in a close agreement with the predicted values. This verified the reliability of the optimization procedure in maximizing the entrapment efficiency of water soluble drugs via microencapsulation process and predicting the dissolution behavior of the prepared microspheres.

$$\begin{aligned} \text{Mean particle size}(Y_1) = & -716.88 + 107.85X_1 + 332.38X_2 \\ & - 1.44X_3 - 2.2X_1^2 - 10.65X_1X_2 \\ & + 0.002X_1X_3 - 18.75X_2^2 \\ & + 0.03X_2X_3 + 0.002X_3^2 \end{aligned} \quad (5)$$

$$\begin{aligned} \text{Entrapment efficiency}(Y_2) = & 70.09 + 7.02X_1 - 18.48X_2 \\ & - 0.16X_3 - 0.33X_1^2 + 0.54X_1X_2 \\ & + 0.005X_1X_3 + 2.1X_2^2 \\ & - 0.01X_2X_3 + 0.0001X_3^2 \end{aligned} \quad (6)$$

$$\begin{aligned} \text{In vitro release}(Y_3) = & 17.42 + 2.86X_1 + 12.29X_2 + 0.03X_3 \\ & + 0.008X_1^2 - 0.34X_1X_2 - 0.001X_1X_3 \\ & - 0.67X_2^2 - 0.003X_2X_3 + 0.000004X_3^2 \end{aligned} \quad (7)$$

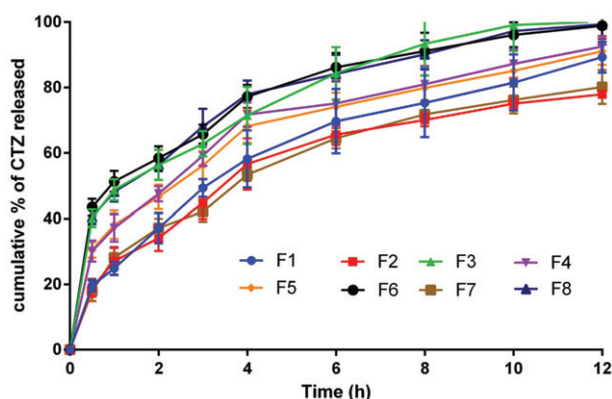


Figure 3. Cumulative % drug release profile of Box-Behnken design batches (F1–F8).

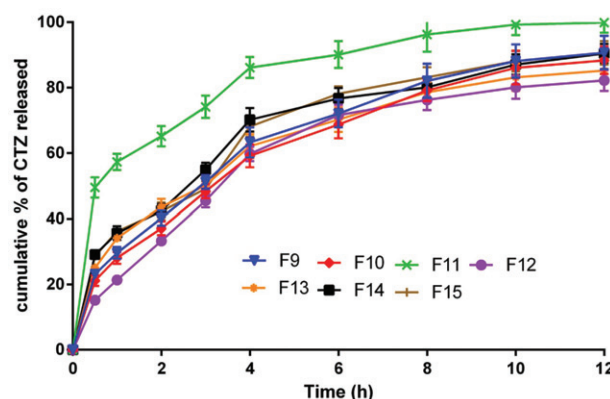
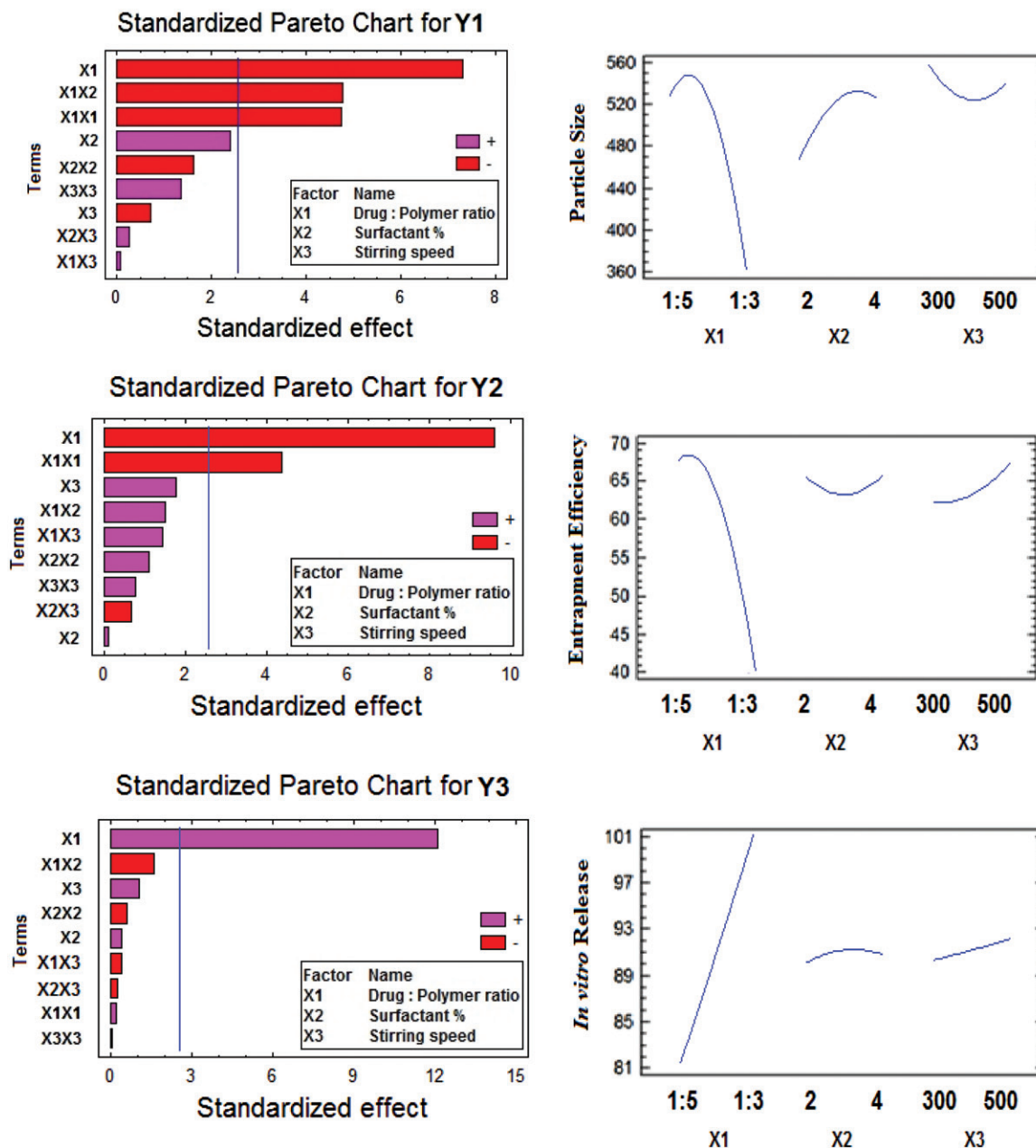


Figure 4. Cumulative % drug release profile of Box-Behnken design batches (F9–F15).

Figure 5. Standardized Pareto charts and main effect plots for Y_1 , Y_2 and Y_3 .

The pharmacokinetic study revealed that administering CTZ as controlled release microspheres can modify its pharmacokinetic profile and improve its bioavailability relative to the marketed oral tablet. Compared with marketed tablet, the $t_{\max}$ of CTZ microspheres was significantly higher ($p < 0.05$) which could be attributed to the preparation of this freely water soluble drug as microspheres that retarded its absorption through the release of the encapsulated CTZ in controlled manner that retard $t_{\max}$ and increase MRT of the drug. The pharmacokinetics of drugs delivered via microsphere formulations are dictated by the properties of the microsphere rather than the physicochemical properties of the drug molecules when the microsphere is administered orally. The increase in the mean $t_{\max}$ and the decrease in the mean $C_{\max}$ compared with the marketed tablet showed the controlled-release effect of the microsphere formulations. The mean $AUC_{0-\infty}$ was found to be 3309 ± 211 ng h/ml for the optimized formula compared with 1974 ± 245 ng h/ml for marketed tablets. From these results, it was found that, the

relative bioavailability of CTZ from optimized formula was 169% compared with marketed tablets. One main reason for such a big difference among controlled-release CTZ microspheres may be due to the slow release of the drug from the microspheres which led to a longer absorption and distribution period for the drug loaded microspheres than that of the free drug.

Conclusions

In this study, the optimized formula deduced by BBD efficiently encapsulates the model water soluble drug, CTZ, using ESE technique. SEM analysis revealed that the microspheres were spherical with smooth surface. This formula showed entrapment efficiency of $\sim 75\%$ and drug release of 81.5% after 12 h. *In vivo* study on human volunteers showed enhanced relative bioavailability by 169% when compared with marketed products that improve the patient compliance.

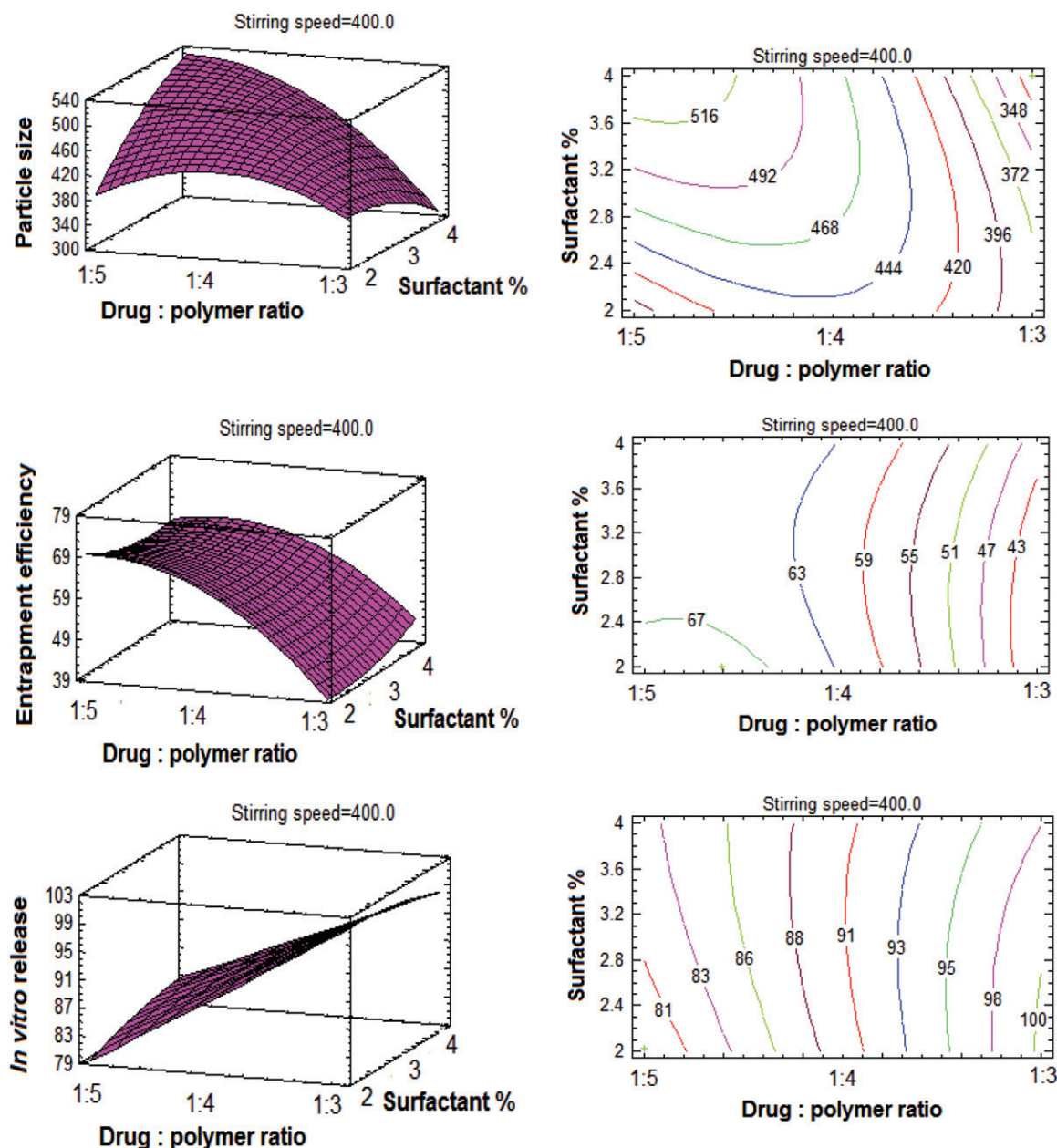


Figure 6. (Left side) 3D Response surface plots showing the effect of X_1 , X_2 and X_3 on the responses. (Right side) Contours of estimated response surface plots showing the relationship between various levels of factors to attain fixed values of responses.

Table 7. Pharmacokinetic parameters $\pm$ SD of CTZ following the administration of a single oral dose (10 mg) of the market Zyrtec[®] tablets, and the optimized formula of CTZ microsphere equivalent to 10 mg.

Pharmacokinetic parameter	Zyrtec [®] 10 mg tablet	Optimized formula
C_{max} (ng/ml)	281 ± 25.38	245 ± 22.75
t_{max} (h)	2 ± 0.195	4.38 ± 0.27
$AUC_{(0-24)}$ (ng h/ml)	1655 ± 237	2828 ± 195
$AUC_{(0-\infty)}$ (ng h/ml)	1947 ± 245	3309 ± 211
$t_{1/2}$ (h)	6.15 ± 0.45	8.59 ± 0.39
K_{el} (h)	0.112 ± 0.043	0.081 ± 0.043
MRT (h)	7.46 ± 1.91	12.58 ± 1.05

Data represent the mean values of $n = 6 \pm$ SD.

Declaration of interest

The authors declare that all the information provided in this article is correct and there are no conflicts of interest.

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