

Original Research Article

Pharmacokinetic Study of a Capsule-based Chronomodulated Drug Delivery System of Salbutamol Sulphate in Rabbits

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Abstract

Purpose: To develop and determine the *in vivo* performance of a capsule-based pulsatile drug delivery system containing salbutamol sulphate

Methods: A controlled pulsatile release of drug after a programmed 4 h lag period was achieved from cross-linked gelatin capsule shells containing salbutamol pellets, and sealed with a suitable mixture of sodium alginate and ethyl cellulose as plug. In order to confirm the utility of developed system for the management of nocturnal asthma, a crossover study was conducted. six male rabbits were fasted overnight and divided into two groups comprised of 3 rabbits each. The individual rabbits were administered the developed pulsatile capsule and immediate release salbutamol capsule as reference, separately. Blood samples were collected from the ear vein of the animals into heparinized tubes and used to determine pharmacokinetic parameters, namely, maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), and area under the plasma concentration - time curve ($AUC_{0-\infty}$) using a validated HPLC method.

Results: It was observed that drug release from the optimized time-controlled capsule stopped for a period of approximately 4.25 h with an average C_{max} and T_{max} of 271.54 ± 58.95 ng/ml and 6.00 ± 0.25 h. The $AUC_{0-\infty}$ of salbutamol after administration of the time-controlled pulsatile system was 2494.73 ± 525.95 ng h/ml while that of the immediate-release formulation was 2352.77 ± 432.51 ng h/ml. Using ANOVA at a significant difference of $p < 0.05$ (CI 95%), there was no significant difference for the $AUC_{0-\infty}$ between immediate release and the pulsatile capsule developed.

Conclusion: The developed system is capable of releasing salbutamol after a 4 h lag period and can be considered as promising delivery system for time-controlled (pulsatile) delivery of the medication for the management of nocturnal asthma.

Keywords: pH-controlled release, Lag time, Pulsatile release, Hydrocolloid plug, Nocturnal asthma.

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INTRODUCTION

Up till the early 1990s, efforts were mainly to design drug delivery systems which will release the drug at fairly constant rate. In fact, these

systems turned to be one of the most successful systems in delivering the drug molecule [1]. But still for many of the drugs, use of such systems is not suitable because of a number of reasons. This is particularly true in cases where the drug is subjected to large metabolic degradation. Due

to 'first pass effect' there will be reduction in the bioavailability of the drug because gradual release can result in greater degradation. Secondly drugs with short half-life need to be administered repeatedly which results in patient non-compliance. Further, in the case of chronic treatment where the drug is given in sustained release dosage form, continuous exposure of the drug to body may lead to adverse effect. Lastly, drugs which exhibit tolerance should not be delivered at a constant rate, since the drug effect decreases with time at constant drug level. In such cases it is preferable to opt for a dosage form which will provide concentration of drug at particular time point only [2].

Chronopharmaceutics, whereby research is devoted to the design and evaluation of drug delivery systems that release a therapeutic agent at a rhythm that ideally matches the biological requirement of a given disease therapy, has attracted some attention in recent times [3,4]. The objective of this study was to determine the *in vivo* behavior of a developed salbutamol pulsatile capsule for the management of nocturnal asthma. Capsule is designed to have a release off period of 4 hour [5,6]. The developed system has already shown positive results during *in vitro* studies [7]. Ideally, an animal model that suitably mimics human physiological system should be chosen for *in vivo* studies. In this regard, animals such as monkeys, pigs and dogs have been suggested [9]. However, since these animals are difficult to handle, rabbit was chosen for use in the present study due to the ease of handling.

EXPERIMENTAL

Materials

Salbutamol sulphate (Ranbaxy Research Laboratories, Gurgaon, India), microcrystalline cellulose (Avicel PH101, Jubilant Organosys, New Delhi India), Eudragit L 100 and S 100 (Rohm Pharma, GmbH Germany) Sodium alginate, HPMC, Polyethylene oxide, Ethylcellulose (Ranbaxy Research Laboratories, Gurgaon, India) were obtained as gifts from the indicated sources. Acetonitrile HPLC grade and methanol HPLC grade were purchased from JT Baker (USA). Salbutamol and standards were purchased from Sigma Aldrich (USA). All other chemicals used were of analytical grade

Development of pulsatile drug delivery system

The pulsatile capsule was aimed to provide a total lag time of approximately 4 h. The basic

design of the proposed dosage form consisted of an insoluble capsule body filled with drug loaded pellets sealed with hydrocolloid plug, and a soluble capsule cap. Hydrocolloid plug was inserted to stop the release of pellets for a period of 2 h. The plugged capsule was further coated with enteric polymer (Eudragit S100: Eudragit L100, 4:1) to provide a additional 2 hour lag time and to minimize the gastric transit time variability. Finally, a total of 4-h lag time was achieved using hydrocolloid plug material together with outer enteric coating of sealed capsule. Enteric coating was expected to be dissolved at pH 6.8. Pellets were prepared by extrusion and spheronization using 2 % w/v of PVP K30 as binder and water as granulating liquid. In order to prepare insoluble gelatin capsule bodies, the empty gelatin capsule bodies were exposed to formalin vapors generated by a reaction between 15 %v/v formaldehyde solution and potassium permanganate. The caps of the capsules were not subjected to the above treatment, leaving them water soluble.

Rapidly disintegrating pellets were prepared by extrusion, spheronization method. Drug and excipients were preblended and sieved through 0.420 mm mesh screen. Ac DiSol (3 %w/w) as disintegrant and 2 % PVP K-30 aqueous solution, as binding agent, were added to mixed powders to achieve proper consistency and kneaded properly. The pellets were evaluated for flow properties, surface morphology and *in vitro* dissolution behavior. Optimized pellets were filled in to insoluble capsule, plugged with hydrocolloid material to provide the desired delay in release and coated with enteric polymer which was required to be dissolved in pH 6.8 to avoid gastric emptying variations [9].

The dissolution behavior of the developed system was studied in pH 1.2 and phosphate buffer pH 6.8 media. For the initial 2 h, the system was exposed to 0.1M HCl after that media was changed to pH 6.8 phosphate buffer to simulate lower intestinal pH.

After *in-vitro* optimization, *in-vivo* studies were carried out in healthy rabbits to validate the developed system. The calibration curves were constructed by spiking drug-free plasma with known amount of salbutamol at concentrations of 5.0 - 400.0 ng/ml. The standard calibration curve ($n = 6$) was found to be linear over the concentration range used with a correlation coefficient of 0.9999. The samples were quantified using peak height ratio of salbutamol over the internal standard.

Animal studies

The pharmacokinetic study was performed in accordance with the guidelines of the National Institutes of Health (10) and was approved by the Ethical Committee for Animal Experimentation of Hamdard University, India. The study was conducted using 6 male rabbits (3.0 - 3.5 kg). The rabbits were fasted overnight for at least 12 h prior to the study. The 6 rabbits were divided into two groups with three rabbits in each group. In the first phase of the study, the rabbits in group 1 received optimized formulation and those in group 2 received immediate release capsule as reference. After a washout period of 7 days, the rabbits were crossed-over and administered the alternate formulation. Both optimized formulation and reference containing equivalent of 5 mg of salbutamol sulphate were administered orally. The animals were fed 4 h after drug dosing and had free access to water throughout the study period. Blood samples of 2 ml were collected through the marginal ear vein into heparinized tubes at regular time intervals. Blood samples were centrifuged at 4,000 rpm (Labofuge 200, Germany) for 5 min. The supernatant was transferred to empty glass tubes and stored at -70 °C until further analysis. Pharmacokinetic parameters, namely, maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), and area under the plasma concentration- time curve ($AUC_{0-\infty}$), were obtained from plasma concentration versus time profiles. C_{max} and T_{max} were derived directly from the *in vivo* data. The area under the plasma salbutamol concentration time curve (AUC_{0-t}) was calculated by the trapezoidal rule. $AUC_{0-\infty}$ was the summation of area under plasma salbutamol concentration-time curve from zero to time t (AUC_{0-t}) and area under plasma salbutamol concentration-time curve from time t to infinity ($AUC_{t-\infty}$). $AUC_{t-\infty}$ was calculated by dividing the last measurable plasma concentration with the terminal elimination rate constant (K_e). The value of K_e was the slope of logarithmic transformation of the terminal plasma concentration-time curve

Plasma treatment and determination of salbutamol concentration

The method reported by Xiao-Li *et al* with a slight modification was used for the determination. Supelco LC-18 solid phase extraction (SPE) columns (1 ml) were used for this purpose. The column was washed with methanol and water 1 ml each. The internal standard solution, bamethan (0.2 µg/ml) and 1 ml of the plasma sample were passed into the column. After 2 min, mild suction was applied to allow the sample

to pass through the column. The suction was increased to expel all the trapped liquid from the column. The column was washed with water three times followed by acetonitrile once. Then 2 ml methanol was passed through the column to elute the internal standard and the drug from the sorbent. The eluted liquid was dried at 40 °C under a gentle stream of nitrogen gas. The residue obtained was reconstituted with 50 µl of mobile phase and 40 µl was injected into the system [10].

The HPLC system comprised of Waters pump (Model 510, Milford, MA, USA) equipped with sample injection port (7725i Rheodyne, Cotati, California, USA) fitted with 50 µl sample loop, a RF- 10AxI fluorescence detector (Shimadzu, Tokyo, Japan) and Chromato-integrator (D-2500, Hitachi, Tokyo, Japan). The analytical column, Luna C18 (2) (5 µm, 250 x 4.6 mm ID) column (Phenomenex, USA) fitted with a refillable guard column (Upchurch Scientific, Oak Harbour, WA, USA) packed with Perisorb RP-18 (30 - 40 µm, pellicular) was used for elution of salbutamol. The analysis was run at a flow-rate of 0.8 ml/min. The mobile phase consisted of phosphate buffer (pH 2.5), methanol and acetonitrile (890:170:20, v: v: v), the excitation and emission wavelengths were 275 nm and 309 nm respectively.

Statistical analysis

Drug plasma concentration and pharmacokinetic parameters were analyzed by Wilcoxon Signed Rank test for paired samples and analysis of variance (ANOVA) at 95 % confidence limit. Difference between two related means was considered statistically significant at $p \leq 0.05$.

RESULTS

Characteristics of pellets

Rapidly disintegrating pellets with good flow properties, smooth surface, a narrow particle size distribution and optimum elasticity and plasticity of the mass were obtained by combining Avicel PH 101 and lactose in the ratio of 70:20, together with 5 % drug, 3 % AcDiSol, and 2 % PVP K30. The yield, flow rate and mean particle size of the optimized pellet formulation were 74.65 %, 21.63 g/sec and 889.6 µm, respectively.

Formulation of hydrocolloid plug

A total of 4 h lag time was intended for this pulsatile capsule. The plug was designed to provide 2 h lag time and the remaining 2 h was aimed to achieved by enteric coating. The

formulation sealed with 10, 20 and 30 mg of sodium alginate as plug material showed lag time of 0.15 ± 0.010 h, 1 ± 0.15 and 1.5 ± 0.25 h, respectively. At the end of 2 h, 87.98 and 42.11 % drug release was observed from formulations plugged with 20 and 30 mg of sodium alginate, respectively. On the basis of plug ejection time and release profile obtained, sodium alginate 20 mg was selected for further study.

Ethyl cellulose (5, 10 and 15 mg) as a release modifier was added to replace the sodium alginate in the mixture to overcome the problem of drug leaching before the actual ejection of the plug. A plug made up of sodium alginate and ethyl cellulose (1:1, 20 mg) was considered as optimized plug since it was able to stop drug release from pellets for the required period of 2 h (Fig 1).

Enteric-coated plugged capsule

In order to achieve the complete lag time of 4 h as indicated above, the capsule plugged with hydrocolloid material was further coated with enteric polymer. On the basis of results obtained after disintegration and dissolution analysis of various enteric polymers and their combinations in 0.1 M HCl and pH 6.8 phosphate buffer, capsules coated with 6 %w/w coat of a mixture of Eudragit S100 and L100 (4:1) was considered to be optimized in terms of providing a lag time to 2 h and a total lag time of 4 h (Table 1).

Animal study for developed pulsatile capsule. The limit of quantification (LOQ) was set at 5.0 ng/ml, which was the lowest concentration used in constructing the standard calibration curve. The limit of detection was 1 ng /ml. Inter-day accuracy ranged between 91.28 and 102.45 % with precision between 1.90 and 9.26 %. Intra-day accuracy ranged between 89.92 and 108.11 % with precision between 0.65 and 10.24 %.

Drug release from the optimized time-controlled capsule was halted for a period of approximately 4 ± 0.25 h with mean C_{max} and T_{max} of 271.54 ± 58.95 ng /ml and 6.0 ± 0.25 h, respectively. Drug

absorption from IR capsule was rapid without lag time. Mean C_{max} and T_{max} values obtained after administration of IR capsule were 260.35 ± 54.19 ng/ml and 2.0 ± 0.25 h, respectively. The $AUC_{0-\infty}$ of salbutamol after administration of time-controlled pulsatile system was 2494.73 ± 525.95 ng h/ml while that of the immediate release formulation was 2352.77 ± 432.51 ng h/ml. There was a statistically significant difference ($p \leq 0.05$) between the T_{max} values of the two formulations. However, there was no significant difference ($p \leq 0.05$) in C_{max} and $AUC_{0-\infty}$ values between the two formulations (Fig 3).

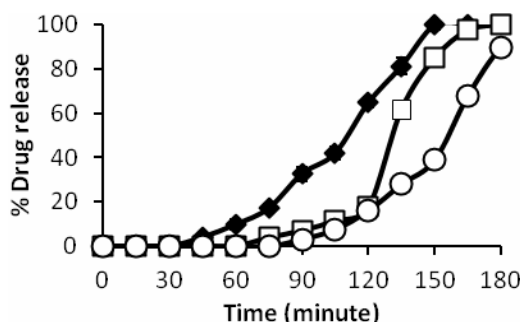


Fig 1: *In vitro* release profile of pulsatile capsule plugged with various amounts of ethyl cellulose (EC) and sodium alginate (SA) mixture; \blacklozenge = 15mg SA and 5 mg EC, \square = 10 mg SA and 10 mg EC, \circ = 5 mg SA and 15 mg EC

Almost 97.65 % drug was released in pH 6.8 in a period of 2.25 h (Fig 2).

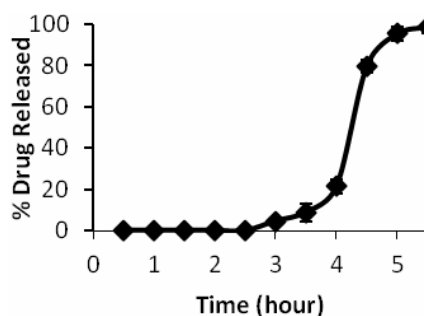


Fig 2: Dissolution profile of optimized dosage form in 0.1M HCl (initial 2 h) and then in pH 6.8 phosphate buffer.

Table 1: Effect of disintegration and dissolution on formulations of various combinations of Eudragit S100 and L 100 with various levels of enteric coating

Parameter	S100			L 100			S100:L100 (1:1)			S100:L100 (2:1)			S100:L100 (4:1)		
	4	6	8	4	6	8	4	6	8	4	6	8	4	6	8
Coating level (% w/w)	4	6	8	4	6	8	4	6	8	4	6	8	4	6	8
Disintegration test	F	P	P	F			F	P	P	F	P	P	P	P	P
Solubility in pH 5.5	--	S	NS	--	S	S	-	S	S	-	S	S	S	NS	NS
Avg. lag time in pH 6.8 (h)	--	1.3	2.8	--	1.3	1.3	--	1.3	1.8	-	1.3		1.9	2.0	2.3

F = Failed, P = Passed, S = Soluble, NS = Not soluble.

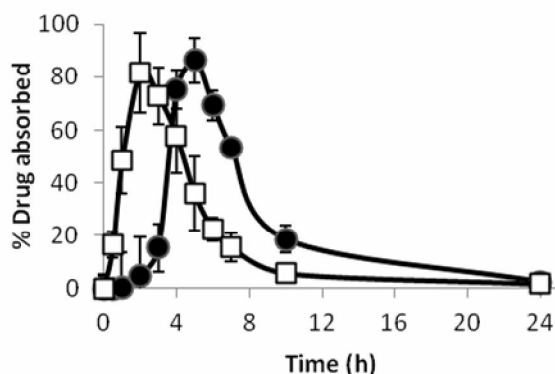


Fig 3: Comparative plasma drug concentration vs. time profiles of immediate release (□) and pulsatile capsules (●) of salbutamol sulphate

DISCUSSION

The developed system is based on the combination of pH sensitive (enteric) coating and time-controlled (plug) delivery.

Schiff's base condensation phenomenon has been employed to modify the solubility of gelatin capsule bodies. On exposure to formalin vapors, amino group present in the gelatin (capsule shell) molecular chain undergoes cross linking with aldehyde group present in formaldehyde molecule as per Schiff's base condensation resulting in decreased aqueous solubility of gelatin [11]. The present study revealed that Avicel PH 101 in combination with lactose formed pellets with smooth surface characteristics. With increase in lactose concentration, a decrease in the yield, increase in friability and generation of fines were observed. At increased Avicel PH 101 content, mean particle diameter decreased and most pellets formed were spherical with even particle size distribution. Since sodium alginate has lower mechanical strength and is sticky, the plug was completely detached from the capsule, leading to complete and immediate release after the desired lag period [12].

A 6 % w/w coat of Eudragit S100 and L100 in ratio of 4:1 was found to be optimum since the coat was intact after 2 h disintegration analysis in 0.1M HCl which indicates the ability of the coat to withstand gastric acid conditions. During dissolution studies it was observed that the coat was completely dissolved in pH 6.8 which confirms the release of the drug in the duodenum part specified region of small intestine to maintain the desired lag time of 2 h. The soluble cap of the capsule had dissolved completely after 15-20 min and thus exposed the polymer plug which absorbed the surrounding fluid, swelled and released the drug through the swollen matrix. Following complete wetting of the plug, it

formed a soft mass which was then easily ejected out of the capsule body by erosion mechanism, thus releasing the pellets into the medium which readily dissolved and complete drug release was achieved after a lag time of 4.25 h.

There was statistically significant difference between the T_{max} values of the two formulations. However, there was no statistical significant difference between the two formulations with regard to C_{max} and $AUC_{0-\infty}$ values. The *in-vitro* results are supported by the *in vivo* data in terms of the drug release and lag time obtained.

CONCLUSION

The *in vivo* study supports the capability of the developed pulsatile release system to delay drug release in the gastrointestinal tract after 4-h lag time. Thus, the developed capsule-based delivery system can be considered a promising formulation for time-controlled delivery of salbutamol for the nocturnal management of asthma.

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