Songklanakarin J. Sci. Technol.
44 (5), 1314–1322, Sep. – Oct. 2022

http://www.sjst.psu.ac.th

Original Article

Domperidone loaded *Caesalpinia pulcherrima* galactomannan-based microspheres for nasal administration: *In-vitro* and *In-vivo* studies

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Received: 2 September 2021; Revised: 17 August 2022; Accepted: 13 September 2022

Abstract

Caesalpinia pulcherrima galactomannan (CPG) based Domperidone (DOM) spray-dried mucoadhesive microspheres for nasal administration were developed using 2^3 FD to avoid first-pass metabolism, with improved therapeutic efficiency for treatment of nausea and vomiting as an alternative therapy to parenterals. Concentration of polymer, inlet temperature, and aspirator speed were selected for manipulated independent variables, whereas %entrapment efficiency and %drug release were the dependent variables. The developed microspheres were evaluated for particle size, entrapment efficiency, zeta potential, swelling ability, *in-vitro* mucoadhesion, *in-vitro* drug release, and DSC and XRD responses. The developed formulation was studied in rabbit for screening nasal absorption potential. The formulation possesses *in vitro* drug release from 62.87% to 94.80%, entrapment efficiency from 89.69% to 98.64%, and mucoadhesion from 73.6 to 82.47% across goat nasal mucosa. *In vivo* study in rabbit showed that DOM microspheres provide quick drug absorption, and enhanced bioavailability of drug upon nasal administration as compared to oral route. Thus, the developed DOM based formulation can be alternative to oral and parenteral formulations.

Keywords: Domperidone, *Caesalpinia pulcherrima* galactomannan, mucoadhesive microspheres, NDDS, spray drying

1. Introduction

Domperidone (DOM) is a potent antiemetic, anti-migraine API. DOM based tablet and suspension give 18% bioavailability with a plasma half-life effect of about 7hrs, whereas rapid IV injection has been shown to cause cardiac arrhythmias (Yadav & Mote, 2008). DOM microspheres can be developed due to short half-life, avoiding first-pass metabolism for increased bioavailability on frequent administration (Deshmukh, Malia, & Ghorpade, 2012).

In the last few decades, nasal mucosa has been widely explored as an alternative route for noninvasive DDS. Nasal mucosa has a large surface area with rich blood supply, which helps to absorb drugs rapidly (Rakesh & Khan, 2015). However, some difficulties with nasal route like mucociliary clearance and ciliary action reduce drug contact time with the mucosa decreasing drug absorption (Dhakar, Maurya, Tilak, & Gupta, 2011).

Mucoadhesive polysaccharide-based formulation facilitates a longer residence time of the drug with increased bioavailability (Duygu *et al.*, 2010). In the current research study CPG was used as a mucoadhesive polysaccharide in the development of spray-dried DOM microspheres. Per a literature survey, *Caesalpinia pulcherrima* from *Leguminosae* has pharmacological activities: antiulcer, antibacterial, analgesic, anti-inflammatory, antimicrobial, antifungal, cytotoxic, and antitumor. CPG is a hydrophilic polysaccharide with high molar mass and high viscosity (Thombre & Gide, 2013). CPG is a natural polymer, biodegradable, nontoxic, and nonirritant to mucous membrane, adhering covalently to the mucosa and facilitating drug transport across mucous membrane due to strong contact of a CPG-based formulation (Ige, Agrawal, & Patil, 2015; Surywanshi, Thakare, More, & Thombre, 2015).

Microspheres have potential for targeted and controlled release drug delivery, but providing mucoadhesive properties to the microspheres brings along additional advantages.

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Microspheres prepared by spray drying have high drug loading capacity and the process is feasible for scale-up, more so than other microsphere fabrication methods (Lena, 1998). Spray-drying provides efficient encapsulation, narrow capsule size distribution, and particles with little residue of an organic solvent. As per the US-FDA regulations, nasal formulations should be manufactured in a sterile area, and the spray-drying operated in aseptic conditions (Chaudhary, Jadhav, & Kadam, 2010).

2. Materials and Methods

2.1 Materials

Domperidone was gifted from Cipla Pvt. Ltd., Mumbai, Maharashtra, India. All other chemicals were of analytical grade. CPG was isolated from seeds collected from Nashik, Maharashtra, India.

2.2 Formulation of microspheres

Domperidone microspheres were formulated using drug to CPG ratios of 1:2 and 1:3 (Table 1). Dispersion of CPG and Domperidone was spray-dried (LU222, Labaltima, India) using 150 °c inlet temperature, 2 mL/min feed rate, and aspirator rate 50 Nm^3/h under magnetic stirring (Surywanshi *et al.*, 2015).

2.3 Experimental design

A 2^3 factorial design (FD) at two levels and three factors was applied to observe response variables at eight possible combinations. Current investigation included as independent variables the amount of polysaccharide (X_1), inlet temperature (X_2), and aspirator speed (X_3). Dependent variables were the %entrapment efficiency (EE)(Y_1) and %drug release (Y_2) (Table 1). All other formulation variables and processing variables were held fixed throughout study.

2.3.1 Optimization data analysis

Statistical validation of polynomial fitted equations was established by using ANOVA and were obtained by using Design Expert® software (version 7.00, Stat-Ease Inc., Minneapolis, MN, USA).

Factorial design (FD) is applied in experiments where conditions or effects of different factors are to be explained. Impacts of numerous features with interactions can be recovered using FD, with N = nk where N is the number of experiments, n is number of levels per factor, and k is the number of factors. FD is an orthogonal design. Estimated effects have good statistical properties and can be estimated separately as advantage of an orthogonal design. Fitting a multiple linear regression model to the 2^3 FD gives a predictor equation incorporating interactions as polynomial terms to response estimates.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3$$

where Y is the measured response related to the factor level combination, b_0 is an intercept symbolizing arithmetic average of all quantitative outcomes, b_1 to b_{123} are regression coefficients computed from experimental values of Y and X_1, X_2 and X_3 are coded levels of the independent variables. X_1X_2, X_2X_3 and X_1X_3 represent interactions. The main effects (X_1, X_2 and X_3) reflect average effects of changing one factor at a time. Interaction terms show how response changes when two factors are changed simultaneously. $X_1X_2X_3$ is the interaction terms for all three factors simultaneously changing. The effects are indicated by magnitude of coefficient with its sign.

ANOVA was applied for checking the adequacy of fitted model, whereas main and interaction effects were assessed from response surface plots. The study was conducted in triplicate for all experiments and the outcomes are reported as mean ± SD; with significance threshold set at $P<0.05$ (Mahajan, Tatiya, & Nerkar, 2012; Patil & Sawant, 2009; Zafar *et al.*, 2021).

2.4 Evaluation of microspheres

2.4.1 Scanning electron microscopy

Particle size of prepared spray-dried DOM–charged CPG microspheres was determined using an optical microscope (Model BH-2, Olympus, Japan) fitted with a stage and ocular micrometer. Particle size, shape and morphological characteristics of spray-dried DOM–charged CPG microspheres were assessed with help of scanning electron microscopy (SEM TEOL5400, Tokyo, Japan), having resolution of 1.0 nm (15 kV) with magnifications of x25 to 1,000,000. Images of the samples were recorded.

Table 1. Experimental full factorial design and formulation compositions of Domperidone loaded CPG microspheres

Formulation code	Drug (mg)	Polymer (mg)	Inlet temperature (°c)	Aspirator rate (Nm^3/h)	Feed rate (ml/min)
B1	660	2640 (+1)	150 (-1)	45 (-1)	2
B2	660	1980 (-1)	150 (-1)	50 (+1)	2
B3	660	1980(-1)	150 (-1)	45 (-1)	2
B4	660	1980 (-1)	160 (+1)	50 (+1)	2
B5	660	2640 (+1)	150(-1)	50 (+1)	2
B6	660	2640 (+1)	160 (+1)	45 (-1)	2
B7	660	2640 (+1)	160 (+1)	50 (+1)	2
B8	660	1980(-1)	160 (+1)	45(-1)	2

+1: High level and -1: Low level

2.4.2 Determination of drug content and encapsulation efficiency

To determine drug content in spray-dried DOM–charged CPG microspheres formulations,15 mg of precisely weighed formulation was dissolved in methanol with continuous stirring overnight. The filtrate was analyzed by UV-visible spectrophotometer (Jasco, V630, India) at 287 nm. Theoretical and experimental drug contents in the developed formulation were established to estimate EE% and drug content (%):

$$EE\% = \frac{\text{Experimental amount of drug in formulation (mg)}}{\text{Theoretical amount of drug in formulation (mg)}} \times 100$$

$$\text{Drug content \%} = \frac{\text{Experimental amount of drug in formulation (mg)}}{\text{Amount of microspheres (mg)}} \times 100$$

With n= 3, both EE% and %drug content were expressed as mean ± standard deviation (SD) (Huh *et al.*, 2010; Shazly *et al.*, 2018).

2.4.3 Percentage yield

The yield of developed spray-dried formulations was detected using a weighing balance (Shimadzu, AY220, Japan).

$$\text{\% yield} = \frac{\text{Total weight of developed formulation}}{\text{Theoretical weight of DOM and polysaccharide}} \times 100$$

2.4.4 Particle size analysis

Spray-dried DOM–charged CPG microsphere formulations were subjected to Malvern Zeta Sizer for particle size distribution study (Malvern Instruments, Malvern, UK) (Gungor, Okyar, Erturk-Toker, Baktir, & Ozsoy, 2010).

2.4.5 Zeta potential determination

Spray dried DOM microspheres suspended in 10 *mM* NaCl were subjected to zeta-potential determination using laser doppler electrophoresis (Nano ZS, Malvern Instruments, UK).

2.4.6 *In-vitro* mucoadhesion

Accurately weighed 100 mg of spray-dried DOM charged CPG microsphere formulation was mounted on fresh, cleaned section of goat nasal mucosa (2 cm^2) with polyethylene support. Simulated nasal electrolyte solution (100mL) was passed on it and desiccated for 25 min at 90% RH, which permits interactions between polysaccharide and membrane at 45°. Phosphate buffer was put on mucosa at the rate of 5 mL/min for 1h. Concentration of DOM from buffer was studied spectrophotometrically. Quantity of spray-dried DOM microspheres equivalent to DOM amount in phosphate buffer was detected. Quantity of stuck microspheres was evaluated from applied microspheres (Cerchira, Luppi, Chidichimo, Bigucci, & Zecchi, 2005).

$$\text{\% Mucoadhesion} = \frac{\text{Amount of DOM in washout phosphate buffer liquid}}{\text{Actual amount of DOM in applied formulation}} \times 100$$

Franz diffusion cell apparatus was applied to determine water absorption capacity of the developed formulation. Polyamide membrane (0.2 μm pore size) permeable to water was positioned between developed formulation (5mg) and receptor cell which was packed with simulated nasal fluid (SNF) and thermostated at 37 °c. Liquid uptake of the developed formulation reduces the level of SNF in a graduated part of Franz diffusion cell, followed by addition of quantity of SNF soaked up by the developed formulation to receptor cell. SNF soaking up by each developed formulation was stated as the quantity of SNF required per mg of developed formulation in 15 min swelling activity.

$$\alpha = \frac{M_t - M_0}{M_0}$$

where, α = degree of swelling, M_t = formulation weight after swelling, M_0 = initial formulation weight (Oliveria, Santana, & Ré, 2005).

2.4.8 Thermal analysis

Pure Domperidone, CPG and optimized DOM nasal microspheres (each 3 mg) were heated at scanning rate of 10 °c/min between 30-300 °c with nitrogen flushing (50 mL/min) and the DSC thermogram was recorded (Shimadzu, DSC-60, Japan).

2.4.9 Powder X-ray diffraction

Crystallinity of Domperidone and DOM-loaded CPG nasal microspheres was studied using X-ray diffractometer (BRUKER D8 advanced, Germany).

2.4.10 *In-vitro* drug release study

Franz diffusion cell apparatus was applied for evaluation of drug release profiles for DOM-nasal microspheres in hydrated environment. Receptor and donor cell were separated by cellophane membrane and equilibrated carefully before putting 15mg of formulation in the donor compartment. 3mL of simulated nasal electrolyte solution was charged in the donor compartment, whereas the receiver compartment was charged with 20mL of phosphate buffer solution (*pH* 6.6) and IPA in ratio of 70:30 and maintained at 37 ± 0.5 °c. Replacement with fresh buffer solution was done after withdrawal of sample from receptor compartment, and the sample was evaluated spectrophotometrically at 283 nm (Brian, 2001; Canan, Gönül, & Erk, 2003; Cheng *et al.*, 2002).

2.4.11 *In-vivo* studies

In vivo rabbit study was approved by the Institutional animal ethical committee at MET's Institute of Pharmacy, Adgoan, Nashik, Maharashtra, India (Registration number: 1344/PO/Re/S/10/CPCSEA under CPCSEA, India).

White New Zealand rabbits (average weight 2 ± 0.3 kg) were isolated in fasting condition for 12h before commencement of the study, and were provided water *ad libitum* throughout the experiment. Blood was treated with heparinized normal saline when collected through a cannula from a marginal ear vein.

1) Method

Rabbit was applied as the model animal due to its large nasal surface area, which determines nasal absorption potential from developed formulations. Oral and developed nasal formulation (5mL) at a dose of 2.5 mg/kg was administered using oral tube in both nostrils. 0.5mL blood samples were collected at predetermined intervals and analyzed for plasma drug concentration by HPLC (Mahajan *et al.*, 2012).

2) Analysis of sample

0.5 mL blood samples were collected after administration of the developed formulations as per a predetermined schedule: every 30 min till 300 min, from the rabbit's marginal ear vein. Plasma was isolated from the collected and mixed blood samples with anticoagulant (heparin) on centrifugation (REMI, C-24BL, India) at 3,000 rpm for 15 min followed by deep freeze at -20 ^{o}c for HPLC study.

Serum samples were extracted with dichloromethane and estimated for drug content. To 100µL of blank serum, 100µL of Domperidone standard solution in mobile phase was added with 0.2, 0.3, 0.5, 1.0 or 5.0 µg/mL of Domperidone. 150ng of propanol in methanol (100µL) as internal standard, 100µL of 0.1 mol L^{-1}NaOH , and 3mL of dichloromethane were added. Mixture was shaken and centrifuged at 3,000xg for 10 min. Collected organic phase was vacuum dried to obtain dry residue. Dried residue was dissolved in mobile phase of 100mL [methanol:10mM KH_2PO_4 Buffer pH :3 (60:40)] and injected into the HPLC column (Jakki, Syed, Kandadi, &Veerabrahma, 2013).

2.4.12 Data treatment and statistical analysis

Drug concentration in plasma versus time was assesses as outcome from the HPLC analysis. T_{max}, C_{max} and AUC were computed as non-compartment pharmacokinetic parameters using Kinetica 5.0 R computer program. AUC outcome for each graph was studied from time zero to end point using trapezoidal rule with extrapolation to infinity. $AUC_{0\text{-}\alpha}$ value achieved from curve was used to study relative bioavailability.

$$\text{Relative bioavailability (\%)} = \frac{\text{AUC (0-}\alpha\text{) nasal}}{\text{AUC (0-}\alpha\text{) oral}} \times \frac{\text{Dose (nasal)}}{\text{Dose (oral)}}$$

Mean ± SD for outcome of *In-vivo* studies was computed and Graph Pad Instat Version 3.01 software was applied for analysis. Unpaired t-test was applied for comparison of pharmacokinetic variables from two dosage forms. Significance was called for p-value< 0.05.

3. Results and Discussion

Spray-dried DOM-loaded CPG nasal microspheres were developed. Spray-drying is a speedy, simple single-step method to make sterile dry porous microparticles with proper encapsulation of an API. Based on pre-trial batches, aspiration flow rate of 50 Nm^3/h was applied to achieve moisture free microspheres. Off-white colored spray-dried DOM-loaded CPG microspheres were found to be discrete, spherical and free flowing.

3.1 Statistical data analysis

The statistical analysis of dataset from experiments was done to identify a mathematical model fit to the data, allowing to optimize the outcome variables by best choice of the manipulated variables. The experimental FD design was applied to study and discover effects and interactions of manipulated variables on optimized formulation.

Concentration of polymer, inlet temperature, and aspirator speed were the independent manipulated variables, each with two factor levels, analyzed for their impacts on %EE and % drug release. One-way ANOVA (P<0.05) was used to assess the fitted models statistically. % EE and % drug release of spray-dried DOM-loaded CPG microspheres were in the ranges 89.67-98.64% and 57.69-87.024%.

Effects of the manipulated variables on EE (Y_1) were modeled with model f-value 22.28 (P< 0.05) indicating statistical significance. The regression fit for %EE was:

$$Y_1 = 92.58 + 2.17X_1 – 1.06X_2 - 0.40X_3 - 1.57X_1X_2 - 7.500E – 003X_1X_3$$

Coefficients b_1 and b_{12} were found to be significant at P<0.05 and hence retained in reduced model. The model was tested to establish whether the coefficients b_2, b_3, b_{13} and significantly contributed to Y_1. The critical f-value for the model was 22.28. Since the calculated value for these coefficients was not meeting the critical value, the terms with b_2, b_3, and b_{13} did not considerably affect estimates of Y_1 and were dropped, giving the reduced model:

$$Y_1 = 92.58 + 2.17X_1 – 1.06X_2 - 1.57X_1X_2$$

This equation reveals that X_1 has an agonistic effect and X_2 an antagonistic effect on %EE.

The model for % drug release (Y_2) had F value of 54.70, found to be significant (P< 0.05). This regression fit was:

$$Y_2 = 73.54 - 10.78X_1 + 0.22X_2 - 2.74X_3 – 0.47X_1X_3 + 0.91X_1X_2X_3$$

Coefficients b_2, b_{23}, and b_{13} were significant at the selected threshold level, while b_1 and b_3 were subject to further study. Thus, set design was studied in required concentration to evaluate whether significance of the coefficients b_2, b_{23}, and b_{13} was relevant to the estimates of Y_2. The critical f-value for this model was 54.74, while the calculated values for these coefficients were lesser, indicating that the terms with b_2, b_{23}, and b_{13} did not significantly affect estimates of Y_2. The reduced model was:

$Y_2 = 73.54 - 10.78X_1 - 2.74X_3$

This reveals that $X_1$and X_3 both had antagonistic effects on % drug release.

Optimized DOM loaded CPG nasal microspheres were developed as per the selected process variables from the statistical analysis, and 2^3 FD was applied for %drug release up to 6h (Figure 1) and from 89.67±0.845% to 98.64±0.434% EE. Desirable ranges of the independent variables were set for development of batch using the optimized setpoint, where CPG(X_1) = 2310 mg, inlet temperature(X_2) =155 ^{o}c and aspirator speed(X_3) = 47Nm^3/h. This gave 95.72±0.078% EE and 73.36±0.08% drug release after 6h, with error from model outcomes of -1.23, -1.75 correspondingly. This error assessment indicates validity of the fitted model equations and the optimized operating point.

3.1.1 Contour plots and analysis of response surfaces

3D response surface plots and 2D contour plots generated by Design Expert® software are shown in Figure1, for the responses EE and %drug release. Figure 1 depicts the combined impact of polymer proportion(X_1) along with inlet temperature(X_2), indicating non-linear effects on EE of the microspheres. Combined effect of polymer proportion(X_1) and aspirator rate(X_3) on %drug release shows a linear effect that is synergistic (Figure 1[C,D]).

3.2 Evaluation of the developed formulation

3.2.1 Morphological evaluation

DOM-loaded CPG microspheres were evaluated by SEM for morphological character, see Figure 2. Spray-dried DOM-loaded CPG microspheres were found to have dented surfaces in the SEM images. Fastest drying of microspheres with well-built shells and no entrapped air caused the surfaces to cave inwards (Anandhanram & Ishwarya, 2015).

3.2.2 Drug content and entrapment efficiency

Developed formulations were found to have actual drug contents and EE in the ranges from 22.55±0.04 to 31.45±0.23% (Table 2) and from 89.67±0.845% to 98.64±0.434%, respectively. As the drug to polymer ratio was increased, a significant increase in EE and decrease in drug content were detected. The developed formulation was found to give a high EE on using spray-drying technology (Canan *et al.*, 2003).

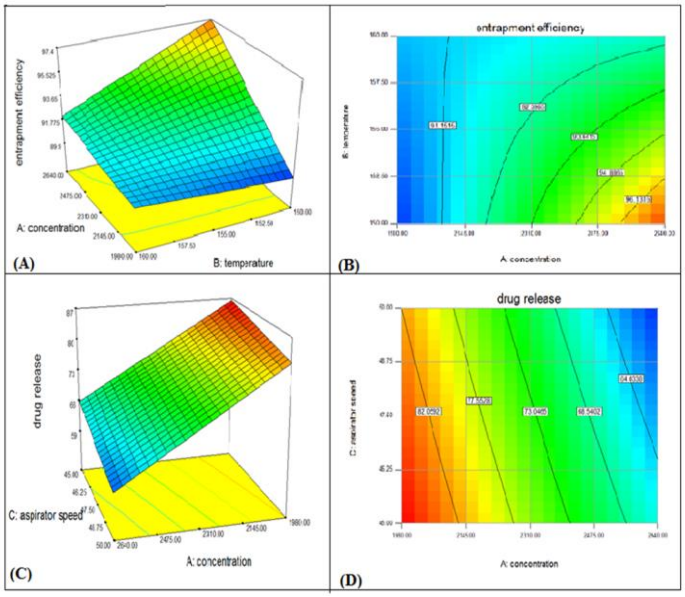

Figure 1. Contour plots and response surfaces of entrapment efficiency (A, B), and % drug release (C, D)

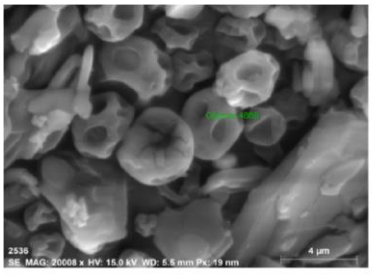

Figure 2(a)

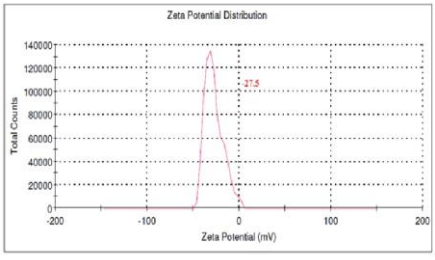

Figure 2 (b)

Figure 2. (a) Scanning electron micrograph of Domperidone loaded microspheres, (b) Zeta potential distribution of Domperidone nasal microspheres

Table 2. Drug content and Entrapment efficiency of prepared Domperidone loaded microspheres

Formulation code	Theoretical drug content (%)	Actual drug content (%)±SD	Entrapment efficiency (%)±SD
B1	33.33	30.89±0.71	98.27±0.434
B2	25	20.84±0.42	89.24±0.854
B3	25	22.55±0.2	90.57±1.173
B4	25	21.74±0.19	92.79±0.73
B5	33.33	31.45±0.3	96.48±0.75
B6	33.33	29.51±0.294	92.05±1.02
B7	33.33	29.82±0.425	92.19±0.928
B8	25	23.83±0.501	91.05±0.045

*All results are expressed as mean ± Std. Dev., n=3

3.2.3 Percentage yield

From 23.7±0.01 to 28.55±0.054% yield of microspheres was detected. A small fraction of the solution being treated by spray-drying caused this low production yield, whereas removal of fine particles from exhaust at time of developing product and sticking of some liquid droplets widely in glass surfaces of cyclone could be reasons for low yield. High consistency feed may block the spray nozzle with moisture entrapment in formulation due to hydrophilic-high molecular weight polysaccharides, having film deposition on cyclone surface. Product yield increases with scale-up to extensive production (Ige *et al.*, 2015; Giunchedi, Juliano, Gavini, Cossu, & Sorrenti, 2002).

3.2.4 Particle size

Particle sizes of the formulated microspheres were in the range from 4.144 to 4.55 μm. According to literature, particle sizes greater than 10 μm will settle by gravity, and inhalation happens with less than 0.5 μm. The developed formulation of 5 to 7 μm sizes gets held in the nasal area, which facilitates permeation (Figure2(a)) (Donovan & Donovan, 1998).

3.2.5 Zeta potential

The formulation had an anionic charge due to the CPG coat, which facilitates good mucoadhesion (Figure 2(b)).

3.2.6 *In-vitro* mucoadhesion

The developed CPG coated microspheres had good mucoadhesion to nasal mucous membrane, due to polysaccharide content, in the range from 72.37±0.094 to 82.47±0.062%. In the experiments, the batch showing 82.47±0.067% mucoadhesion was considered the best suited batch with superior mucoadhesion. A high percentage of hydroxyl functional groups in the developed formulation could be one factor contributing to superior mucoadhesion.

3.2.7 *In-vitro* swelling study

Degree of swelling of the CPG loaded microspheres was studied, and was in the range from 0.631±0.47 to 0.961±0.51%. The results showed that as polymer concentration increased the degree of swelling also increased. The developed formulation appeared as a thick consistency slurry when in contact with nasal mucous membrane, due to absorption of water from mucosa and swelling of the microspheres. Water holding capacity of polysaccharide with presence of carboxyl chains was responsible for thick consistency slurry formation, which diminishes the rate of ciliary secretions and extends retention at the nasal mucous layer.

3.2.8 Thermal analysis

Probable reactions between API, polysaccharides, and their behavior at various temperatures, were studied using DSC analysis. As per Figure 3, the CPG in amorphous state showed a sharp endothermic peak at 250.88 °c. Polymer thermogram indicated 72.77 °c as the glass transition temperature T_g. Drug loaded formulation had thermogram showing that, after processing of the polymer, its T_g was 50.26 °c. During spray-drying, the observed reduction in Tg might be due to the dissolution and heating of polysaccharides, whereas characteristics of polysaccharides were stagnant with endothermic peak at 252 °c as the melting point of API. Per the DSC results, polysaccharides and drug loaded formulation did not react with each other.

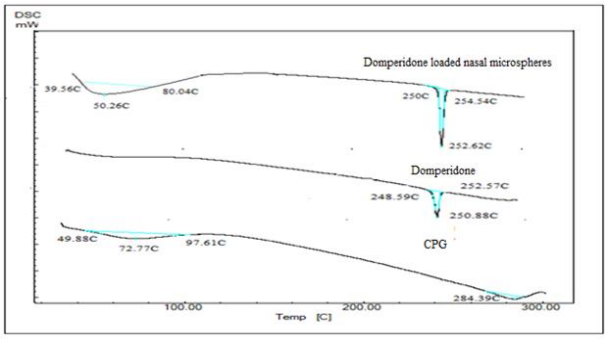

Figure 3. DSC responses of Domperidone, CPG, and DOM-loaded nasal microspheres

3.2.9 Powder X- diffraction study

XRD spectra (Figure 4) for API, polysaccharides without drug, and the developed DOM-charged CPG micro formulation, showed characteristic intense peak with crystalline nature from 2 υ from 10 to 30 for Domperidone. Reduced intensity of peak for blank microspheres was

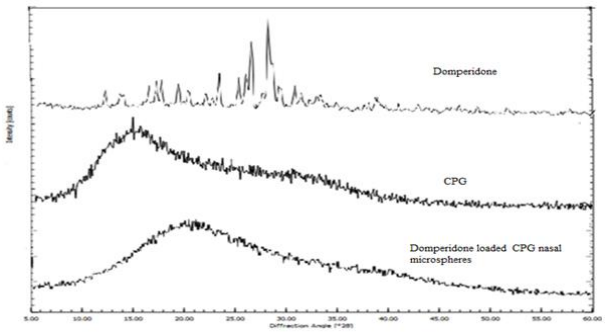

Figure 4. X-ray diffractograms of Domperidone, CPG, and Domperidone-loaded nasal microspheres

detected, with complete entrapment of API using spray-drying.

3.2.10 *In-vitro* drug release study

In-vitro drug release profile of DOM-loaded CPG microsphere formulations is shown in Figure 5. Release profiles of formulations with drug to polysaccharide ratios 1:2 and 1:3 were respectively higher and lower. An increase in polymer concentration decreased the drug release rate from the formulation.

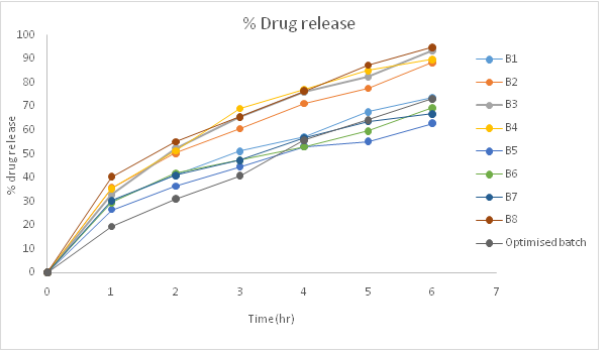

Figure 5. *In vitro* drug release of Domperidone from microspheres

In-vitro drug release results were subjected to various kinetic models for selecting a suitable kinetic model from among zero order, first order, second order and Higuchi models. The developed formulation followed Higuchi model in release from the matrix (Table 3). A linear relationship was detected between % drug release and time (Figure 5). Gel diffusion layer due to swelling on imbibition by the developed microspheres in buffer solution as matrix was detected, which maintained the sustained release (Lim, Martin, Berry, & Brown, 2000).

3.2.11 *In-vivo* study

Anesthesia was not preferred as it could influence API *in vivo* absorption. Effective mucociliary effect was detected due to conscious state of the rabbit. Plasma drug concentration against time was studied using HPLC. Kinetics 5.0® computer program was applied for estimating the non-compartmental pharmacokinetic parameters, such as C_{max} ,T_{max} and AUC. Trapezoidal rule was found useful for calculating AUC from zero and extrapolating to infinity. The $AUC_{0-\alpha}$ value from %relative bioavailability and relative bioavailability was computed.

Pharmacokinetic parameters $C_{max(ng/mL)}$, T_{max}(min) and AUC(ng/mL min) detected in rabbit plasma after microsphere administration, and on oral administration, are detailed in Table 4.

Table 4. Comparative pharmacokinetic parameters of Domperidone following administration orally (solution) or nasally (microspheres) in rabbits

Parameter	Oral solution	Nasal microspheres
C_{max}(ng/mL)	245.32±15.75	450.46±28.43
T_{max}(min)	30±0.78	39±0.25
$AUC_{0-\infty}$(ng/mL)	50.44±2.89	268.98±1.24
AUC_{0-300}(ng/mL)	37.84±3.33	95.54±6.04

*All results are expressed as mean ± Std. Dev., n=3

On nasal administration the mean C_{max} of API was found to be superior to that with oral administration (Figure 6). Statistically significantly ($p<0.05$) the level was better for AUC_{0-300} on nasal administration as compared to oral administration. Statistical significantly the level of $AUC_{0-\alpha}$ was better on nasal administration than with oral administration. Non-significant level was detected for C_{max} due to the slow rate on nasal administration as compared to oral administration. *In vivo* performance of the developed formulation had superior bioavailability (AUC_{0-300} and $AUC_{0-\alpha}$ reaching 5.54±6.04 and 268.98±1.24) while oral administration of solution gave AUC_{0-300} and $AUC_{0-\alpha}$ as 37.84±3.33 and 50.44±2.89, possibly due to escaping 1st pass metabolism. Relative bioavailability of Domperidone nasal

Table 3. *In vitro* release kinetics of Domperidone from CPG microspheres: goodness of alternative model fits

Batch	Zero order	First order	Higuchi model	Best fitted
B1	0.9435±0.014	0.9694±0.087	0.992±0.054	Higuchi
B2	0.9791±0.078	0.9876±0.078	0.9975±0.021	Higuchi
B3	0.9731±0.024	0.9891±0.587	0.998±0.05	Higuchi
B4	0.9552±0.048	0.9754±0.058	0.9953±0.04	Higuchi
B5	0.9594±0.047	0.975±0.0278	0.9905±0.09	Higuchi
B6	0.9829±0.0.78	0.9892±0.078	0.9863±0.037	Higuchi
B7	0.9892±0.12	0.9943±0.021	0.9916±0.062	Higuchi
B8	0.9851±0.056	0.9921±0.047	0.9975±0.023	Higuchi

*All results are expressed as mean ± Std. Dev., n=3

microspheres was 290.271 ± 54% as compared to oral administration. These preliminary results support the development of poorly water-soluble drug Domperidone into a formulation for microparticulate nasal drug delivery system using CPG.

4. Conclusions

The current study demonstrated potential of CPG based Domperidone loaded formulation for application via the intranasal route, as a possible alternative to available conventional formulations. Nasal microspheres loaded with DOM were optimized using a full factorial experimental design with a response surface approach, and this gave well-performing model fits. In the current study, microspheres with a smallish particle size (4µm diameter) were obtained, which facilitate viscous gel formation on contact with nasal mucosa by interacting with cations present in the nasal secretions. This prolongs nasal mucosal retention and decreases nasal ciliary clearance. Bioavailability of the encapsulated drug was found to be improved due to enhanced mucoadhesion of the CPG based microcapsule formulation in the nasal route.

Acknowledgements

Authors are thankful to Cipla Pvt. Ltd. Mumbai, Maharashtra, India for providing gift sample of Domperidone. Authors are also grateful to trustees, Bhujbal Knowledge City, MET's Institute of Pharmacy, Nashik, Maharashtra, India for providing necessary facilities to conduct the current research work.

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