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Mebeverine Hydrochloride Loaded Chitosan Microspheres as Potential Treatment Targeting Irritable Bowel Syndrome: Box-Behnken Design Optimization

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ABSTRACT

Objective: Mebeverine hydrochloride is an antispasmodic agent that has a direct musculotropic action on the smooth muscles of the gastrointestinal tract; especially the colon.Therefore, the current study aimed at formulating and optimizing colon targeted mebeverine hydrochloride microspheres for treatment of chronic gastrointestinal disorder. **Methods:** Mebeverine hydrochloride-loaded chitosan microspheres were formulated adopting emulsion cross-linking method using glutaraldehyde as a cross linking agent. A 3^3 Box Behnken design was utilized in formulating the microspheres and investigating the effect of different formulation factors such as drug: polymer ratio (X_1), stirring speed (X_2) and the surfactant concentration (X_3) on particle size (Y_1), the entrapment efficiency percentage (Y_2) and the cumulative release percentage of mebeverine hydrochloride after 8 h (Y_3). **Result:** The particle size and entrapment efficiency were significantly affected by tested formulation parameters. The release of mebeverine hydrochloride from optimized formula was pH dependent. In simulated gastric fluid, less than 10% of entrapped mebeverine hydrochloride was released, while, a relatively high amount of the drug (> 65%) was released in simulated colonic fluid (pH 7.4). The *in vivo* pharmacokinetic study revealed that the optimized formula of microspheres exhibited increased oral absorption of mebeverine hydrochloride, compared to free drug (C_{max} 168.51±20.05 ng/ml vs. 126.45±29.46 ng/ml, respectively). In addition, the optimized formula exerted a remarkably higher systemic bioavailability, compared to the free drug. **Conclusion:** These results underscore the applicability of cross-linked chitosan microspheres as a promising carrier for colon targeted delivery of mebeverine hydrochloride for treating diseases associated with the colon such as irritable bowel syndrome.

Key words: Box-Behnken design, Chitosan, Irritable bowel syndrome, Mebeverine hydrochloride, Microspheres.

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INTRODUCTION

Irritable bowel syndrome (IBS), a chronic gastrointestinal disorder, is characterized by abdominal pain and an erratic bowel habit.[1,2] Mebeverine hydrochloride (MBH) is an antispasmodic agent that has a direct musculotropic action on the smooth muscles of the gastrointestinal tract; especially the colon.[3] For many years, MBH has been considered the drug of choice for the management of IBS.[4,5] Nevertheless, following oral administration, MBH is rapidly absorbed from the upper part of gastrointestinal tract and undergoes extensive hepatic metabolism.[6] This adversely hinders the delivery of MHB to the site of action; colon, in appropriate concentrations. Accordingly, development of drug delivery systems that are capable of delivering MBH to the diseased organ (colon) in adequate concentrations is urgently needed in order to enhance the overall therapeutic efficacy along with reducing the incidence of adverse side effects.

Recently, colon targeted drug delivery systems have gained enormous attention as means for delivering drugs specifically into the colon.[7-9] Targeting of drugs to colon is valuable approach for treating diseases associated with the colon such as colorectal cancer, ulcerative colitis, Crohn's disease, amebiosis, inflammatory bowel disease and irritable bowel syndrome.[10,11] Nevertheless, for successful targeted drug delivery to the colon, the delivery vehicle should protect the drug from degradation, release and absorption in stomach and small intestine and should allow the selective/controlled release in the proximal colon. This can be accomplished by the use of well-designed delivery vehicles that can shield the drug during its transit to the colon.

Polymeric micro-particles represent one of the promising delivery vehicles that have been recognized for their potential as therapeutic carriers to the colon.[12,13] They can be prepared using different kinds of polymers. Among them, naturally occurring biodegradable polymers, especially polysaccharides, have been extensively explored for their potential in colon-specific drug delivery.[14,15] Polysaccharides, such as chitosan, pectin, inulin, dextran and guar gum, show the potential to be retained intact in the environment of the stomach and small intestine, while being degraded by polysaccharidases upon arrival in the colon. Chitosan is one of the non-toxic biodegradable polysaccharide that is obtained from the alkaline deactivation of chitin.[16] Chitosan shows a propensity to dissolve in acidic pH of the stomach but get swollen in the intestinal pH. This gelling property retards drug release from the dosage form, making it more susceptible to degradation in the colon.[17,18] Consequently, chitosan serves as an effective polymer for the preparation of colon-specific drug delivery systems.

The aim of the present study was, therefore, to formulate and optimize chitosan microspheres loaded with MBH for colon-specific drug targeting using a 3-factor, 3-level Box Behnken design. In addition, the *in vivo* fate of the optimized MBH chitosan microspheres was evaluated in rabbits following oral administration.

MATERIALS AND METHODS

Materials

Mebeverine hydrochloride (MBH) was generously supplied by EPICO (10th of Ramadan city, Egypt). Chitosan low molecular weight (MW 10031), was purchased from Sigma Aldrich. Hydrochloric acid, light liquid paraffin, span80, glutaraldehyde and glacial acetic acid were supplied from El-Nasr Pharmaceutical Chemical Company (Cairo, Egypt). Other chemicals and materials were of analytical grade.

Preparation of mebeverine hydrochloride-loaded chitosan microspheres

Chitosan microspheres were formulated using the emulsion cross-linking method.[19] Briefly, different amounts of chitosan and a definite weight of MBH (100mg) were dissolved in 20 ml of 1% v/v aqueous acetic acid solution. The resulting drug-polymer dispersion was emulsified into an external phase of light liquid paraffin containing different concentrations of span 80. After 30 min, 1.5 ml of the glutaraldehyde (1.25 %) was added and further stirring was continued for 3 h for cross-linking and stabilization. The formed microspheres were filtered, washed repeatedly with petroleum ether for removing residual liquid paraffin and then dried in hot air oven at 50°C.

3^3 level Box Behnken Experimental Design

A 3-factor, 3-level Box–Behnken design (Statgraphics Centurion version 18 software; Stat Point Technologies Inc., VA, USA) was adopted for optimizing chitosan microspheres of MBH and to explore the effect of different formulation variables, namely; drug: polymer ratio (X_1), stirring speed (X_2) and surfactant concentration (X_3) on product characteristics, namely; particle size (Y_1), % entrapment efficiency (Y_2) and % cumulative drug release at 8 h (Y_3). A total of fifteen runs were prepared (Table 1).

Physicochemical and morphological characterization of microspheres

Scanning Electron Microscopy

Scanning Electron Microscope (SEM, JEOL JSM-5400LV Jeol, Tokyo, Japan) was used to examine the shape and surface morphology of the prepared microspheres.

Particle size analysis

The average particle size of the prepared microspheres was analyzed using dynamic light scattering (Zetasizer Nano ZS, Malvern, United Kingdom). Suspensions of microspheres in distilled water were used for the measurement. All measurements were conducted in triplicate at 25°C.

Entrapment efficiency

Accurately weighed amount (50 mg) of MBH microspheres was crushed and dispersed in 20 ml phosphate buffer pH 7.4. The dispersion was continuously agitated on a shaker at 37°C for 24 h. The dispersion was then filtered and drug content of the filtrate was determined spectrophotometrically at λ_{max} of 263 nm. The percentage drug entrapment efficiency (% EE) was estimated using the following formula:

$$\% \text{ EE} = \text{Actual drug content/Theoretical drug content} \times 100$$

Percentage yield

The dried microspheres were accurately collected and weighted to obtain the yield of the prepared microspheres. The percentage yield was computed using the following formula:

$$\% \text{ Yield} = (\text{Actual weight of microspheres/Total weight of drug and polymer}) \times 100$$

In vitro release study

In vitro release of MBH from chitosan microspheres was conducted in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) for 2 and 22 hrs, respectively, at 37 ± 1°C. Both SGF and SIF were prepared as per "British Pharmacopeia 2014" without the addition of digestive enzymes. Chitosan microspheres containing MBH equivalent to 3 mg were accurately weighed, filled into dialysis bags and suspended in 100 ml dissolution medium. Release studies were carried out in SGF (pH 1.2) for 2 h followed by SIF (pH 6.8 and 7.4) for 3 h and 19 h, respectively. Samples of 3 ml were withdrawn at different time intervals (1, 2, 3, 4, 5, 6, 8, and 24 h) and replaced with an equal volume of fresh medium to maintain a constant volume. The drug concentration in each aliquot was analyzed spectrophotometrically at λ_{max} of 263 nm.

Pharmacokinetic study of optimized MBH-loaded chitosan microsphere

Male albino rabbits (weighing 2 to 2.25 kg) were randomly categorized into three groups (n=4). The first group received an oral capsule containing free drug. The second group received the optimized chitosan microsphere formula (FO). MBH dose was 20 mg/kg. The third group received saline instead of MBH and served as control. All animal experiments were approved by the Institutional Animal Ethics Committee, Faculty of Pharmacy, Zagazig University, Egypt (approval number: ZU-IACUC/3/F/45/2019).At predetermined time points (0.5, 1, 2, 3, 4, 6, 8 and 24 h post-dose), blood samples (500 µl) were collected into heparinized tubes. The plasma fraction was obtained by centrifuging blood samples at 4000 rpm and 4°C for 10 min and then stored at -20°C until analysis. Drug concentration in each plasma sample was quantified by HPLC using a mobile phase consisted of a mixture of 50 mM KH_2PO_4, acetonitrile and tetrahydrofuran (THF) (63:35:2%; v/v/v) and equipped with a UV-VIS detector set at a wavelength of 263 nm. Pharmacokinetic parameters (C_{max}, t_{max}, K_{el}, $AUC_{0\text{-}24h}$ and MRT) were estimated from the individual plasma concentrations versus time profiles using the

Table 1: Composition of the formulated batches of MBH microspheres and the obtained responses.

Run	X_1	X_2	X_3	Y_1	Y_2	Y_3
FC1	1:4	1000	1.5	39±1.0	11.66±0.19	100±0.68
FC2	1:4	1600	1.5	32±0.45	12.85±0.33	100±5.30
FC3	1:8	1000	1.5	36.4±1.15	36.15±1.41	68.31±1.32
FC4	1:8	1600	1.5	40.7±1.53	48.07±1.72	60.16±1.75
FC5	1:4	1300	1	33±1.0	17.5±0.07	100±0.22
FC6	1:4	1300	2	38.6±0.51	11.88±0.42	100±3.23
FC7	1:8	1300	1	41.3±0.57	47.16±1.15	57.36±1.19
FC8	1:8	1300	2	46.4±1.13	33.84±1.32	59.94±0.89
FC9	1:6	1000	1	40.2±1.53	23.63±0.7	100±2.40
FC10	1:6	1000	2	42±0.57	30.75±2.84	100±0.03
FC11	1:6	1600	1	34±1.0	35.66±0.44	100±3.40
FC12	1:6	1600	2	35.7±1.43	31.7±2.12	90.4±4.80
FC13	1:6	1300	1.5	40.7±3.05	18.87±2.06	100±3.19
FC14	1:6	1300	1.5	43.65±1.31	20.18±1.23	100±0.97
FC15	1:6	1300	1.5	39.85±2.33	18.45±0.76	100±2.09

Data are represented as mean ± SD

pharmacokinetic software PK solver and the relative bioavailability was computed using the following equation:

Relative bioavailability (%) = ($AUC_{0\text{-}24\ (test\ formulation)}$ / $AUC_{0\text{-}24(pure\ drug)}$ × 100.

Statistical analysis

Student's *t*-test and one-way ANOVA was adopted to assess the significance of the difference between different formulationsusing Graph-Pad Prism versions 5.02. Values were represented as the mean ± SD.

RESULTS

Effect of formulation variables on physicochemical characteristics of MBH-loaded microspheres

Mebeverine hydrochloride (MBH)-loaded chitosan microspheres were formulated and optimized by the three-factor, three-level Box–Behnken design (BBD). The quantitative effects of the independent variables (X_1, X_2 and X_3) on the dependent variables (Y_1, Y_2 and Y_3) were fitted into regression analysis and second-order polynomial equations were obtained to explain the mathematical relationships between the dependent and independent variables (equations 1-3).

$$Y_1 = -1.3625 - 0.758\ X_1 + 0.056\ X_2 + 8.2677\ X_3 - 0.316\ X_1^2 + 0.005\ X_1X_2 - 0.125\ X_1X_3 - 0.00003\ X_2^2 - 0.0002\ X_2X_3 - 1.25\ X_3^2 \quad (1)$$

$$Y_2 = 128.52 - 3.73\ X_1 - 0.145\ X_2 - 38.473\ X_3 + 0.647\ X_1^2 + 0.004\ X_1X_2 - 1.925\ X_1X_3 + 0.00006\ X_2^2 - 0.018\ X_2X_3 + 23.362\ X_3^2 \quad (2)$$

$$Y_3 = -64.189 + 48.044\ X_1 + 0.031\ X_2 + 46.33\ X_3 - 4.519\ X_1^2 - 0.003\ X_1X_2 + 0.645\ X_1X_3 + 0.000002\ X_2^2 - 0.016\ X_2X_3 - 10.385\ X^2 \quad (3)$$

The significance and magnitude of the studied dependent variables on the investigated responses was explained by Pareto charts (Figure 1). ANOVA test was used to test their significance. A positive sign indicates a synergistic effect while a negative sign indicates an antagonistic effect of the factor on the selected response.

Effect on the particle size (Y_1)

The particle size distribution of MBH-loaded chitosan microspheres is presented in Table 1. The mean particle size of the prepared microspheres was in the range of 32 ± 0.45 to 46.4 ± 1.13 µm. It was obvious that both drug: polymer ratio (X_1) and the interaction effect X_1X_2 exerted a significant synergistic effect on the particle size (Y_1) of the prepared microspheres. On the other hand, neither the stirring speed (X_2) nor surfactant concentration (X_3) exerted any effect on the particle size (Y_1) as represented in Table 2 and Figure 1A. At the same levels of X_2 and X_3, increasing drug: polymer ratio (X_1) from 1:4 to 1:8 resulted in an increase in the average particle size of the formulated microspheres from 32±0.45µm (F2) to 40.7±1.53µm (F4), from 33±1.0µm (F5) to 41.3±0.57µm (F7) and from 38.6±0.51µm (F6) to 46.4±1.13µm (F8).

Effect on drug entrapment (Y_2)

The percentage entrapment efficiency of MBH-loaded chitosan microspheres was in the range of 11.66±0.19% to 48.07±1.72% (Table 1). It was noticed that drug: polymer ratio (X_1) and stirring speed (X_2) exerted a significant synergistic effect on the percentage entrapment efficiency (Y_2) of the prepared microspheres. On the other hand, surfactant concentration (X_3) and the interaction effects (X_1X_2), (X_1X_3) and (X_2X_3) exerted insignificant effect on the percent drug entrapped (Y_2) as presented in Pareto chart (Figure 1B). Similarly, at the same levels of X_1 and X_3, stirring speed (X_2) exerted a positive effect on the percentage entrapment efficiency (Y_2).

Effect on the cumulative percentage of drug release after 8 h (Y_3)

The *in vitro* release of MBH from the prepared chitosan microspheres was conducted using buffer change method to mimic the GIT environment. As shown in Figure 2, the drug release for the initial 2 h in SGF (pH 1.2) was found to be low in all formulations. Then, the drug release increased markedly (% cumulative drug releaseranged from 57.36% to 100% at the end of 8 h) depending on the level of factors in the formulations. In addition, it was noticed that both drug: polymer ratio (X_1) and stirring speed (X_2) possessed a significant antagonistic effect on the cumulative amount of MBH release after 8 hours (Y_3). On the other hand, surfactant concentration (X_3) failed to affect MBH release from chitosan microspheres at any of the studied concentrations ($p < 0.05$) (Table 2 and Figure 1C).

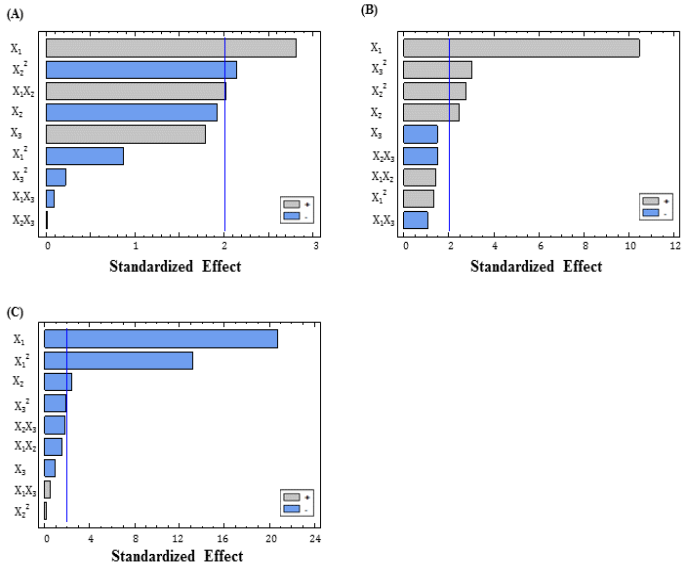

Figure 1: Standardized Pareto charts revealing the significance of the independent variables on the investigated dependent variables.Positive sign means synergistic effect while negative sign means antagonistic effect.

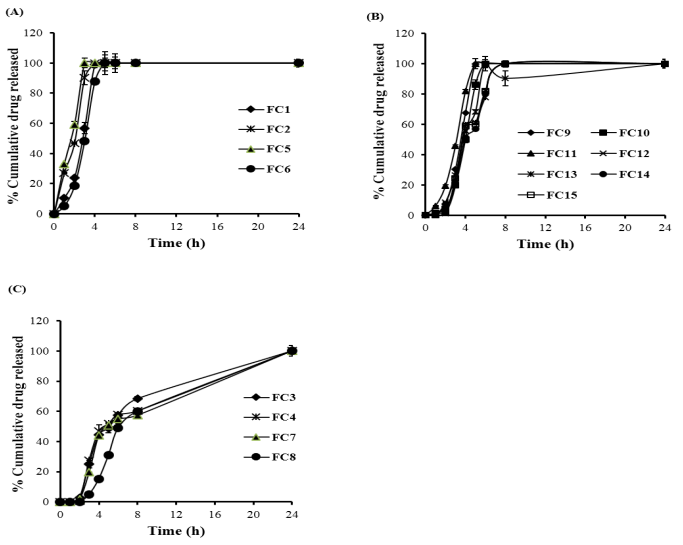

Figure 2: In vitro release profile of MBH from chitosan microspheres. Release of MBH from microspheres prepared with drug:polymer ratio (A) 1:4 (B) 1:6 (C) 1:8 at different stirring speed and different surfactant concentration. Data represents mean±SD.

Table 2: Estimated effects of factors and associated *P*-values for dependent variables (Y_1, Y_2 and Y_3).

Factor	Y_1		Y_2		Y_3	
	Factor effect	P- value	Factor effect	*P*- value	Factor effect	*P*- value
X_1	5.55	0.0374*	27.8325	0.0001*	-38.5575	0.0000*
X_2	-3.8	0.1121	6.5225	0.0579*	-4.4375	0.0622*
X_3	3.55	0.1319	-3.945	0.1984	-1.755	0.3875
X_1^2	-2.525	0.4244	5.17583	0.2438	-36.1575	0.0000*
X_1X_2	5.65	0.0988*	5.365	0.2134	-4.075	0.1810
X_1X_3	-0.25	0.9321	-3.85	0.3534	1.29	0.6437
X_2^2	-6.225	0.0850*	10.8558	0.0393*	0.3925	0.8913
X_2X_3	-0.05	0.9864	-5.54	0.2011	-4.8	0.1268
X_3^2	-0.625	0.8381	11.6808	0.0308*	-5.1925	0.1156
			Analysis of Variance			
R^2	82.79		96.53		99.20	
Adj. R^2	51.81		90.28		97.77	
SEE	2.79		3.76		2.62	
MAE	1.29		1.88		1.18	

X_1 is the polymer-drug ratio ; X_2 is the stirring speed ; X_3 is the surfactant concentration ; X_1X_2, X_1X_3, X_2X_3 are the interaction terms between the factors; X_1^2, X_2^2, X_3^2 are the quadratic terms of the factors; Y_1 is the particle size; Y_2 is the entrapment efficiency percentage ; and Y_3 is the percentage of MB HCL cumulative release after 8 hrs. *Significant effect of factors on individual responses.

Selection of optimized Formula

After analyzing the effect of selected variables on the targeted responses, the optimized formula for MBH were obtained at a drug: polymer ratio of 1:8, stirring speed of 1600 rpm and surfactant concentration of 1% w/v. The observed particle size, % EE and % drug release after 8 h of optimized formula were 40.55µm, 58.11% and 60.62%, respectively, which were close to the predicted values (38.88 µm, 59.19% and 58.64%, respectively) for the optimized formula. The optimized formula (FO) fulfilled the targets of the mathematical experimental design in having smaller particle size, optimum entrapment efficiency and controlled drug release in the colonic environment.

Surface morphology of the optimized MBH-loaded microsphere formulation

SEM studies of the optimized MBH-loaded chitosan microspheres (FO) indicate that the prepared microspheres exhibit a discrete spherical shape with nearly smooth surface (Figure 3).

In vitro release study of optimized MBH-loaded chitosan microspheres

The *in vitro* release of MBH from the optimized formula (FO) was carried out in SGF (pH 1.2) and SIF (pH 6.8 and pH 7.4) as abovementioned. The release of MBH from the optimized formula was slow with less than 35% drug released in both simulated gastric fluid and intestine fluid (pH 6.8). However, a high amount of MBH (> 65%) was released from optimized formula in colonic environment (pH 7.4); emphasizing the applicability of our formulated chitosan microspheres for achieving site specific delivery to the colon.

In vivo Pharmacokinetic study of optimized MBH-loaded chitosan microsphere

The *in vivo* pharmacokinetic of the optimized formula (FO) was evaluated and compared with that of free drug to address whether formulating MBH within chitosan microspheres could improve drug systemic bioavailability (Figure 4). The mean pharmacokinetics parameters (C_{max}, t_{max}, K_{el}, AUC and MRT) for optimized formula (FO) and free drug are summarized in Table 3. The optimized formula (FO) exhibited a higher peak plasma concentration compared to that of free drug (168.51 ± 20.05 ng/ml vs. 126.45 ± 29.46 ng/ml, respectively). In addition, the t_{max} for the optimized formula FO was significantly longer than that of free drug, indicating a delayed absorption of MBH from chitosan microspheres. Furthermore, the optimized formula showed higher area under the curve (AUC_{0-24}) than free drug (1349.17±231.59 ng/ml.h vs. 402.8744±89.88 ng/ml.h, respectively), reflecting higher extent of MBH absorption from chitosan microspheres.

DISCUSSION

Mebeverine hydrochloride (MBH) is an antispasmodic agent that is commonly used for the treatment of irritable bowel syndrome (IBS).[4,5] Nevertheless, following oral administration, MBH is rapidly absorbed from the upper part of gastrointestinal tract and undergoes extensive first pass metabolism[6] and thereby, shows a poor oral bioavailability. Accordingly, site specific delivery of MBH to the colon might represent a promising strategy for delivering MBH specifically in the colon in a reproducible and/or controlled manner.[20,21]

Nevertheless, for achieving colon targeting, the delivery vehicle should protect the drug from degradation, release and absorption in stomach and small intestine and should allow the selective/controlled release in the proximal colon. In the present study, therefore, MBH was loaded onto chitosan microspheres prepared with cross linking method using gluteraldehyde as a cross linking agent. Cross-linked chitosan is relatively stable in acidic medium but rapidly swell and gradually release its entrapped drug in an alkaline medium.[22,23] Accordingly, by loading MBH onto cross-linked chitosan microspheres, only a little amount of MBH is expected to be released in the stomach. Whilst, at the intestinal pH, the cross-linked chitosan will get swollen. This gelling property is anti-

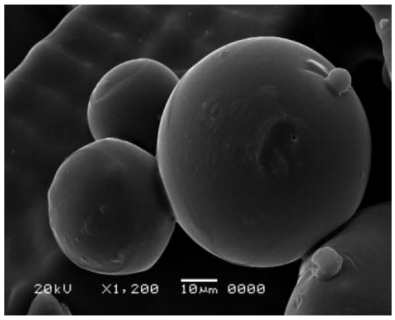

Figure 3: Scanning electron microscopy image of optimized MBH-loaded chitosan microsphere.

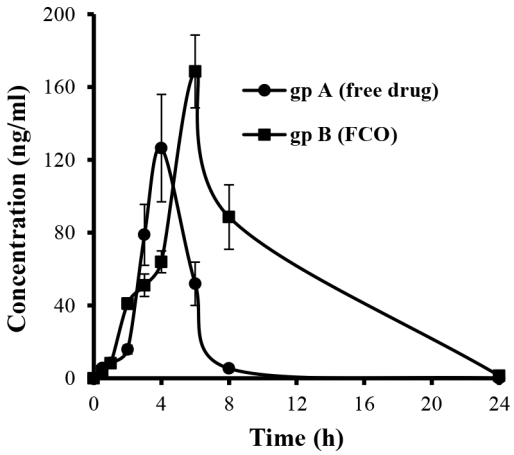

Figure 4: Mean plasma concentration–time profiles following oral administration of optimized formula (FCO) and free MBH.
Rabbits were orally treated with either free MBH or MBH-loaded chitosan microspheres (FCO) at a dose of 20 mg MBH/kg. Data represent mean ± SD ($n = 4$).

Table 3: Pharmacokinetics parameters after oral administration of different formulations of Mebeverine HCl.

Parameter	Formula	
	FCO	Free drug
C_{max} (ng/ml)	168.51 ± 20.05	126.45 ± 29.46
T_{max} (hr)	6.00 ± 0.002	4.00 ± 0.004
K_{el}(hr^{-1})	0.29 ± 0.08	0.79 ± 0.02
AUC $_{0-24}$ (ng/ml.hr)	1343.40 ± 226.11	402.8744 ± 89.88
AUC $_{0-\infty}$ (ng/ml.hr)	1349.17 ± 231.59	409.86 ± 92.14
MRT (hr)	6.99 ± 0.26	4.31 ± 0.02

Data represent mean ± S.D (n = 4).

cipated to retard MBH release in the small intestine until reach the site of action (colon).

A three-level Box-Behnken design was employed to optimize the formulation parameters of MBH microspheres for maximum entrapment percent, optimum particle size and controlled percent drug release. Increasing drug: polymer ratio from 1:4 to 1:8 resulted in an increase in the average particle size of the formulated microspheres (Table 1). These findings might be attributed to the increment in the viscosity of the dispersed phase upon increasing polymer concentration, which in turn, favors the formation of larger droplets and consequently larger microspheres.[24] Similarly, increasing drug: polymer ratio exerted a synergistic effect on entrapment efficiency percentage (Figure 1). Such synergistic effect was ascribed to the increase in aqueous phase viscosity of aqueous phase upon increasing polymer concentration, which in turn, stabilize droplets and inhibit the leakage of drug during the hardening phase.[25] On the other hand, drug: polymer ratio was found to exert an antagonistic effect on percentage drug released after 8 h (Figure 1). Such antagonistic effect might be attributed, on the one hand, to the fact that increasing polymer concentration increases the density of polymer matrix and triggers the formation a thicker matrix wall of microspheres with less number of pores, which in turn, results in the prolongation of diffusion path for the drug and thereby hindering drug release from microspheres. On the other hand, at higher drug: polymer ratio (1:8), the number of NH_2 groups of chitosan available for cross-linking with – COO group of glutaraldehyde will be much higher than those at lower drug: polymer ratios (1:4 or 1:6), which favors more cross-linking reaction. Increasing cross-linking density is reported to increase the hydrophobicity of chitosan matrix and prolong the time for hydration resulting in lower and/or controlled drug release from microspheres.[26]

Site specific drug delivery to the colon has recently gained increased attention as a mean for delivering drugs specifically in the colon in a reproducible and/or controlled manner.[7-9] In this study, the feasibility of cross-linked chitosan microspheres as a vehicle for MBH site specific delivery to the colon was investigated. *In vitro* release study of optimized formula revealed a controlled site specific delivery of the majority of entrapped drug at the colonic environment. In addition, *in vivo* evaluation of optimized MBH-loaded chitosan microspheres proved that entrapment of MBH within chitosan microspheres significantly enhances drug oral bioavailability compare to free counterpart (Figure 4 and Table 3). The bioavailability of MBH from the optimized formula (FO) was ≈ 3 times greater than that of free drug. These results underscore the potential use of MBH-loaded chitosan microspheres for treating diseases associated with the colon such as irritable bowel syndrome.

CONCLUSION

In the present study, MBH-loaded chitosan microspheres were prepared, optimized and evaluated for their effectiveness for achieving site specific delivery of MBH to the colon. *In vitro* release studies verified the site-specific drug release at the colonic environment; with more than 60% of entrapped drug released at the colon. In addition, pharmacokinetic studies confirmed the highest rate and extent of drug absorption from the optimized formula, compared to free drug, leading to a significant enhancement in drug bioavailability. Collectively, MBH-loaded chitosan microspheres might represent a potential alternative for conventional oral dosage forms for treating diseases associated with the colon such as irritable bowel syndrome.

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CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

ABBREVIATIONS USED

IBS: Irritable bowel syndrome; **MBH:** Mebeverine hydrochloride; **EE:** Entrapment efficiency; **SGF:** simulated gastric fluid; **SIF:** simulated intestinal fluid; **C_{max}:** Peak plasma concentration; **t_{max}:** Time of maximum

concentration; **Kel:** Elimination rate constant; **AUC:** Area under the curve; **MRT:** Mean residence time; **SEM:** Scanning electron microscope.

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