

Okara Gum: A natural polysaccharide based carrier for preparation of microbially triggered colon targeted drug delivery system

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Abstract

Colon targeted pulsatile systems are best for drugs which follow chronopharmacological behavior where, night time dosing is required, drugs have high first-pass effect and specific site of absorption in GIT. In this study, colon targeted pulsatile release tablets of atenolol was prepared using press coating technique to treat hypertension in the early hours of the morning. Pulsatile release of atenolol in colon was achieved by press coating of atenolol core tablet using biodegradable polysaccharides. A biodegradable polymer is a polymer in which the degradation results from the action of naturally occurring microorganisms such as bacteria, algae or fungi. The inability of GIT enzymes to digest certain plant polysaccharides (pectin, guar gum, okara gum) is taken as an advantage to develop colon specific drug delivery systems. The aim of this study is to find out the suitability of newer polysaccharide okara gum for colon targeting of atenolol by compression coating atenolol core tablet with okara gum. The minimum coat weight of okara gum required for colon targeting is optimized. OG4 press coated tablet having 250 mg coat weight of okara gum showed 6 hr lag time and near 7 hr $T_{85\%}$, which is applicable pulsatile drug delivery of atenolol for treating early morning hypertension.

Keywords

Colon targeting, pulsatile system, press coating, biodegradable polymer, Okara gum, hypertension

INTRODUCTION

In order to achieve the chronopharmaceutical design for the time controlled pulsatile type of colon targeted preparations, formulation design to control the lag time is prior to the immediate release of drug. These systems have a peculiar mechanism of delivering the drug rapidly and completely after a lag time (a period of no drug release). Though most delivery

systems are designed for constant drug release over a prolonged period of time, pulsatile delivery systems are characterized by a programmed drug release, as constant blood levels of a drug may not always be desirable. Pulsatile time release systems are designed in a manner that the drug is available at the site of action at the right time in the right amount. These systems are beneficial for drugs having high first pass

effect, drugs administered for diseases that follow chronological behavior, drugs having specific absorption site in GIT, targeting to colon, where night time dosing is required^[1].

Atenolol, a β -blocker, is prescribed widely in diverse cardiovascular diseases like; Hypertension, angina pectoris, arrhythmias, and myocardial infarction. In case of cardiovascular diseases, several functions (e.g. BP, heart rate, stroke volume, cardiac output, blood flow) of the cardiovascular system are subject to circadian rhythms. It has been reported that more shocks and heart attacks occur during morning hours^[2]. The level of cortisol is higher in the morning hours, and its release is reported to decline gradually during the day. Blood pressure is also reported to be high in the morning till late afternoon, and then drops off during night^[3]. On oral administration of colon targeted pulsatile drug delivery system at bed time, releases atenolol after a desired lag time of about 6 – 6.5 hr which corresponds with peak levels of cortisol, capillary resistance, platelet aggregability and vascular reactivity in the morning hours, which leads to hypertension in the early hours of the morning.

The natural polymers can be proteins and polysaccharides in chemical origin. With Greentree Group

this natural polymers will not shows any interactions with the API. Biodegradable natural polymers are highly desirable in their conditions as they degrade in the body to biologically inert and compatible molecules and cleared by the body^[4]. Biodegradable natural polymers are attractive class for controlled drug delivery since they are: derived from natural sources, easily available, relatively cheap, free of leachable impurities, produce degradation by-products that must be tolerated with little or no adverse reactions within the biological environment and chemically inert.

Several approaches have been investigated to targeting drug to colon. Targeting of drugs to the colon following oral administration has been done by using biodegradable polysaccharides. A biodegradable polymer is a polymer in which the degradation results from the action of naturally occurring microorganisms such as bacteria, algae or fungi. The inability of GIT enzymes to digest certain plant polysaccharides (pectin, guar gum, okara gum) is taken as an advantage to develop colon specific drug delivery systems^[5]. The anaerobic bacteria of colon only produce the polysaccharidase enzyme which degrade the carrier polysaccharides and release the contents for

localized or systemic absorption through colon^[6]. Various biodegradable polysachharides used for colon targeting are guar gum, locust bean gum, inulin, pectin, amylose, chondroitin sulfate, cyclodextrin, chitosan, dextran, alginate, xanthan gum etc.

With the increase in demand for natural polysachharides, it has become necessary to explore the newer sources of polysachharides to meet the industrial demands^[7]. With this belief, the aim of this study is to find out the suitability of newer polysaccharide okara gum for colon targeting of atenolol by compression coating atenolol core tablet with okara gum. The minimum coat weight of okara gum required for colon targeting is optimized.

MATERIALS AND METHODS

Atenolol was supplied as a gift sample by Zydus Cadila Healthcare Ltd., Ahmedabad, India. Sodium starch glycolate IP was supplied as a gift sample by Maruti Chemicals, Ahmedabad, India. Fresh immature fruits of *Hibiscus esculentus* Linn. were purchased from local market of Ahmedabad. Polyvinyl pyrrolidone K 30 (PVP) was purchased from S. D. Fine Chemicals Ltd., Mumbai, India. Microcrystalline cellulose, Talcum powder, magnesium stearate and sodium hydroxide

were purchased from Chemdyes Corporation, Rajkot, India. Potassium dihydrogen phosphate was purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Acetone and Methanol were purchased from Ranbaxy Fine Chemicals Ltd., New Delhi, India.

Evaluation of flow property of powder blends

Powder blends used for preparation of atenolol core tablets and compression coated tablets were evaluated for flow property by measuring bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose.

Preparation of atenolol core tablets

The core tablets of atenolol (AT2) were prepared by direct compression method. An optimized core tablet was formulated using various concentrations of dry binder and super disintegrant as described in table 1. An accurately weighed quantity of atenolol, microcrystalline cellulose, polyvinyl pyrrolidone K30 (PVP), sodium starch glycolate, talc (2% w/w) and magnesium stearate (1% w/w) were passed through 22# sieve and mixed by triturating in a mortar and pestle for 10 min. The resultant powder mixtures were compressed into tablets (average tablet weight = 80 mg) by 6 mm standard concave plain punches using rotary

tableting machine (Hardik Engineering Works, Ahmedabad, India) and compression force was controlled to produce more than $3 \pm 0.5 \text{ kg/cm}^2$ tablet hardness. The prepared atenolol core tablets were tested for weight variation, hardness, thickness, drug content, disintegration time, friability and *in vitro* dissolution study^[8,9].

Figure 1: Cross sectional photograph of atenolol press coated tablet (OG)



Extraction of okara gum mucilage

The fresh immature fruits of *Hibiscus esculentus* Linn. were collected, washed with water to remove debris and dried. The fruits were then sliced, homogenized with ten times its weight of water and then heated at 80°C for 10 min to inactivate any enzymes present and to completely extract the active constituents into solvent. The heated solution was then filtered using a muslin cloth and the filtrate was centrifuged at 4000 rpm for 15 min, it produced clear, viscous solution. Three volumes of acetone were then added into viscous solution contained in a separating funnel to

precipitate out the mucilage. Okara gum mucilage was then washed with diethyl ether to remove any impurities and the obtained cream colored product, was dried in hot air oven at 60°C . A light brown colored powder was obtained after complete removal of moisture. Dried okara gum powder milled in disintegrating mill to produce fine powder^[10,11].

Table 1: Composition of atenolol core tablet

Ingredients	Quantity for each tablet (mg)
Atenolol (API)	45 mg
Sodium Starch Glycolate (SSG) (5%)	4 mg
Polyvinyl pyrrolidone K30 (PVP K30) (5%)	4 mg
Microcrystalline Cellulose (MCC)	24.6 mg
Talc (2%)	1.6 mg
Mg Stearate (1%)	0.8 mg
Total Weight	80 mg

Preparation of granules of okara gum

Preparation of granules of okara gum

Okara gum powder exhibited poor flow properties and compressibility. A wet granulation method was used to prepare the okara gum granules using starch paste as binder. Various concentrations of starch paste (as shown in table 3) were made by dissolving soluble starch into boiling water

by continuous stirring which upon cooling formed thick paste. The dump mass of okara gum powder was prepared using starch paste and passed through sieve 20# to obtain the granules. The granules were dried in hot air oven at about 60°C for 24 hours and stored in airtight container and used as press coating material to prepare atenolol press coated pulsatile tablets^[12].

Table 2: Independent variables with levels for central composite design

Independent variable (Factor)	Levels of factors				
	- α (-1.414) (Extreme Low)	-1 (Low)	0 (Center Point)	+1 (High)	+ α (+1.414) (Extreme High)
A: percentage of starch paste (%)	2.06	3	5	7.5	8.43
B: Amount of okara gum granules (mg)	129.28	150	200	250	270.74

Preparation of atenolol press coated (OG) tablets

To study the suitability of okara gum for colon targeting to allow its release only in the colon, the atenolol core tablets were compression coated with powder blend containing different weight ratio of okara gum granules (shown in table 3). Weigh accurately required quantity of okara gum granules, add talc (2% w/w) (40#) into it and

mix in double cone blender for 10 min. Add magnesium stearate (1% w/w) (40#) into granular blend and mix in double cone blender for 5 min. Forty three percentage of weight of granular coating material was first placed into die cavity (diameter 9 mm); then, the core tablet was carefully placed on it manually at the centre of the die. The remaining fifty seven percentage of the granular coating material was added into the die and the coating material was then compressed around the core tablet by 9 mm standard concave plain punches using rotary tableting machine (Cadmach Machinery, Ahmedabad, India). The prepared compression coated atenolol tablets were evaluated for weight variation, hardness, thickness, drug content, friability and *in vitro* dissolution study^[13,14].

In this study, central composite design (CCD) was used to optimize the coat weight of barrier layer (okara gum granules) and concentration of granulating agent (starch paste). This design is suitable for exploring main effect, interaction effect, and quadratic effect by constructing polynomial equation incorporating polynomial terms used to evaluate the response.

Figure 2 Dissolution profile of atenolol press coated (OG) tablets in absence of rat cecal content

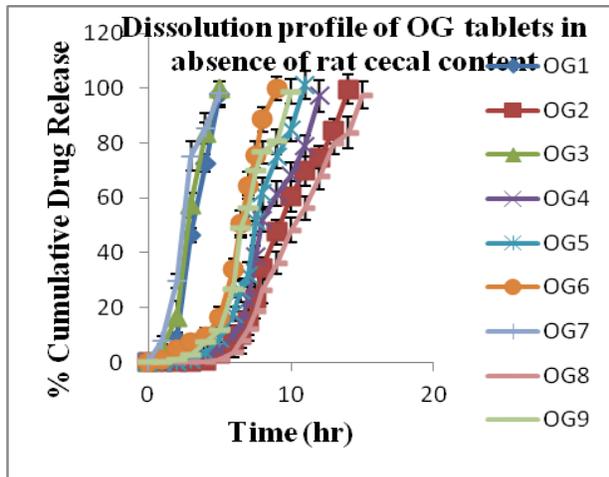
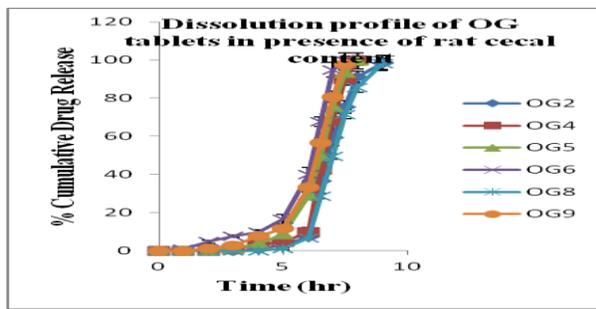


Figure 3 Dissolution profile of atenolol press coated (OG) tablets in presence of rat cecal content



$$Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 \dots \dots (1)$$

Where, Y is the dependent variables, b_0 is the arithmetic mean response of the nine runs, b_1 is the estimated coefficient for the factor A, b_2 is the estimated coefficient for the factor B and b_3 is the estimated coefficient for the factor C. b_{12} , b_{13} , and b_{23} are coefficients of interaction. b_{11} , b_{22} and b_{33} are coefficients of quadratic terms.

Table 3 CCD for optimization of formulation of atenolol press coated (OG) tablets

Formulation code	Core tablet	Independent variables coded value		Independent variables actual value	
		Factor 1 (A)	Factor 2 (B)	Factor 1 (A)	Factor 2 (B)
OG1	AT2	-1	-1	3	150
OG2	AT2	-1	1	3	250
OG3	AT2	1	-1	7.5	150
OG4	AT2	1	1	7.5	250
OG5	AT2	-1.414	0	2.06	200
OG6	AT2	1.414	0	8.43	200
OG7	AT2	0	-1.414	5	129.28
OG8	AT2	0	1.414	5	270.74
OG9	AT2	0	0	5	200

Where, A = percentage of starch paste (%), B = Amount of okara gum ranules (mg)

The coded value of $\pm\alpha$ represents the distance of the star points from the center point and is calculate using equation 2 and total numbers of experimental runs required in CCD is determined using equation 3.

$$\alpha = (2^K \text{ or } 2^{K-r})^{1/4} \dots \dots \dots (2)$$

$$\text{Experimental runs} = 2^K \text{ or } 2^{K-r} + 2K + N \dots \dots \dots (3)$$

Where, K = number of variables, r = fractional of full factorial, N = replicated center point experiment

Table 4 Flow property study for powder blends of atenolol press coated (OG) tablets

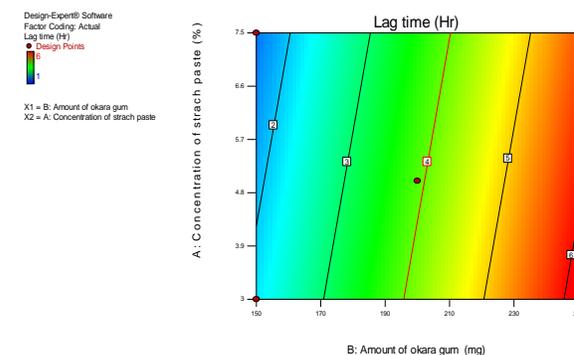
Formula Code	Bulk Density (g/cm ³) (Avg. ± SD)	Tapped Density (g/cm ³) (Avg. ± SD)	Carr's Index (%) (Avg. ± SD)	Hausner's Ratio (H _R) (Avg. ± SD)	Angle of Repose (θ) (Avg. ± SD)
AT2	0.280 ± 0.03	0.33 ± 0.04	15.15 ± 0.65	1.178 ± 0.12	29.74 ± 0.40
OG1	0.550 ± 0.04	0.640 ± 0.03	14.06 ± 0.20	1.163 ± 0.11	28.84 ± 0.15
OG2	0.550 ± 0.04	0.640 ± 0.03	14.06 ± 0.20	1.163 ± 0.11	28.84 ± 0.15
OG3	0.563 ± 0.05	0.644 ± 0.02	12.57 ± 0.25	1.143 ± 0.13	27.82 ± 0.20
OG4	0.563 ± 0.05	0.644 ± 0.02	12.57 ± 0.25	1.143 ± 0.13	27.82 ± 0.20
OG5	0.548 ± 0.03	0.639 ± 0.04	14.24 ± 0.15	1.166 ± 0.14	28.93 ± 0.12
OG6	0.568 ± 0.05	0.647 ± 0.02	12.21 ± 0.18	1.139 ± 0.10	27.56 ± 0.14
OG7	0.559 ± 0.04	0.643 ± 0.03	13.06 ± 0.30	1.150 ± 0.15	28.15 ± 0.12
OG8	0.559 ± 0.04	0.643 ± 0.03	13.06 ± 0.30	1.150 ± 0.15	28.15 ± 0.12
OG9	0.559 ± 0.04	0.643 ± 0.03	13.06 ± 0.30	1.150 ± 0.15	28.15 ± 0.12

Thus, for two factor CCD, coded value of $\alpha = (2^2)^{1/4} = 1.414$ and number of experimental runs = $2^2 + 2(2) + 1 = 9$ runs

In CCD, amount of okara gum granules (A) and percentage of starch paste (B) were selected as independent variables. The lag time or times required for start drug release (hr) and time required for 85% drug dissolution ($t_{85\%}$) were selected as dependent variables. The independent variables with their levels are described in the table 2,

while the experimental design with corresponding formulation outline in table 3.

Figure 4 Contour plot for effect of starch paste and okara gum on lag time



EVALUATION OF ATENOLOL CORE AND PRESS COATED TABLETS

Weight Variation

Twenty tablets from each batch were individually weighed using electronic digital balance (Shimadzu BL – 220 H) and average weight was calculated. Individual weights of the tablets were compared with the average weight according to the official method in Indian Pharmacopoeia, 2007^[15].

Hardness

Six tablets from each batch were selected and tested for tablet hardness using Monsanto hardness tester. The tablet was placed in contact between the plungers and the handle was pressed, the force of the fracture that causes the tablet to break was recorded^[16].

Thickness

The thickness of ten tablets from each batch was determined using vernier calipers as per indian pharmacopoeia, 2007.

Friability

The friability of the twenty tablets from each batch was determined using Roche friabilator (Indosati Scientific Lab. Equipments). This device subjects the tablets to the combined effect of abrasions and shock in a plastic chamber revolving at 25 rpm and dropping the tablets at a height of 6 inches in each revolution. A pre-weighed sample (20 tablets) was placed in the friabilator and was subjected to 100 revolutions^[17]. Tablets were dedusted and reweighed. The % friability (F) was calculated using following formula:

$$F = (W_1 - W_2 / W_1) \times 100 \quad (4)$$

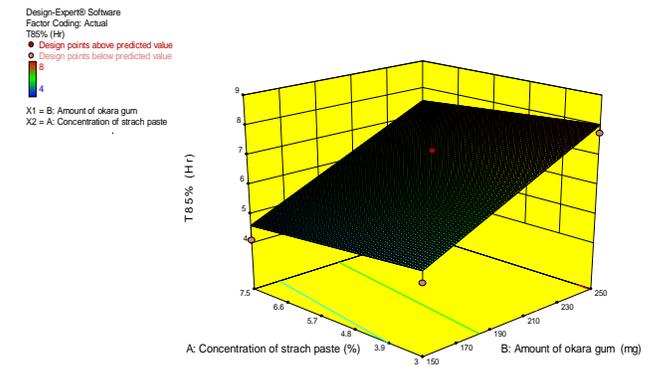
Where, W_1 is the initial weight of the sample of twenty tablets before the test; W_2 is the weight of the tablet after the test

Drug content

For determination of drug content, ten tablets were crushed into powder and powder equivalent to 45 mg of atenolol was weighed and dissolved in methanol then filtered through syringe filter (Axiva SFCA25X, 0.45 μ m). Solution was analyzed for atenolol content by spectrophotometrically by UV spectrophotometer (Thermo Scientific Greentree Group

Evolution 201) at wavelength of 225 nm using methanol as blank^[18].

Figure 5 3-D response plot for effect of starch paste and okara gum on $T_{85\%}$



Position of Core Tablet

Compression coated tablet is cut vertically and cross sectional photographs were taken to evaluate the position of core tablet in the compression coated tablet^[19].

% Swelling studies

One tablet from each press coated formulation was randomly selected, weighed individually (W_1) and placed separately in petridishes containing 20 ml of phosphate buffer pH 7.4. After 6 h, the tablets were carefully removed from petridishes and excess water was removed using filter paper. The swollen tablets were reweighed (W_2) and swelling index of each tablet was calculated using the equation 5 and expressed in percentage^[20].

$$\% \text{ Swelling index} = (W_2 - W_1) / W_1 * 100$$

..... (5)

***In vitro* drug release study of microbially triggered atenolol press coated tablets without rat cecal content**

In vitro drug release studies were carried out using USP type II dissolution apparatus (Electrolab, TDT-08L) in a 900 ml of dissolution media at a temperature of $37 \pm 1^\circ\text{C}$ at 100 rpm. In order to simulate the pH changes along the GI tract, multimedia dissolution studies were performed. Three dissolution media with pH 1.2, 6.8 and 7.4 were sequentially used. Initially dissolution study was performed using 0.1 N HCl (pH 1.2) as dissolution medium for 2 hrs (since the average gastric emptying time is 2 hrs), than dissolution medium was discarded and replaced with phosphate buffer pH 6.8 and dissolution study was continued for next 3 hrs (average small intestinal transit time is 3 hrs). After 3 hrs, the dissolution medium was removed and replaced with phosphate buffer pH 7.4 for subsequent hours. At regular time intervals, 10 ml of samples were withdrawn and same amount replaced by fresh medium. Samples were suitably diluted and filtered through syringe filter (Axiva SFCA25X, $0.45\mu\text{m}$). Drug amount released was analyzed spectrophotometrically by UV

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spectrophotometer (Thermo Scientific Evolution 201) at wavelength of 225 nm. All studies were carried out in triplicates^[18]. The time for which the tablet does not show any release of the drug is known as its lag time. The lag time of tablets was estimated.

Table 5 Post-compression parameters of atenolol press coated (OG) tablets

Formulation Code	Hardness (Kg/cm ²) (Avg. \pm SD)	Friability (%) (Avg. \pm SD)	Drug content (%) (Avg. \pm SD)	Weight variation (mg) (Avg. \pm SD)	Tablet Thickness (mm) (Avg. \pm SD)
AT2	3.25 \pm 0.15	0.12 \pm 0.05	99.97 \pm 1.3	79.57 \pm 2.57	3.20 \pm 0.05
OG1	3.50 \pm 0.35	0.20 \pm 0.09	99.95 \pm 1.5	150.20 \pm 1.45	5.4 \pm 0.07
OG2	4.00 \pm 0.75	0.17 \pm 0.06	99.80 \pm 1.7	249.60 \pm 1.40	6.2 \pm 0.07
OG3	4.00 \pm 0.50	0.13 \pm 0.07	99.75 \pm 1.9	149.35 \pm 1.85	5.4 \pm 0.05
OG4	5.50 \pm 0.25	0.06 \pm 0.08	99.85 \pm 1.2	249.20 \pm 1.44	6.2 \pm 0.04
OG5	3.50 \pm 0.25	0.21 \pm 0.08	99.55 \pm 1.0	200.10 \pm 2.10	5.8 \pm 0.05
OG6	5.00 \pm 0.75	0.09 \pm 0.07	100.0 \pm 1.8	199.56 \pm 1.55	5.9 \pm 0.01
OG7	3.50 \pm 0.25	0.29 \pm 0.05	99.35 \pm 1.9	128.68 \pm 1.20	4.9 \pm 0.05
OG8	5.50 \pm 0.50	0.07 \pm 0.04	100.1 \pm 1.4	270.20 \pm 2.05	6.8 \pm 0.02
OG9	4.00 \pm 0.35	0.15 \pm 0.07	99.75 \pm 1.6	199.57 \pm 1.30	5.8 \pm 0.03

***In vitro* drug release study of microbially triggered atenolol press coated tablets with rat cecal content**

Dissolution study of microbially triggered press coated tablets was carried out without and with rat cecal content to assess the susceptibility of okara gum coats to enzymatic action of colonic bacteria. For dissolution study of microbially triggered press coated tablets with cecal content, initially dissolution study was performed using 0.1 N HCl (pH 1.2) as dissolution medium for 2 hrs, then dissolution medium was discarded and replaced with phosphate buffer pH 7.4 and dissolution study was continued for next 3 hrs. After 3 hrs, study performed with slight modifications. A glass beaker (capacity 250 ml) containing 200 ml of phosphate buffer pH 6.8 containing 4% w/v rat cecal content, was immersed in water contained in the 1000 ml vessel, which was, in turn, in the water bath of the apparatus. The tablets were placed in the vessel of the apparatus containing rat caecal contents. The experiment was carried out with continuous CO₂ supply into the beakers to simulate anaerobic environment of the caecum. At regular time intervals, 10 ml of samples were withdrawn and same amount replaced by fresh medium. Samples were suitably diluted and filtered through syringe filter (Axiva SFCA25X, 0.45µm). Drug

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amount released was analyzed spectrophotometrically by UV spectrophotometer (Thermo Scientific Evolution 201) at wavelength of 225 nm^[16,21].

Table 6 Lag time and % swelling index of atenolol press coated (OG) tablets

Formulation Code	Lag Time (hr)	% Swelling Index
OG1	2	95.35 ± 4.50
OG2	6	165.35 ± 5.25
OG3	1	108.35 ± 7.75
OG4	6	178.46 ± 6.64
OG5	5	130.26 ± 6.57
OG6	4	148.29 ± 4.65
OG7	1	78.90 ± 5.60
OG8	6	189.27 ± 4.37
OG9	4	139.53 ± 6.45

Preparation of rat cecal content medium

The Institutional animal ethical committee approved the experimental protocol under strict compliances of CPCSAE guidelines for the use of experimental animals. Wistar rat/Albino rat weighing 150–200 g were kept on a normal diet and administered 1 ml of 1-2% w/v aqueous dispersion of okara gum with the help of teflon tubing directly into stomach region via oral cavity. The treatment was continued for 7 days to induce enzyme responsible for okara gum degradation. Thirty minutes before the commencement of drug release studies, seven rats were killed by spinal traction. The abdomen was opened, the cecal were isolated, ligated at both the ends, dissected,

and immediately transferred into phosphate buffer pH 6.8 previously bubbled with CO₂. The cecal bags were opened, their contents were individually weighed, pooled, and suspended in the phosphate buffer pH 6.8 bubbled continuously with CO₂ to maintain anaerobic condition to give final cecal dilution of 4% w/v (cecal content equivalent to 8 g were added to 200 ml phosphate buffer pH 6.8 to give a final cecal dilution of 4% w/v). All the above procedures were carried out under CO₂ in order to maintain anaerobic conditions^[21,22].

RESULTS AND DISCUSSION

Flow property study of powder blend

The bulk density, tapped density, angle of repose, hausner's ratio and carr's index of powder blend for atenolol core tablets were 0.280 ± 0.03 , 0.33 ± 0.04 , $29.74^{\circ} \pm 0.40$, 1.178 ± 0.12 and 15.15 ± 0.65 respectively as shown in table 4. The results of indicated that powder blend has good flow property with good compressibility and suitable for direct compression method. Okara gum powder showed 18.05 ± 0.25 carr's index, 1.22 ± 0.18 hausner's ratio and angle of repose $37.56^{\circ} \pm 0.30$, which indicated that okara gum powder showed poor flow property and compressibility. To improve the flow property and compressibility, okara

gum granules were prepared using starch paste. The angle of repose, hausner's ratio and carr's index of okara gum granules used for coating of core tablets were ranged from $27.56^{\circ} \pm 0.14$ to $28.93^{\circ} \pm 0.12$, 1.139 ± 0.10 to 1.166 ± 0.14 and 12.21 ± 0.18 to 14.24 ± 0.15 respectively. The values of pre-compression parameters indicated that okara gum granules had good free flowing property and were suitable for direct compression method. Flow property of okara gum granules was increased as the concentration of starch paste was increased in the granulation. Okara gum granules were prepared using starch paste containing highest concentration of starch showed excellent flow property.

Post compression study of core tablets and press coated tablets

The data obtained from post-compression study of core and press coated tablets such as weight variation, hardness, friability, and drug content are shown in table 5. The hardness of core tablets of atenolol was 3.25 ± 0.15 , indicated that core tablets had good crushing strength. The friability, drug content, weight variation and thickness of atenolol core tablets were 0.12 ± 0.05 %, 99.97 ± 1.3 %, 79.57 ± 2.57 and 3.20 ± 0.05 respectively, which indicated that atenolol core tablets passed the post compression

study. In all press coated formulations, the hardness test indicated good mechanical strength. Hardness was ranged from 3.5 to 5.5 Kg/cm². Friability was ranged from 0.06 ± 0.08 to 0.21 ± 0.08. Friability is less than 1% which indicated that tablets had good mechanical resistance and able to withstand pressure and remain intact during transportation. Drug content was found to be high (>99.54 %). It was ranged from 99.35 ± 1.9 to 100.15 ± 1.4 % and uniform in all tablet formulations. In weight variation test, none of tablets showed more than 7.5 % weight variation from average weight. So, all formulations passed the weight variation test as per IP. Tablet thickness varied from 4.9 to 6.8 mm.

Position of Core Tablet

Cross sectional photograph in figure 1 of atenolol compression coated tablet showed that atenolol core tablet centrally placed into compression coated tablet.

% swelling study

Table 6 describes the % swelling ratio of different batches of OG1 to OG9. On exposure to water, the okara gum absorbs water, gets hydrated and forms a swollen gel. Swelling study of press coated tablets of different OG batches indicated that as the coat weight of okara gum granules was

increased, mass of swollen gel around the core tablet was increased. OG8 tablets containing highest 270.74 mg coat weight of okara gum granules were showed highest % swelling of 189.27 ± 4.37%.

***In vitro* dissolution study of atenolol press coated tablets**

In time controlled press coated tablets, the different batches (OG1 to OG9) showed a variable lag time depending on the concentration of starch paste and coat weight of okara gum in the outer coating layer. Lag time is defined as time period during which dosage form release less than 10% of drug. On exposure to dissolution fluids, the okara gum got hydrated and formed a viscous gel layer. The presence of a surface gel layer; delayed both dissolution fluids penetration into and diffusion of drug out of the okara gum coat. The hydration of okara gum seemed to be not affected by the pH of the dissolution medium^[18]. Drug release after a specific lag time occurred due to diffusion of drug through the swellable okara gum gel layer. The lag time of the compression coated OG tablets are given in table 6. OG1, OG3 and OG7 tablets having lower coat weight of okara gum and was not able to retain around the atenolol core tablets. But, as the coat weight of okara gum was increased, lag time also was increased.

For ideal press coated pulsatile drug release lag time is 6 hr. OG2, OG4 and OG8 tablets showed lag time 6 hr.

During dissolution study, it was observed that lower coat weight of okara gum (129.28 mg and 150 mg) was not able to retain on core tablets and showed lower lag time, lower $T_{85\%}$ and release drug in upper GIT. The coat of okara gum remained intact when coat weight of okara gum was ≤ 200 mg. High Coat weight of okara gum retarded drug release more significantly in conditions of the upper GIT due to a higher swelling. Dissolution study of OG tablets in phosphate buffer pH 7.4 without rat cecal content (figure 2) showed lower drug release as compared to dissolution study in presence of rat cecal content (figure 3) because okara gum swellable layer was intact, can't eroded and retain biodegradability; when dissolution study was performed without rat cecal content. Natural polysaccharides are remain undigested in the stomach and the small intestine and are degraded by the vast anaerobic microflora of the colon, for example, *bacteroides*, *bifidobacteria*, *eubacteria*, to smaller monosaccharides, which are then used as energy source by the bacteria^[23]. The susceptibility of okara gum coatings to the enzymatic action of colonic bacteria, was assessed by continuing the

drug release studies in phosphate buffer pH 7.4 medium containing rat caecal content for 4 h after 5 h of testing in 0.1 N HCl and phosphate buffer pH 6.8. Tablets with okara gum coat ratio of 200 mg (OG5, OG6, OG9), 250 mg (OG2, OG4) and 270 mg (OG8) were selected for dissolution study in presence of rat cecal content.

Figure 3 shows that the presence of rat caecal contents in the dissolution medium resulted in a significant increased in drug release, when compared to without rat cecal content indicating that polysaccharidases metabolizing okara gum were present in rat caecal contents. The ability of the enzyme to degrade the coat was sufficiently and allowed the rapid drug release from the core. The cumulative percent drug released after 7 h from OG2, OG4, OG5, OG6, OG8 and OG9 press coated tablets were increased from 15.34%, 22.74%, 32.01%, 64.59%, 12.31% and 56.22% respectively in the absence of rat caecal contents to 59.44%, 67.95%, 75.43%, 94.9%, 49.37% and 80.74% respectively; when dissolution study was performed in phosphate buffer pH 7.4 containing rat cecal content. As the coat weight of okara gum was decreased from 270 mg (OG9) to 200 mg (OG8), the coat might have been more hydrated and subsequently degraded by the caecal

enzymes at a faster rate, explaining the relatively higher drug release 80.74% (OG9) as compared to 49.37% (OG8). The more swollen okara gum coat observed during the dissolution study of OG8 tablets, might be due to too high coat weight and hence reduced the drug release from the tablets. A rat caecal content concentration higher than 4% w/v might be required to provide the bacterial population necessary for using this polysaccharide mixture as a substrate and carrying out its hydrolysis^[24]. Thus, okara gum in the form of coat was capable of protecting the drug from being released completely in the physiological environment of stomach and intestine. Drug release profile varied as coat weight of okara gum was changed. Results of *in vitro* dissolution study of atenolol press coated tablets shown in figure stated that OG4 press coated tablet having 250 mg coat weight of okara gum showed 6 hr lag time and near 7 hr T_{85%}, which is applicable pulsatile drug delivery of atenolol for treating early morning hypertension.

Press coated tablets were coated by compression coating of okara gum granules on atenolol core tablets. Okara gum granules were prepared by wet granulation method using different concentration of starch paste. The dissolution study showed that starch

paste concentration had a great impact on lag time and percentage cumulative drug release of atenolol press coated tablets (figure 2). OG6 tablets press coated with okara gum granules were prepared using 8.43 % starch paste showed 4 hr lag time, as compared to OG5 tablets press coated with okara gum granules were prepared using 2.06 % starch paste showed 5 hr lag time. Percentage cumulative drug release was increased as the concentration of starch paste used to prepare okara gum was increased. OG5, OG6 and OG9 tablets prepared from okara gum granules made up using starch paste concentration of 2%, 5% and 8.43% respectively, showed 28.70%, 40.01% and 33.11% drug release respectively after 6 hr. This might be because of difference in hydration of outer barrier layer. The soluble starch which was used in wet granulation method; is hydrophilic in nature which was achieved rapid hydration leads to rapid penetration of dissolution medium through outer barrier layer^[12].

Statistical analysis for optimization of formulation of atenolol press coated (OG) tablets

Central composite design (CCD) was used to optimize the coat weight of barrier layer (okara gum granules) and concentration of

granulating agent (starch paste). CCD used to check effect of two factors on response in polynomial equation. Table 7 showed that values of responses lag time (Y1) and $T_{85\%}$ (Y2) for the OG tablets. It was logically decided to obtain 6 hr lag time and 7 hr $T_{85\%}$ from the formulated products.

Response 1: Lag time

Polynomial equation in terms of coded factors

$$\text{Lag time (Y}_1\text{)} = +3.88 - 0.29 * A + 2.01 * B \dots\dots\dots (6)$$

In equation 6, coefficients of A is with negative sign (-0.29), indicated that as concentration of starch past was increased, lag time was decreased, while B is with a positive sign (+2.01) indicated on increased the coat weight of okara gum granules, lag time of OG tablets was increased. The magnitude of coefficient of B is more than coefficient of A, described that amount of okara gum granules were affected lag time more strongly than concentration of starch paste. ANOVA study of CCD was performed to identify whether the factors affecting the response significantly or not. The p-value of independent variables of A and B was 0.1940 and 0.0001 respectively. p-value of B is lower than 0.001, indicated that amount of okara gum granules was

highly significantly affect the lag time, while p-value of A is more than 0.05 indicated that effect of concentration of starch paste on lag time was not significant. ANOVA study for linear model showed the p-value of model is 0.0002 which is less than 0.05 indicated model terms are significant and value of correlation coefficient (R^2) is 0.970 which indicated that a goodness of fit of model. The relationship between the dependent and various independent variables was further elucidated using contour plots (figure 4). Logically, it was predecided to obtain the lag time 6 hr for the formulated products. In contour plots, various color regions indicated variable lag time. A red color area in plots showed lag time near to 6 hr.

Response 2: $T_{85\%}$

Polynomial equation in terms of coded factors

$$T_{85\%} \text{ (Y}_2\text{)} = +6.32 - 0.24 * A + 1.47 * B \dots\dots\dots (7)$$

In equation 7, coefficients of A is with negative sign (-0.24), indicated that as concentration of starch past was increased, $T_{85\%}$ was decreased, while B is with a positive sign (+1.47) indicated on increased the coat weight of okara gum, $T_{85\%}$ of OG tablets was increased. The magnitude of

coefficient of B is more than coefficient of A, described that amount of okara gum was affected $T_{85\%}$ more strongly than concentration of starch paste. ANOVA study of CCD was performed to identify whether the factors affecting the response significantly or not. The p-value of independent variables of A and B was 0.3436 and 0.0008 respectively. p-value of B is lower than 0.001, indicated that amount of okara gum was highly significantly affect the $T_{85\%}$, while p-value of A is more than 0.05 indicated that effect of concentration of starch paste on $T_{85\%}$ was not significant. ANOVA study for linear model showed the p-value of model is 0.0022 which is less than 0.05 indicated model terms are significant and value of correlation coefficient is (R^2) 0.9788 which indicated that a goodness of fit of model. The relationship between the dependent and various independent variables was further elucidated using 3-D response plot (figure 5). Logically, it was predecided to obtain the $T_{85\%}$ 7 hr for the formulated products. 3-D response plot, various color regions indicated variable $T_{85\%}$. A red color area in plots showed $T_{85\%}$ near to 7 hr.

CONCLUSION

Optimization of result

For the optimization of atenolol press coated (OG) tablets, constraints were fixed for all factors and response. Constraints were set according to formulation of atenolol press coated OG tablet using minimum amount of okara gum granules, which would give desired response. It was predecided to obtain the 6 hr lag time and 7.0 hr $T_{85\%}$ for the optimized formulated products. Figure 6 showed the overlay plot for independent factors with desirability 0.977. When the desirability value 1.0 or near to 1.0, it indicated optimum formulation. As per optimization result, optimized formulation containing 231.34 gm of okara gum granules were prepared using 5.84% of starch paste should showed theoretically 5.84 hr lag time and 6.99 hr $T_{85\%}$. Validation of optimization technique was done by preparing checkpoint batch containing optimized value of independent factors and responses were evaluated. The check point batch was showed 6 hr lag time and 6.89 hr $T_{85\%}$. The practically response value observed in the checkpoint batch was closet to theoretical value obtained from polynomial equation.

The microbiologically triggered colon targeted pulsatile release of atenolol is successfully achieved by press coating of

okara gum granules on atenolol core tablets. Okara gum in the form of coat was capable of protecting the drug from being released completely in the physiological environment of stomach and intestine. OG4 press coated tablet having 250 mg coat weight of okara gum was showed 6 hr lag time and near 7 hr $T_{85\%}$, which was applicable pulsatile drug delivery of atenolol for treating early morning hypertension. Thus, the dosage forms can be taken at bedtime, so, that the content will be released in the morning hours, i.e., at the time of symptoms. The release of drug was rapid and complete after the lag time. Lag time was greatly affected by coat weight of okara gum and concentration of starch paste used for wet granulation.

Table 7 Results of lag time and $T_{85\%}$ for atenolol press coated (OG) tablets

Formulation code	Core tablet	Independent variables				Dependent Variables	
		coded value		actual value		Y_1 (Lag time) (hr)	Y_2 ($T_{85\%}$) (hr)
		Factor 1 (A)	Factor 2 (B)	Factor 1 (A)	Factor 2 (B)		
OG1	AT2	-1	-1	3	150	2	4.7
OG2	AT2	-1	1	3	250	6	7.75
OG3	AT2	1	-1	7.5	150	1	4.09
OG4	AT2	1	1	7.5	250	6	7.16
OG5	AT2	-1.414	0	2.06	200	5	7.25
OG6	AT2	1.414	0	8.43	200	4	6.75
OG7	AT2	0	-1.414	5	129.28	1	4
OG8	AT2	0	1.414	5	270.74	6	8
OG9	AT2	0	0	5	200	4	7.25

Where, A = percentage of starch paste (%), B = Amount of okara gum ranules (mg)

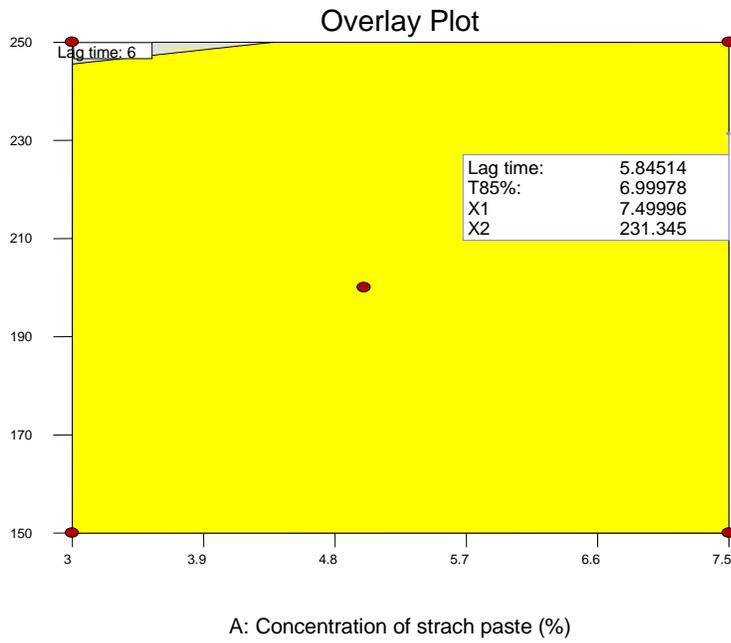
Figure 6 Overlay plot for optimize formulation of atenolol press coated (OG) tablets

Design-Expert® Software
Factor Coding: Actual
Overlay Plot

Lag time
T85%
● Design Points

X1 = A: Concentration of strach paste
X2 = B: Amount of okara gum

B: Amount
of okara gum
(mg)



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