Pharmaceutical Development and Technology, 2011, 1–11, Early Online

ISSN 1083-7450 print/ISSN 1097-9867 online
DOI: 10.3109/10837450.2011.557730

informa
healthcare

RESEARCH ARTICLE

Development of novel sustained release matrix pellets of betahistine dihydrochloride: effect of lipophilic surfactants and co-surfactants

Rehab Nabil Shamma, Emad B. Basalious, and Raguia Shoukri

Faculty of Pharmacy Cairo University, Cairo, Egypt

Abstract
Sustained release matrix pellets of the freely water soluble drug, betahistine dihydrochloride (BH), were prepared using freeze pelletization technique. Different waxes and lipids (cetyl alcohol, beeswax, glyceryl tripalmitate (GTP) and glyceryl tristearate) were evaluated for the preparation of matrix pellets. A D-optimal design was employed for the optimization and to explore the effect of drug loading (X_1), concentration of lipophilic surfactant (X_2), concentration of co-surfactant (X_3) and wax type (X_4) on the release extent of the drug from matrix pellets. The entrapment efficiency (Y_1), pellet diameter (Y_2), and the percentage drug released at given times were selected as dependent variables. Results revealed a significant impact of all independent variables on drug release from the formulated pellets. The lipophilic surfactant significantly increased both the entrapment efficiency and the *in vitro* drug release and significantly decreased the pellet size. The optimized BH-loaded pellets were composed of 19.95% drug loading, 9.95% Span® 80 (surfactant), 0.25% Capmul® (co-surfactant) using glyceryl tripalmitate as a matrix former. The release profiles of the drug from hard gelatin capsule containing optimized pellets equivalent to 32 mg BH was similar to that of target release model for once-daily administration based on similarity factor. It could be concluded that a promising once-daily capsule containing sustained release pellets of BH was successfully designed.

Keywords: Betahistine, D-optimal design, pellets

Introduction

Betahistine, a histamine analogue, is claimed to improve the microcirculation of the labyrinth resulting in reduced endolymphatic pressure. It is used to reduce the symptoms of vertigo, tinnitus, and hearing loss associated with Ménière's disease.[1] Betahistine comes in the form of dihydrochloride salt. Betahistine dihydrochloride (BH) is a freely water soluble drug. BH is rapidly and completely absorbed. The mean plasma half-life is 3–4 h.[1] The usual initial dose is 16 mg three times daily taken preferably with meals; maintenance doses are generally in the range of 24–48 mg daily. The very high solubility of the drug requires specific technologies in order to control the release in oral dosage forms.

Oral controlled-release dosage forms can be broadly classified into two groups: Single unit dosage forms, such as tablets or capsules, and multiple unit dosage forms, such as beads, and pellets.[2] Multiple unit controlled-release dosage forms can be further classified into reservoir and matrix systems. In the first case, a drug-containing core is surrounded by a membrane, which controls the release rate of the drug out of the dosage form. Although coated pellets are widely used in the pharmaceutical industry, their preparation is often complex, time consuming and expensive. On the other hand, the production of controlled-release matrix systems is generally much easier. In these systems, the drug is embedded within a carrier material, which controls the release rate of the drug out of the device. The physicochemical nature of the matrix determines the underlying drug release mechanisms and the resulting release patterns.[3]

Different techniques have been used to obtain controlled-release matrix pellets including extrusion spheronisation,[4,5] melt agglomeration, melt pelletization,[6–8] and melt solidification.[9] The freeze pelletization technique is a simple technique for preparing

Address for correspondence: Rehab Nabil Shamma, Faculty of Pharmacy Cairo University, Cairo, Egypt. E-mail: rehab_shamma@hotmail.com

(Received 15 November 2010; revised 03 January 2011; accepted 06 January 2011)

spherical drug loaded matrix pellets.[10] In this technique, molten solid carriers together with a dispersed drug are introduced as droplets into an inert and immiscible column of liquid. These droplets can move either upward or downward depending on their density with respect to the liquid in the column and then solidify into spherical pellets. Different carrier solids and liquids can be used as pellet forming material and column liquids in this freeze pelletization process.[11] The freeze pelletization technique had been investigated by Cheboynia et al. to prepare matrix pellets for controlled and immediate release of drugs.[11]

Recently, there has been increasing interest in using lipid excipients for the formulation of oral sustained release dosage forms. Windbergs et al.[12] studied the influence of varying the composition of tristearin and glyceryl monostearate on the dissolution profiles of solid lipid extrudates. In another study, Schulze and Winter examined the feasibility of using glyceryl tristearate (GTS) and glyceryl tripalmitate (GTP) in the preparation of lipid extrudates as a sustained release carrier for pharmaceutical proteins.[13]

Literature lacks any data about the formulation of BH as sustained release dosage form. The short half-life (3–4 h), frequent dosing (three times daily), and very high solubility of BH require specific technologies in order to control its release in oral formulations. Design of a new formulation of BH would be advantageous if it allows a once-daily administration. Thus, the aim of this work is the formulation of BH multiple unit sustained release dosage form for once-daily administration. D-optimal design was applied to optimize and study the effect of lipophilic surfactant and co-surfactant on the drug release, encapsulation efficiency and pellet diameter of BH-loaded matrix pellets. The main effects, the interaction effects and the quadratic effects of different variables on drug release, encapsulation efficiency and pellet diameter were investigated. The optimized formulation exhibiting promising *in vitro* drug dissolution is anticipated to control the delivery of the drug.

Materials

BH was kindly donated by Chemipharm, Egypt. Cetyl alcohol (CA), Glycerol, Beeswax, hydrochloride (HCl), and Na_3PO_4 were purchased from El-Nasr pharmaceutical company, Egypt. GTS, GTP, Span® 80 were obtained from Sigma-Aldrich Co., St. Louis, MO. Aeroperl® 300 pharma (granulated silicon dioxide) were obtained from Degussa, Germany. Capmul® MCM C8 (glyceryl monocaprylate) was obtained from Abitec Corp. (Janesville, WI).

Methods

Preparation of BH-loaded matrix pellets

Pelletization apparatus was built in-house with borosilicate glass tubes, as described by Cheboyina et al.[10] In this apparatus, the lengths of initial and cooling columns were 30 and 50 cm, respectively. These columns have an inner diameter of 2 cm.

The molten matrix was prepared by dispersing BH (10%) in a molten wax containing 5% Aeroperl®, maintained at 75°C using thermostatically controlled magnetic stirrer (Thermolyn Corporation, Dubuque, IA). Different matrix formers were examined; namely CA, Beeswax, GTS and GTP. The molten wax matrix was then withdrawn using a glass syringe having a beveled needle tip (Tuberculin screw type syringes). This molten matrix was slowly injected as droplets into a column containing an 80% (w/w) glycerol solution.[10] The initial column jacket temperature was maintained at 75°C, and the cooling column jacket temperature was kept at 15°C for all the studies, using thermostatically controlled water circulators (Falc Instruments, Lurano, Italy). The pellets were collected at the top of the column, washed with deionized water, and then air dried at 25°C for 24 h.

Evaluation of the prepared pellets

Determination of encapsulation efficiency of BH-loaded matrix pellets

Samples weighing ~100 mg were accurately weighed and transferred into a 100 mL volumetric flask containing 50 mL 0.1 N HCl (pH = 1.2). The flasks were immersed in a water bath maintained at 75°C for 2–3 min and shaken vigorously until all the pellets were completely melted. The flasks were cooled and 0.1 N HCl was added to bring the volume to 100 mL. The resulting suspensions were filtered through a 0.45 µm syringe filter and the drug content was spectrophotometrically analyzed at 261 nm (UV 160 1 PC UV-Visible, Shimadzu, Japan).[10]

Determination of the pellet diameter

For different batches of the pellets prepared, around 30 pellets were randomly selected, and their diameters were measured using a vernier caliper.

In vitro drug release studies from BH-loaded matrix pellets

Drug release from different BH-loaded matrix pellets was conducted according to USP 30 Apparatus II (paddle method). BH-loaded matrix pellets (200 mg) were placed in the dissolution medium maintained at 37 ± 0.5°C and mixed at 50 rpm (Pharma Test Dissolution Tester, Germany). The dissolution medium used in this study was 250 mL 0.1 N HCl (pH 1.1–1.2) for the first 2 h followed by addition of 100 mL 0.2 M Na_3PO_4 to raise the pH to 7.4 for the rest of 24 h. Samples were withdrawn at different time intervals, replaced by equal volumes of fresh medium, and the concentration of BH in the samples was measured spectrophotometrically at 261 nm.

Study of the effect of formulation parameters on properties of the prepared BH-loaded matrix pellets using D-optimal design

The drug loading (X_1), concentration of Span® 80 (surfactant) (X_2), concentration of Capmul® (co-surfactant) (X_3)

and wax type (X_4) were selected as the independent variables. The encapsulation efficiency (Y_1), pellet diameter (Y_2), and the percentage drug released at 1, 6 and 12 h (Y_3, Y_4, and Y_5, respectively) were selected as the dependent variables. The design parameters and experimental conditions are shown in Table 1. The responses of all model formulations were treated by Design-Expert® software (version 7; Stat-Ease Inc., Minneapolis, MN). Suitable models for D-optimal design include linear, two factor interaction, and quadratic models. These models provide several comparative measures for model selection. Predicted R^2 (multiple correlation coefficient) which gives a correlation between the experimental response and the predicted response, should be high for the model to be significant. Adjusted R^2 (adjusted multiple correlation coefficient) gives a similar correlation after ignoring the insignificant model terms, and should be in good agreement with predicted R^2 for the model to be fit. Predicted and adjusted R^2 should be within 0.20 of each other.[14] PRESS (predicted residual sum of square) indicates how well the model fits the data. PRESS, for the chosen model, should be small relative to the other models under consideration.[15]

D-optimal design was selected since it minimizes the variance associated with the estimates of the coefficients in the model.[16] The software selected a set of candidate points as a base design, such as factorial points (high and low level from the constraints on each factor, centers of edges, constraint plane centroids, axial check point, and an overall center point). The base design consisted of 36 runs. Table 2 shows the experimental runs, with independent variables and the measured responses of different BH-loaded matrix pellets.

Kinetic analysis of *in vitro* release data

The mean *in vitro* drug release data were fitted to Korsemeyer–Peppas equation[17] to evaluate the kinetics of drug release from the prepared matrices. To analyze the release mechanism of the drug from these matrices, the release data obtained were fit to a simple power equation:[17]

$$M_t/M_\infty = K\,t^n$$

Where M_t/M_∞ is the fraction of drug released at time t and k denotes the constant incorporating structural and geometrical characteristics of the drug/polymer system and the n is the diffusion exponent related to the mechanism of the drug release. For non-Fickian (anomalous) release from spheres, the *n* value falls between 0.43 and 0.85 (where release is controlled by a combination of diffusion and polymer relaxation) while for Fickian (Case I) diffusion, $n \leq 0.43$ ($t_{1/2}$ dependence) and for zero-order release (Case II transport), $n = 0.85$ where the drug release rate is independent of time and involves polymer relaxation and chain disentanglement.[18] It is important to note that for determination of the exponent n, only the initial portion of the release curve ($M_t/M_\infty \leq 0.6$) must be used.[17]

The value of K and n were estimated by linear regression of log (M_t/M_∞) on log (t) where log k is the intercept and n is the slope of the straight line.

$$\text{Log } M_t/M_\infty = \log K + n \log t$$

Morphological examination of the optimized BH-loaded matrix pellets

Morphological examination of the optimized BH-loaded matrix pellets was carried out using an optical computer microscope (Intel Corp., Santa Clara, CA).

Effect of storage on the BH-loaded matrix pellets

The optimized BH-loaded matrix pellet formulation was stored in at temperature of 40°C and 75% relative humidity according to the ICH guidelines for a period of 3 months. The relative humidity was maintained using sodium chloride saturated salt solution. Stability was assessed by comparing the results of *in vitro* dissolution studies before and after storage. The results were checked for statistical significance using the one-way analysis of variance (ANOVA) *F*-test for testing the equality of several means. A *p* value >0.05 was considered statistically insignificant.

Results and discussion

The aim of this work was to develop new sustained release matrix pellets of BH, a highly water soluble drug, to sustain the drug release for once-daily administration. An ideal drug release profile (i.e. 8% in the first h and a constant drug release thereafter releasing 100% in 18 h) was considered as a target release profile suitable for once-daily administration of the drug.

Preparation of BH-loaded matrix pellets

In the freeze pelletization process, a suspending agent was considered necessary to maintain the homogeneity of drug distribution in the wax suspensions,[10] therefore, 5% Aeroperl® was added as a suspending agent in all the molten suspensions. Glycerol solution 80% (w/w) was chosen as a suitable column liquid for producing spherical pellets based on a previous study

Table 1. Design parameters and experimental conditions for D-optimal design.

Independent variables	Levels of variables
X_1: Drug loading %	10% 15% 20%
X_2: Span 80%	0% 5% 10%
X_3: Capmul %	0% 2.5% 5%
X_4: Wax type	CA Beeswax GTP GTS
Responses	Constraints
Y_1: Encapsulation efficiency	80–100%
Y_2: Pellet diameter	3–4 mm
Y_3: % BH released after 1 h	$5\% \leq Y_3 \leq 11\%$
Y_4: % BH released after 6 h	$27.5\% \leq Y_4 \leq 42\%$
Y_5: % BH released after 12 h	$60\% \leq Y_5 \leq 75\%$

Table 2. Formulations of the experimental design and their response results.

Run	X_1: Drug loading (%)	X_2: Span® 80 concentration (%)	X_3: Capmul® concentration (%)	X_4: Wax type	Y_1 (%)	Y_2 (mm)	Y_3 (%)	Y_4 (%)	Y_5 (%)
1	15	10	0	CA	96.65	3.49	9.12	53.82	66.26
2	20	5	2.5	GTP	105.29	3.62	13.80	41.86	57.26
3	20	5	5	CA	53.23	3.39	26.94	69.41	74.08
4	20	10	0	GTP	109.80	3.74	13.52	35.70	53.10
5	20	0	0	GTS	24.70	3.65	3.77	19.69	27.27
6	20	0	2.5	Beeswax	23.50	3.45	1.04	7.92	11.28
7	20	10	5	GTS	101.44	3.74	18.47	39.91	50.55
8	15	5	5	Beeswax	58.85	3.60	2.11	9.40	15.58
9	15	10	0	Beeswax	49.88	3.01	1.42	9.87	15.65
10	10	0	0	CA	25.00	3.47	4.16	9.72	23.36
11	15	0	0	GTP	44.27	3.70	4.76	13.58	17.33
12	15	5	2.5	GTS	54.92	3.61	18.64	37.50	46.55
13	20	0	5	GTP	78.31	3.41	12.18	32.44	45.69
14	20	10	2.5	CA	65.30	3.30	24.55	66.47	73.97
15	20	10	5	GTS	93.40	3.80	18.52	37.75	48.05
16	15	0	5	CA	43.93	3.41	15.14	44.73	57.90
17	10	10	5	GTS	102.00	3.46	10.06	24.50	29.80
18	10	0	5	GTS	85.00	4.17	3.98	12.37	19.06
19	10	0	5	GTP	20.79	4.14	1.83	3.66	18.47
20	20	10	5	Beeswax	104.65	3.29	3.63	9.63	16.63
21	10	10	0	GTS	101.00	3.54	6.18	18.44	30.26
22	20	0	2.5	Beeswax	33.50	3.28	2.00	10.00	14.00
23	15	10	5	GTP	92.12	3.56	31.25	61.85	72.47
24	10	10	0	GTS	98.00	3.48	8.14	22.34	32.26
25	10	10	0	GTP	104.20	3.85	9.44	23.52	31.60
26	10	10	5	CA	102.00	3.00	16.68	62.49	71.69
27	10	0	5	Beeswax	72.77	3.55	2.24	8.56	13.28
28	10	0	0	Beeswax	13.69	3.60	0.80	8.71	13.26
29	10	5	2.5	GTP	100.90	3.67	7.22	18.89	27.29
30	10	10	2.5	Beeswax	104.90	3.55	4.70	13.00	19.90
31	10	10	5	CA	99.00	3.10	15.88	63.75	71.93
32	20	0	0	CA	63.68	3.44	8.37	44.92	60.16
33	20	5	0	Beeswax	75.47	3.34	5.35	13.99	21.37
34	15	0	0	GTP	42.00	3.80	3.48	13.72	18.43
35	10	0	5	GTS	82.00	4.20	4.45	13.32	22.17
36	10	0	0	CA	29.00	3.50	4.16	9.72	23.36

by Cheboyina et al.[10] They observed that in low viscosity column liquids (<70% w/w glycerol solutions), the spherical shape of the droplets was distorted as they rapidly ascend the column resulting in disc shaped particles upon solidification. Their results concluded that as the viscosity of column liquid increased, convection of the liquid medium was minimized and also the droplets ascend the column at a slower rate, which helped in preserving the spherical shape of the pellets. However, at much higher viscosities (>80% w/w glycerol solutions), movement of the droplets was very slow and led to the agglomeration of pellets.

Evaluation of the prepared BH-loaded matrix pellets

Table 3 shows the average pellet diameter, and average encapsulation efficiencies of BH in matrix pellets prepared using four different matrix formers. The pellet diameter ranged between 3.4 and 3.9 mm. The highest encapsulation efficiency was obtained with BH-loaded matrix pellet of GTP showing a value of 37.5%.

Table 3. The average pellet diameter and the average encapsulation efficiencies of BH in matrix pellets prepared using four different matrix formers.

Matrix former	Particle diameter (mm)	Encapsulation efficiency (%)
CA	3.47 ± 0.26	25.17 ± 5.54
GTP	3.85 ± 0.18	37.50 ± 0.48
GTS	3.87 ± 0.23	8.86 ± 0.55
Beeswax	3.60 ± 0.23	13.69 ± 4.12

Data are mean values ± SD.

The release profiles of BH from matrix pellets prepared using four different matrix formers are graphically illustrated in Figure 1. The *in vitro* release profiles indicated that the release of BH significantly depended on the type of wax used as a matrix former.

The cumulative percentages BH released in 24 h were 56.69, 15.53, 36.93, and 24.35% from CA, Beeswax, GTP, and GTS based pellets, respectively. The release data shows the impact of the fatty acid chain length. This difference in the release profiles can be attributed to the chemical nature and the relative hydrophobicity of the waxes.[6,19,20] CA is an aliphatic alcohol (C_{16}) with one-OH group, therefore is more susceptible to hydration by the dissolution media. Accordingly, the release of BH was found to be much higher for CA pellets when compared to the release obtained for other wax pellets. GTS and GTP are fatty acid esters of glycerol. GTS (C_{18}) chain length is longer compared to GTP (C_{16}). Beeswax primarily consists of various esters of straight-chain monohydric alcohols with even-numbered carbon chains (C_{24}–C_{36}) esterified with straight-chain acids. According to Cheboyina et al., as the hydrophobicity of the wax increased, the percentage drug released at different time intervals decreased.[21]

Statistical analysis of D-optimal experimental design

Preliminary studies were performed to study the effects of lipophilic surfactants (Span® 80) and/or co-surfactant (Campul®) on the *in vitro* drug release from matrix pellets. It was observed that the presence of Span® 80 and Campul® in the pellet matrix remarkably increased the drug release (data not shown). The positive effect of lipophilic surfactant and co-surfactants on the *in vitro* drug release from matrix pellets encouraged us to study their effects on encapsulation efficiency, pellet size, and *in vitro* drug release. Sorbitan monooleate, known as Span® 80, is a nonionic surfactant. It is variously used as a dispersing agent, emulsifier, and stabilizer. Capmul® (glyceryl monocaprylate), added as a co-surfactant, is a medium chain monoglyceride. It was reported that medium chain monoglycerides (polar lipids) promote water penetration and self dispersibility of lipid formulations and have good solvent capacity for drugs.[22] Moreover, Capmul® is likely to increase the interfacial fluidity of surfactant boundaries in the micelles because of the entrapment of Capmul® in the surfactant enhancing the emulsification process upon dilution with the aqueous medium.[23]

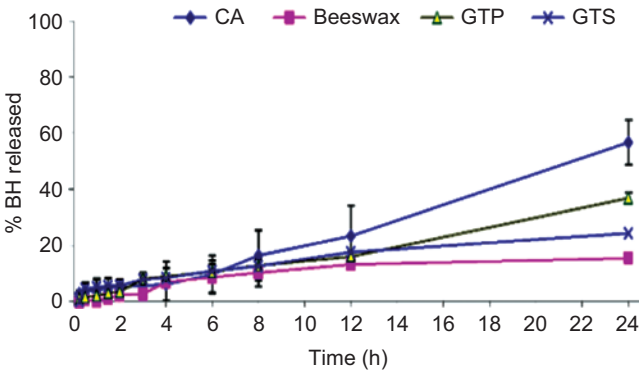

Figure 1. Release profile of BH from BH matrix pellets prepared using four different matrix formers. (See colour version of this figure online at www.informahealthcare.com/phd)

D-optimal experimental design was used to study the effect of concentrations of drug, lipophilic surfactant (Span® 80) and co-surfactant (Capmul®) on the different responses of the different wax formers. D-optimal design enables us to optimize BH-loaded matrix pellets and conclude mathematical equations correlating all the previously mentioned formulation variables with the different properties of matrix pellets. Table 4 shows the regression results of the measured responses. The approximation of response values of the encapsulation efficiency, and pellet diameter, percentage BH released after 1 h based on the linear model was the most suitable because it showed high values for R^2, and good agreement for the adjusted and predicted values of R^2, and the lowest value for PRESS, while the quadratic model, and the two factor interaction model were selected for the approximation of response values of the percentage BH released after 6 h, and 12 h, respectively. It can be observed that the values of R^2, predicted R^2 and adjusted

Table 4. Regression results of the measured responses.

Model		Y_1	Y_2	Y_3	Y_4	Y_5
Linear	SD	20.3100	0.1900	4.5500	9.7876	9.4200
	R^2	0.5905	0.5630	0.7206	0.8013	0.8381
	Predicted R^2	0.5163	0.4023	0.5748	0.6893	0.7498
	Adjusted R^2	0.5657	0.5067	0.6628	0.7601	0.8046
	PRESS	16086.33	1.60	913.38	4342.32	3975.75
2FI	SD	19.8796	0.1522	3.6258	5.6996	5.7900
	R^2	0.7980	0.8526	0.8960	0.9605	0.9578
	Predicted R^2	–0.0968	0.0828	0.1671	0.6620	0.8402
	Adjusted R^2	0.5841	0.6965	0.7858	0.9187	0.9261
	PRESS	36474.02	2.45	1789.46	4723.94	2539.13
Quadratic	SD	18.6670	0.1648	3.1635	4.4400	5.3587
	R^2	0.8533	0.8578	0.9348	0.9760	0.9747
	Predicted R^2	–0.6779	–0.5425	–0.0167	0.8236	0.6604
	Adjusted R^2	0.6332	0.6444	0.8370	0.9506	0.9368
	PRESS	55799.69	4.12	2184.47	2466.24	5395.61

R^2 are in good agreement resulting in a reliable model. The initial model was refined by including in the model only those terms for which the level of significance was below or equal to $p \leq 0.05$.

Effect of formulation factors on the encapsulation efficiency

ANOVA test was performed to evaluate the level of significance of the tested factors on the encapsulation efficiency of different BH-loaded matrix pellets. Figure 2 shows the response surface plot of the effect of Span® 80 concentration (X_2), and Capmul® concentration (X_3) on drug encapsulation efficiency of BH-loaded matrix pellets. Results show that only the Span® 80 concentration (X_2) had a significant effect on the encapsulation efficiency ($p < 0.0001$). Increasing the Span® 80 concentration from 0% to 10 % resulted in significant increase in the encapsulation efficiency. The increase in the drug encapsulation with increase in the Span® 80 concentrations could be ascribed to the solubilizing effect of the surfactant which helped the drug to be finely dispersed and embedded in the polymer matrix prior to encapsulation.[24] Similar results were obtained by Khoee and Yaghoobian in a study on investigating the role of surfactants on the properties of polymeric nanocapsules.[25] They found that the yield of encapsulation of penicillin-G double emulsions increased from 21.85% to 59.22% with increasing the concentration of Span® 60 in the internal phase from 1.4% to 7%.

Effect of formulation factors on the pellet diameter:

Statistical analysis using ANOVA showed that the wax type had a significant impact on the pellet diameter ($p < 0.0001$). It is clear that GTS matrix pellets showed the largest size followed by GTP, beeswax, and finally CA (Table 2). A significant impact of the concentration of lipophilic surfactant (Span® 80) on the pellet diameter was demonstrated. Increasing the Span® 80 concentration from 0% to 10 % resulted in significant decrease in the pellet size ($p = 0.0045$).

Similar results were obtained by Cheboyina and Wyandt in a study on investigating the formulation variables affecting pellet characteristics manufactured by the freeze pelletization technique.[10] They found that a reduction in the surface/interfacial tension can be achieved, by the addition of a surfactant, which may result in smaller pellets. Their results showed that Precirol® pellets containing 0% and 2% (w/w) Brij® 76 were significantly larger than the pellets containing 10% (w/w) Brij® 76. As Brij® concentration increases in the matrix, the density of the matrix also increases because Brij® replaces some parts of Precirol® and the density of Brij® is higher than that of Precirol®. This increase in matrix density decreases the density difference between the matrix and the glycerol solution. Based on their mathematical model, as the density difference decreases, pellet size decreases only if the interfacial tension decreases.[26] Therefore, the decrease in pellet size with increase in surfactant concentration was due to the decrease in the interfacial tension between the matrix and the glycerol solution. Similar results were also obtained by Khoee and Yaghoobian in a study on investigating the role of surfactants in controlling the particle size of polymeric nanocapsules. They found that increasing Span® 20 and Span® 60 concentrations from 1.4% to 7%, the particle sizes reduces from 638 nm to 416 nm and from 356 nm to 240 nm, respectively. They observed that the particle size depends on the balance between type and concentration of the internal surfactant.[25] According to Khoee and Yaghoobian, the same parameters that could increase the encapsulation efficiency, could also decrease the particle size.[25]

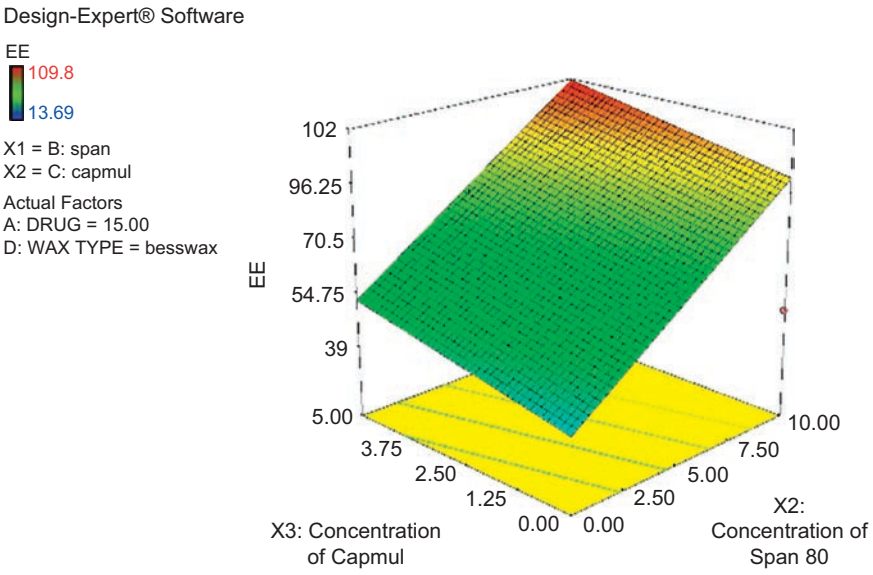

Figure 2. Response surface plot of the effect of concentration of Span® 80 (X_2), and concentration of Capmul® (X_3) on the encapsulation efficiency of BH matrix pellets. (See colour version of this figure online at www.informahealthcare.com/phd)

Effect of formulation factors on the drug release

ANOVA test was performed to evaluate the level of significance of the tested factors on the percentage BH released from different matrix pellets at 1, 6, and 12 h as well as the interactions between these factors. Figure 3 shows the impact of the tested factors on the percentage BH released from the matrix pellets after 1 h (Y_3) (similar responses were obtained after 6 h (Y_4), and 12 h (Y_5), data not shown).

A significant impact of the drug loading on the drug release was demonstrated. Increasing the drug loading from 10% to 20% resulted in significant increase in the percentage BH released after 1, 6, and 12 h ($p = 0.002$, $p \square 0.0001$, and $p \square 0.0001$, respectively). Similar results were obtained by Cheboyina and Wydant in a study on preparing sustained release matrix pellets of diltiazem HCl.[21] They found that the drug release increased significantly as diltiazem HCl loading increased in Precirol® pellets. Precirol® pellets containing 10% and 20% (w/w) diltiazem HCl released >90% of the drug in about 9 h and 6 h, respectively, compared to only 87% of the total drug released at the end of 24 h from pellets containing 2% (w/w) diltiazem HCl.[21] The effect of increasing the drug loading on enhancing the drug release was more pronounced for CA pellets, followed by GTP, and GTS. However, it has no remarkable effect on beeswax pellets (Table 2).

A significant impact of the concentration of lipophilic surfactant (Span® 80) on the drug release was demonstrated. Increasing the Span® 80 concentration from 0% to 10% resulted in significant increase in the percentage BH released after 1, 6, and 12 h ($p = 0.0002$, $p \square 0.0001$, and $p \square 0.0001$, respectively). Similar results were obtained by Cheboyina and Wydant in a study on preparing theophylline sustained release matrix pellets.[21] They found that Precirol® pellets containing 5 and 10% (w/w) Brij® 76 released 100% theophylline by the end of 24 h. However, only 21 and 51% of the total drug were released at the end of 24 h from Precirol® pellets containing 0 and 2% (w/w) Brij® 76, respectively.[21] This result correlates well with the results of BH encapsulation efficiency, where increasing the concentration of lipophilic surfactant (Span® 80) resulted in increasing the drug encapsulation efficiency, increasing the concentration gradient of the drug in

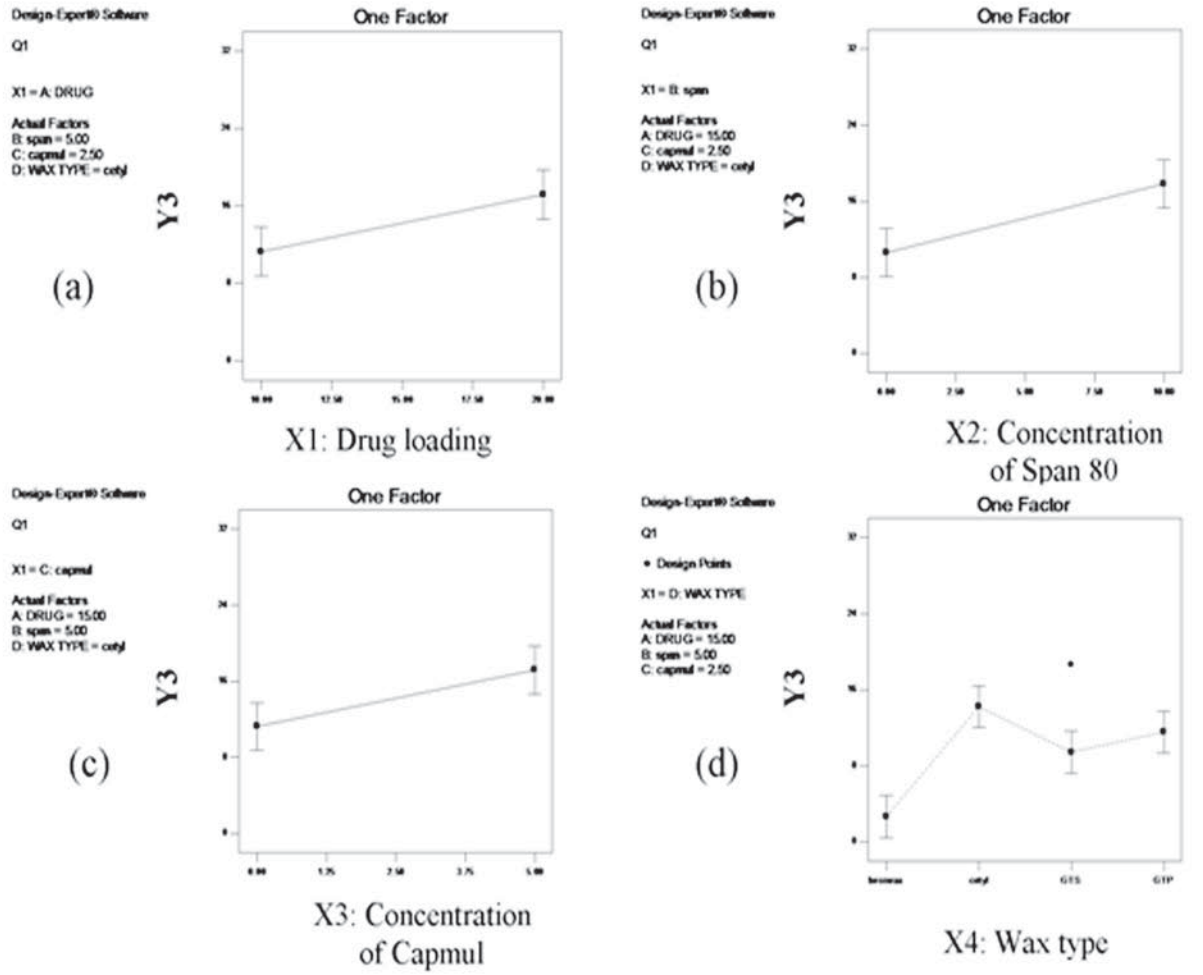

Figure 3. Effect of the independent variables on the percentage BH released after 1 h. (A) X_1: Drug loading % (B) X_2: Concentration of Span® 80%. (C) X_3: Concentration of Capmul® % (D) X_4: Wax type.

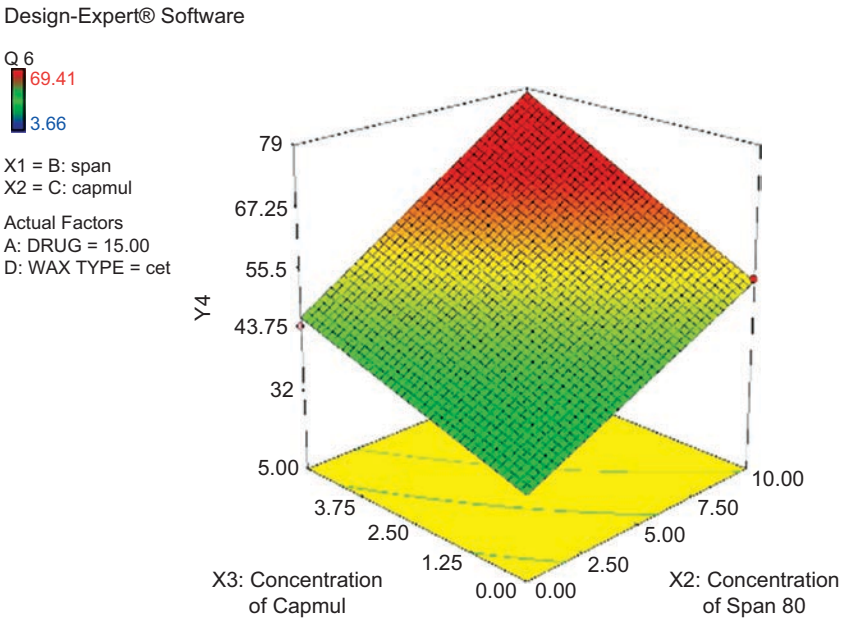

Figure 4. Response surface plot of the effect of the concentration of Span® 80 (X_2), and concentration of Capmul® (X_3) on the percentage BH released after 6 h. (See colour version of this figure online at www.informahealthcare.com/phd)

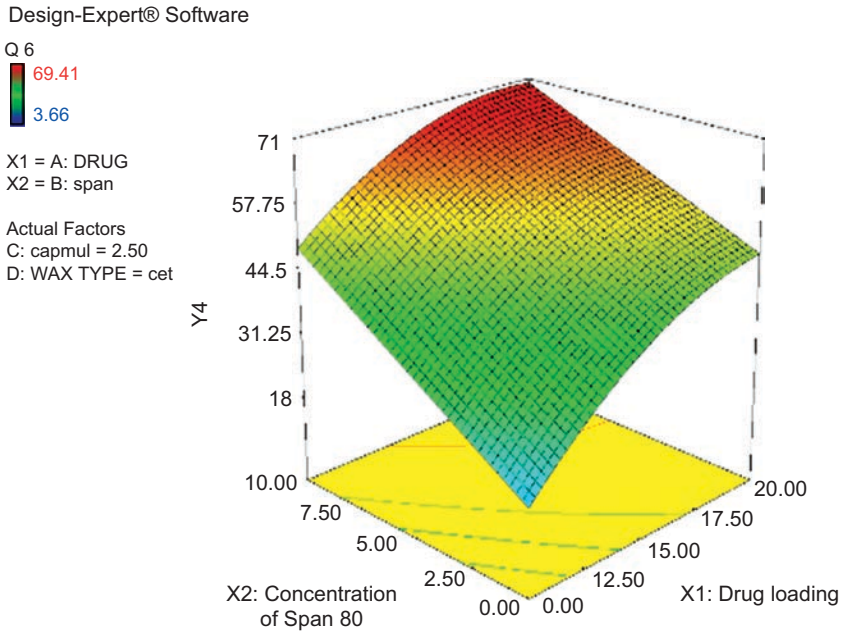

Figure 5. Response surface plot of the effect of drug loading (X_1), and concentration of Span® 80 (X_2) on the percentage BH released after 6 h. (See colour version of this figure online at www.informahealthcare.com/phd)

the matrix pellets, and hence increasing percentage BH released after 1, 6, and 12 h. This result correlates also with the results of pellet diameter measurement, where increasing the concentration of lipophilic surfactant (Span® 80) resulted in decreasing the pellet diameter, increasing the surface area available for drug release, and hence increasing percentage BH released after 1, 6, and 12 h. The effect of increasing the concentration of lipophilic surfactant on enhancing the drug release was more pronounced for CA pellets, followed by GTP, and GTS. However, it has no remarkable effect on beeswax pellets (Table 2).

A significant impact of the concentration of co-surfactant (Capmul®) on the drug release was demonstrated. Increasing the Capmul® concentration from 0% to 5% resulted in significant increase in the percentage BH released after 1, 6, and 12 h ($p = 0.0016$, $p \square 0.0001$, and $p \square 0.0002$, respectively). The effect of increasing the concentration of Capmul® on enhancing the drug release was more pronounced for CA pellets, followed by GTP, and GTS. However, it has no remarkable effect on beeswax pellets (Table 2).

Figure 4 shows the response surface plot of the effect of the Span® 80 concentration (X_2), and Capmul®

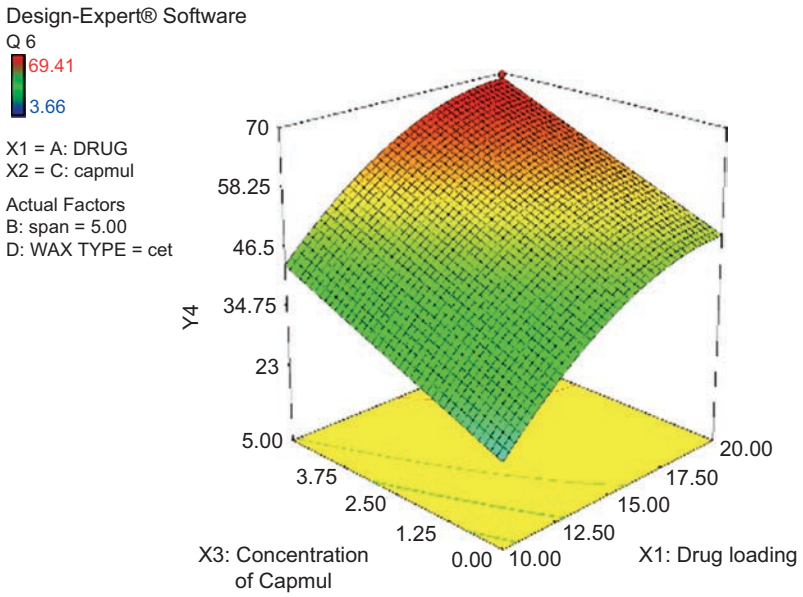

Figure 6. Response surface plot of the effect of drug loading (X_1), and concentration of Capmul® (X_3) on the percentage BH released after 6 h. (See colour version of this figure online at www.informahealthcare.com/phd)

concentration (X_3) on the percentage BH released after 6 h (Y_4). Increasing Span® 80 concentration increased with greater extent the percentage BH released for matrix pellets with high Capmul® concentration than those with low Capmul® concentration. This is attributed to the synergistic effect of the lipophilic surfactant and co-surfactant on increasing the release of the drug from the matrix pellets.

Figures 5 and 6 show the response surface plots of the effect of the drug loading (X_1)/Span® 80 concentration (X_2) and the drug loading (X_1)/Capmul® concentration (X_3) on the percentage BH released after 6 h (Y_4). It is obvious that the highest drug release was obtained at the highest levels of drug loading, surfactant and co-surfactant.

Kinetic analysis of in vitro release data

The kinetic analysis of the *in vitro* release data of BH from BH-loaded matrix pellets are presented in Table 5. The values of n were >0.43 and <0.85 indicating non-Fickian (anomalous) transport for BH-loaded pellets prepared according to formulae R1, R2, R5, R7, R14, R26, R28, R29, R31, and R35, while the rest of formulae showed Fickian transport.

Optimization

In order to find the level of each independent variable that will lead to an optimized formulation, the optimization process was performed for X_1, X_2, X_3 and X_4 using the following target ranges; encapsulation efficiency (Y_1) 80–100%, and pellet diameter (Y_2) 3–4 mm, 8%≤ Y_3 ≤16%; 35%≤ Y_4 ≤ 50% and 60%≤ Y_5 ≤75%,. The target ranges of Y_3, Y_4, Y_5 were determined based on the target release model deduced form zero-order dissolution profile of BH for once-daily administration. The optimum values of the variables were obtained by graphical and numerical analyses using the Design-Expert® software and based on the criterion of desirability.[22] The optimized formulation was composed of 19.95% drug loading, 9.95% Span® 80, 0.25% Capmul® using GTP as a matrix former. The optimized formulation was prepared to confirm the validity of the optimization procedure. Pellets of the optimized formulation equivalent to 32 mg BH were filled into hard gelatin capsule, and subjected to *in vitro* release study. The predicted and observed responses of the optimized formulation of BH matrix pellets are presented in Table 6. Results show that the observed values of the new batch were mostly similar to the predicted values. The release profiles of the optimized formulation and the target release model are presented in Figure 7. These dissolution profiles were compared using the similarity factor ($f2$). The calculated value of $f2$ was 52, indicating that the dissolution profile of the optimized pellet formulation is similar to that of the target release model.[27] Kinetic analysis of the *in vitro* dissolution study of the optimized formulation showed Fickian (Case I) diffusion transport (n = 0.381).

Morphological examination of the optimized BH-loaded matrix pellets

The appearance and the surface morphology of the prepared optimized BH-loaded matrix pellets were examined. Figure 8 shows a photograph of the optimized matrix pellets. The prepared pellets were found to be discrete, non-aggregated, and spherical in shape.

Effect of storage on BH-loaded matrix pellets

Figure 9 shows the release profiles of the optimized BH-loaded matrix pellets before and after storage. Stability studies showed that there is no significant difference in the *in vitro* dissolution studies of the selected formulation after storage for 3 months (p > 0.05) com-

Table 5. Kinetic analysis of the *in vitro* release data of BH from BH-loaded matrix pellets.

Formulae	R^2	*n*	Release order
R1	0.977382	0.55171	Non-Fickian
R2	0.999626	0.502681	Non-Fickian
R3	0.989894	0.386489	Fickian
R4	0.998606	0.347351	Fickian
R5	0.987894	1.028914	Non-Fickian
R6	0.928451	0.391543	Fickian
R7	0.977349	0.552064	Non-Fickian
R8	0.983458	0.279079	Fickian
R9	0.963682	0.234836	Fickian
R10	0.989819	0.282964	Fickian
R11	0.97314	0.422483	Fickian
R12	0.996324	0.311979	Fickian
R13	0.996771	0.409086	Fickian
R14	0.990012	0.812476	Non-Fickian
R15	0.994684	0.239328	Fickian
R16	0.997956	0.409876	Fickian
R17	0.983716	0.26795	Fickian
R18	0.994618	0.30361	Fickian
R19	0.862387	0.329367	Fickian
R20	0.997764	0.211971	Fickian
R21	0.995791	0.366865	Fickian
R22	0.895537	0.326214	Fickian
R23	0.998166	0.378008	Fickian
R24	0.997281	0.359549	Fickian
R25	0.986803	0.243792	Fickian
R26	0.954726	0.571799	Non-Fickian
R27	0.996574	0.248661	Fickian
R28	0.86101	2.245479	Non-Fickian
R29	0.99833	0.293628	Fickian
R30	0.986734	0.118327	Fickian
R31	0.96941	0.530868	Non-Fickian
R32	0.978966	0.48878	Non-Fickian
R33	0.999924	0.280861	Fickian
R34	0.99984	0.243097	Fickian
R35	0.995247	0.43629	Non-Fickian
R36	0.906068	0.295185	Fickian

Table 6. Predicted and observed responses of the optimized formulation of BH-loaded matrix pellets.

Response	Predicted values	Observed values	Error %
Y_1	90.07%	95.00%	5.47
Y_2	3.59 mm	3.50 mm	2.50
Y_3	15.86%	16.13%	1.70
Y_4	43.92%	47.11%	7.26
Y_5	61.42%	63.85%	3.95

pared to as before storage. The stored pellets maintained its shape and appearance during storage.

Conclusion

A novel multiple unit sustained release matrix pellets of BH with satisfactory release characteristics were successfully prepared. A mathematical model was developed connecting the important formulation variables with the measured

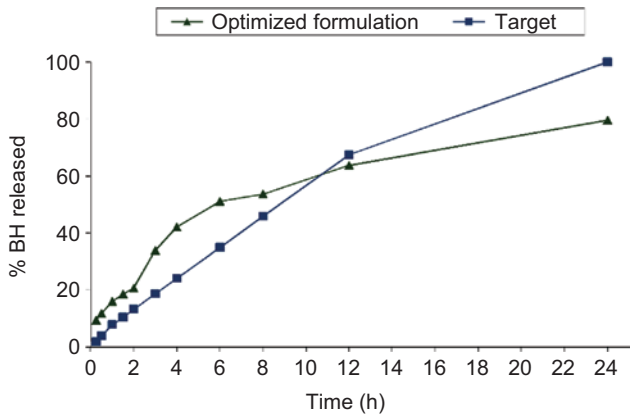

Figure 7. Release profiles of the optimized formulation and the target model. (See colour version of this figure online at www.informahealthcare.com/phd)

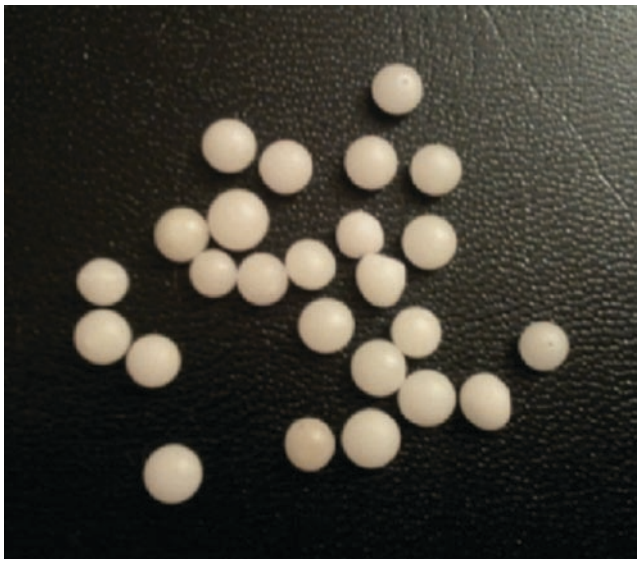

Figure 8. A photograph of the optimized BH-loaded matrix pellets. (See colour version of this figure online at www.informahealthcare.com/phd)

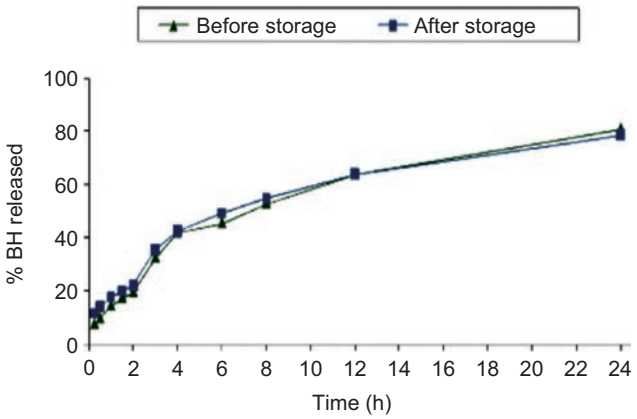

Figure 9. Release profiles of the selected BH-loaded matrix pellet formulation before and after storage. (See colour version of this figure online at www.informahealthcare.com/phd)

responses. The release profile of the optimized BH-loaded matrix pellets, composed of 19.95% drug loading, 9.95% Span® 80, 0.25% Capmul® using GTP as a matrix former, was comparable to that of the target release model deduced form zero-order dissolution profile of BH for once-daily administration. Further studies for *in vivo* evaluation of controlled-release solid dosage forms of the optimized BH-loaded matrix pellets are presently investigated.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

References

1. Sweetmant SC. MartinDale: The Complete Drug Reference: Pharmaceutical Press; 2009.
2. Lopes CM, Lobo JM, Pinto JF, Costa P. Compressed mini-tablets as a biphasic delivery system. Int J Pharm 2006;323:93–100.
3. Gandhi R, Lal Kaul C, Panchagnula R. Extrusion and spheronization in the development of oral controlled-release dosage forms. Pharm Sci Technol Today 1999;4:160–170.
4. Dupont G, Flament MP, Leterme P, Farah N, Gayot A. Developing a study method for producing 400 microm spheroids. Int J Pharm 2002;247:159–165.
5. Flament MP, Dupont G, Leterme P, Farah N, Gayot A. Development of 400 microm pellets by extrusion-spheronization: application with Gelucire 50/02 to produce a "sprinkle" form. Drug Dev Ind Pharm 2004;30:43–51.
6. Thomsen LJST, and Kristensen HG. Prolonged release matrix pellets prepared by melt pelletization II. Hydrophobic substances as meltable binders. Drug Dev Ind Pharm 1994;20:1179–1197.
7. Vergote GJ, Vervaet C, Van Driessche I, Hoste S, De Smedt S, Demeester J et al. *In vivo* evaluation of matrix pellets containing nanocrystalline ketoprofen. Int J Pharm 2002;240:79–84.
8. Hamdani J, Moës AJ, Amighi K. Development and evaluation of prolonged release pellets obtained by the melt pelletization process. Int J Pharm 2002;245:167–177.
9. Maheshwari M, Ketkar AR, Chauhan B, Patil VB, Paradkar AR. Preparation and characterization of ibuprofen-cetyl alcohol beads by melt solidification technique: effect of variables. Int J Pharm 2003;261:57–67.
10. Cheboyina S, Wyandt CM. Wax-based sustained release matrix pellets prepared by a novel freeze pelletization technique I. Formulation and process variables affecting pellet characteristics. Int J Pharm 2008;359:158–166.
11. Cheboyina S, Chambliss WG, Wyandt CM. A novel freeze pelletization technique for preparing matrix pellets. Pharm Technol 2004;28:98–110.
12. Windbergs M, Strachan CJ, Kleinebudde P. Influence of the composition of glycerides on the solid-state behaviour and the dissolution profiles of solid lipid extrudates. Int J Pharm 2009;381:184–191.
13. Schulze S, Winter G. Lipid extrudates as novel sustained release systems for pharmaceutical proteins. J Control Release 2009;134:177–185.
14. Gonnissen Y, Gonçalves SI, De Geest BG, Remon JP, Vervaet C. Process design applied to optimise a directly compressible powder produced via a continuous manufacturing process. Eur J Pharm Biopharm 2008;68:760–770.
15. Huang YB, Tsai YH, Yang WC, Chang JS, Wu PC, Takayama K. Once-daily propranolol extended-release tablet dosage form: formulation design and in vitro/*in vivo* investigation. Eur J Pharm Biopharm 2004;58:607–614.
16. Holm R, Jensen IH, Sonnergaard J. Optimization of self-microemulsifying drug delivery systems (SMEDDS) using a D-optimal design and the desirability function. Drug Dev Ind Pharm 2006;32:1025–1032.
17. Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers. Pharm Acta Helv 1985;60:110–111.
18. Harland RS, Gazzaniga A, Sangalli ME, Colombo P, Peppas NA. Drug/polymer matrix swelling and dissolution. Pharm Res 1988;5:488–494.
19. Adeyeye CM, Price JC. Development and evaluation of sustained-release ibuprofen-wax microspheres. II. *In vitro* dissolution studies. Pharm Res 1994;11:575–579.
20. Duclos R, Bourret E, Brossard C. Rheology of polyol behenates and drug release from matrix monolithic capsules. Int J Pharm 1999;182:145–154.
21. Cheboyina S, Wyandt CM. Wax-based sustained release matrix pellets prepared by a novel freeze pelletization technique II. *In vitro* drug release studies and release mechanisms. Int J Pharm 2008;359:167–173.
22. Basalious EB, Shawky N, Badr-Eldin SM. SNEDDS containing bioenhancers for improvement of dissolution and oral absorption of lacidipine. I: development and optimization. Int J Pharm 2010;391:203–211.
23. Taha EI, Al-Saidan S, Samy AM, Khan MA. Preparation and *in vitro* characterization of self-nanoemulsified drug delivery system (SNEDDS) of all-trans-retinol acetate. Int J Pharm 2004;285:109–119.
24. Shivakumar HN, Patel R, Desai BG. Formulation optimization of propranolol hydrochloride microcapsules employing central composite design. Indian J Pharm Sci 2008;70:408–413.
25. Khoee S, Yaghoobian M. An investigation into the role of surfactants in controlling particle size of polymeric nanocapsules containing penicillin-G in double emulsion. Eur J Med Chem 2009;44:2392–2399.
26. Cheboyina S, O'Haver J, Wyandt CM. A mathematical model to predict the size of the pellets formed in freeze pelletization techniques: parameters affecting pellet size. J Pharm Sci 2006;95:167–180.
27. Moore JW, Flanner HH. Mathematical comparison of dissolution profiles. Pharm Tech 1996;20:64–74.