DESIGN AND DEVELOPMENT OF CHRONOPHARMACEUTICAL DRUG DELIVERY OF METOPROLOL SUCCINATE

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ABSTRACT

Aiming to design the chronopharmaceutical drug delivery of Metoprolol Succinate (MPS) into the colon in the current study. By sealing the micro particles inside a gelatin capsule which was made up of erodible hydrogel plug, a time delayed capsule was prepared. The microparticles were formulated by counter-ion elicited aggregation methodology. Poly-cations such as Chitosan a natural polymer and poly anions such as small molecular electrolytes like sodium sulphate, sodium citrate and sodium tripolyphosphate were selected. The formulated aggregate microparticles were tested for size distribution, surface morphology, unharness (*in-vitro*) and drug-excipient compatibility study. Optimized microparticles were carefully chosen on the basis of their dissolution performance studies. Enteric coating was applied to the entire device, and a hydrogel plug was put in the capsule entrance. The pulsatile-capsule performed well enough to delay drug release in the intestinal (small) fluid while also ejecting the plug into the colonic fluid, resulting in a 5 hour delay in the release of the microparticles in-to the colonic fluid. To replicate the pH variations in the gastrointestinal system, pH 1.2, 6.8, and 7.4 dissolving medium were utilized. No such interaction between polymer and medication was discovered in FT-IR investigations. Amongst-all the formulations, Metoprolol Succinate formulated with sodium tripolyphosphate shown protracted release of drug for 12 hours. The results showed that the device can delay medication delivery for a preset period of time. The findings also show that they can prevent a considerable rise in blood pressure, especially in the early morning hours, when hypertension is most prevalent.

Keywords Microparticles; Counterion aggregation; Metoprolol Succinate (MPS); Pulsatile

INTRODUCTION

Pulsatile drug delivery systems deliver the medicine in the exact amount and at the exact moment to the site of action. This is advantageous for medications having a high first-pass effect. This is especially advantageous for pharmaceuticals taken in diseases with chrono-pharmacological behavior, such as diabetes, where nighttime

administration is essential, and for drugs having a specific absorption site in the gastro-intestinal tract, such as treatments that target the colon (1). In the morning, the cardiovascular events are more common (2). Ambulatory blood pressure (BP) varies from day to day as blood pressure rises in the morning (3). The surge in morning blood pressure was known to be related with high risk of ischemic, hemorrhagic stroke and cardiac death (4). Myocardial infarction, angina pectoris, hypertension, and cardiac arrhythmias can all be treated with MPS, a cardio selective β1 blocker (5). MPS is readily absorbed after an oral dosage and undergoes significant first-pass metabolism, resulting in 50% oral bioavailability. Metoprolol is said to have a half-life of 3 to 4 hours. Usual anti-hypertensive dose of MPS ranges from 25-100 mg / day (6). It requires frequent administration due to its low bioavailability and short biological half-life. As a result, creating a time-bound drug delivery system (DDS) synchronizes drug delivery with circadian variation, especially during times of higher risk. This is best for people who have high blood pressure. The goal of this study is to develop and test a chronopharmaceutical DDS including MPS for the treatment of high blood pressure (7). The drug is delivered at definite time as per physiological needs of the disease thereby improving the patient compliance and therapeutic efficacy.

The formulation can be administered before going to bed. By proportioning drug concentrations, this DDS can release medicine at 3 a.m (8). Early in the morning, free cholesterol levels are higher (9). Hydrogel plugs are used to control the intention of delaying drug absorption for 5 hours.

MATERIALS AND METHODS:

Ranbaxy Laboratories Limited in India supplied a free sample of metoprolol succinate. Chitosan, Sodium sulphate, Sodium citrate, Sodium Tripolyphosphate were procured from Loba Chemicals, India. Analytical grade chemicals were used. Experiments were conducted with the approval of the institution's animal ethics committee (Ref: IAEC/IX/10/ACOP/ CPCSEA, Dated 21-12-2018).

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Formaldehyde-exposed Gelatin Capsules Manufacturing: A total of over 100 hard gelatin capsules in size "0" were chosen. Capsules bodies and caps are separated. To create formalin fumes, desiccators were filled with 15% formaldehyde (v/v) and a pinch of potassium permanganate. The empty bodies of capsule contained in the wire mesh were exposed to formaldehyde vapours. To make them water-soluble, the caps were not exposed to the vapours. Tightly closing the desiccators for 12 hours for the reaction to take place (10). To complete the reaction between formaldehyde vapours and hard gelatin capsules, The bodies were taken from the capsule and dried for 30 minutes at 50 degrees (11). Dry the bodies at room temperature to remove any formaldehyde residues (12). Sealing of hard gelatin capsule bodies was done using untreated caps. Use polythene bag for storage.

Preparation of Hydrogel Plug:

The capsule bodies were sealed using plugs that were prepared by compressing equal amount of Methocel K100M: Lactochem, CMC Sodium: Lactochem, Carbopol 934: Lactochem and Methocel A4CP: Lactochem. With different hardness and thickness values of tablet plugs, a rotary tablet press with 7 mm punches and dies was used (13).

Manufacturing of microparticles:

The counter ion induced aggregation approach was used to make all of the microparticle preparations (14) and the composition was shown in **Table 1**. Dissolve exactly weighed amount of chitosan in 2% acetic acid solution, add drug and dissolve it. Until

homogeneous blend was formed the blend was stirred. Formulation of 20 percent Salt Solution: 20g of salt (Sodium Sulphate/ Sodium Citrate/ Sodium Tripolyphosphate) was precisely weighed and dissolved in 100 mL of water. Continuously stir

to obtain a clear solution. Add prepared drug polymer mixture to salt solutions using needle (24 Guage/0.55 mm diameter) to form microparticles. Remove excess salt solution by placing in hot air oven at 35-40^{0}C for 72 hrs.

Table 1: Formulation Composition ratio of Metoprolol Succinate microparticles

Cross linking agents					
Sodium TPP		Sodium sulphate		Sodium citrate	
Composition Code	Core (Polymer): Coat (Crosslinking agent)	Composition Code	Core (Polymer): Coat (Crosslinking agent)	Composition Code	Core (Polymer): Coat (Crosslinking agent)
MS1	1.00:0.50	MS4	1.00:0.50	MS7	1.00:0.50
MS2	1.00:0.75	MS5	1.00:0.75	MS8	1.00:0.75
MS3	1.00:1.00	MS6	1.00:1.00	MS9	1.00:1.00

Designing of Pulsin cap:
The Pulsin cap was formulated by filling microparticles of MPS 50mg into the bodies that were treated using formaldehyde with hand filling method. The capsules comprising the microparticles were sealed using optimized hydrogel plug. To attach the capsule body and cap, a 5% ethanolic solution of ethyl cellulose was employed (15). A dip coating method (5 percent CAP in a 5: 5 (v/v) blend of acetone: ethanol plasticized with 0.75 percent dibutyl phthalate) was used to completely coat the sealed capsules to prevent variable gastric emptying. Coating was repeated until a weight increase of 8 to 12 percent was achieved. Calculate the formaldehyde-exposed

capsules' % weight growth before and after coating (16).

Hydrogel Plug Physico-Chemical Evaluation:
The consistency of weight, lag-time, thickness, and hardness of the prepared Hydrogel-Plugs were assessed (17).

Uniformity of content:
The encapsulated microparticles equal to 50mg of MPS were grounded in a mortar. Dissolve and solubilize the crushed powder in pH 6.8 phosphate buffer. UV-spectrophotometer at 224nm was used to analyze the filter (18).

***In-vitro* drug dissolution studies for pulsatile capsule:**
Dissolution rate test apparatus (USP XXIII Apparatus 2, 37°C±0.5°C, 100 rpm), drug release

studies of pulsincaps were performed for two hrs in 0.1M HCl (900mL) because the typical time for gastric emptying is approximately 2hrs. Because this is the normal small intestinal transit time, the dissolving medium was changed with pH 7.4 phosphate buffer (900mL) for 3 hours. Post five hours, switch to pH 6.8 phosphate buffer (900mL) and test for the following hrs. At all times, keep 900mL of dissolving medium on bowl. Stirring speed was kept constant at 100 rpm and the temperature was kept constant at 37±0.5°CAt predetermined time intervals, 5mL of dissolving media was removed and replaced with fresh aliquot dissolution media. A UV-spectrophotometer set to 224 nm was used to examine the samples. Calculate the cumulative drug release as a percentage over the sample time points (19).

FT-IR analysis:

The KBr pellet approach was used to analyze the pure drug, excipients, and formulations using a BRUKER Fourier transform IR Spectrophotometer (20) at a resolution rate of $4cm^{-1}$. The wave number range 380 - $4368cm^{-1}$ was used to integrate the spectrum in transmittance mode (21).

SEM analysis:

The surface morphology was determined using scanning electron microscopy (SEM) (Hitachi S-3700 N). The microparticles were fixed in slabs and sputter coated with gold or palladium.

In-Vivo Evaluation:

Subject selection: In this study, 12 male healthy rabbits having age range of 8 to 12 weeks and average body weight range of 2.8 to 3.2 kg were used. Six rabbits in each group were made to fast overnight (22). No stress was given to the animals. Use one cage to group one set of rabbits. For different sampling times, divide the rabbits randomly into two groups. During the experiment, at all times water and food were made available to the animals. Crossover design manner methodology was used between the two experiments with a two week washout period. The dose of the animal for MPS was estimated with relevance to the dose of human by using the below formula. Gastric intubation method was used to administer the dosage form (23).

Human dose (HD) of MPS = 50 milligram.

$$\text{Animal dose} = \frac{HD \times \text{Weight of the animal}}{\text{Average Human weight}}$$

= 50 x 3 / 70 = 2.14 milligram = 2.5 mg

Blood sampling: Withdraw blood samples of 1 mL from the rabbit's tracheal lobular vein and kept in plastic tubes that have a screw top and are heparinized. The blood sampling time was 0 min (Predose), 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24 and 48 hours. Post centrifuging for 5 minutes at 4000 rpm, the plasma was aspirated and kept at -20°C until it was analysed using the LC-MS/MS technique (24).

Mass Spectroscopy (MS) method/ Liquid Chromatography (LC) - MS technique:

Chromatographic parameters:

The mass spectrometric and chromatographic conditions are given below [11]:

HPLC	:	PerkinElmer
Mass-spectroscopy	:	Sciex API 2000
Run-time	:	3.00min
Detection-ions		
MPS	:	267.400amu [parent], 115.30amu [product]
Propranolol HCl	:	263.500amu [parent], 57.0amu [product]

Ion-source	:	Heated Nebulizer
Column-oven temperature	:	35 °C
Mobile phase	:	0.1% HCOOH : CH_3OH: C_2H_3N (pH: 6.0 ± 0.1) (10:45:45)
Flow rate	:	1.00mL/min
Volume of injection	:	20μL
Retention-time	:	Propranolol HCl : 1.5-2.3 min MPS: 1.6-2.4 min
Polarity	:	Positive ion mode

MRM Conditions

Curtain-Gas	:	10 psi
Collision-Gas	:	8 psi
Nebulizer-current	:	3 Volts
Temperature	:	550°C
GAS 1	:	40 psi
GAS 2	:	60 psi
Entrance-Potential	:	10 Volts
Collision-Cell Exit-Potential	:	3 Volts

MRM parameters

Parameters	MPS	Propranolol HCl
Declustering-Potential	33.0	60.0
Focusing-Potential	400	280
Collision-Energy	41.0	25.0

A. Preparation of reagents:

1. Preparation of 0.1% formic acid buffer:

585-μL of formic acid was added in 500 mL of water and the solution was subjected to sonication. From that solution, 200 mL was taken and adjusted its pH to 6.0±0.1 with liquor ammonia.

2. Preparation of mobile phase:

To 100-mL of the above 0.1 % formic acid buffer (pH 6.0±0.1), 450-mL acetonitrile, 450-mL of methanol were added and mixed them by sonication.

3. Preparation of reconstitution solution:

To 50-mL of the above 0.1 % formic acid buffer (pH not adjusted), 225mL of acetonitrile & 225mL of methanol were added and mixed them by sonication.

4. Rinsing solution preparation:

To 500mL of purified water, 250mL acetonitrile & 250mL methanol were added and mixed by sonication.

5. Preparation of 60% methanol in water:

To 60-mL of methanol, add 40-mL of water and mix by sonication.

B. Working standard solutions preparation:

I. MPS stock solution preparation:

10 mg of MPS was weighed and added into volumetric flask of 10-mL as a working standard. Solubilized in methanol and made up the volume. By considering the purity of Metoprolol, the conc. of the final solution was calculated and stored at 5±3°C. A 60 percent methanol in water solution was used to dilute the stock solution to a concentration of 1 μg/mL.

II. Propranolol HCl as internal standard stock solution preparation:

10mg of Propranolol HCl was measured and added into a volumetric flask of 10-mL as a working standard. Solubilized in methanol and made up the volume. By considering the purity of Propranolol, the conc. of the final solution was calculated and stored at 5±3°C.
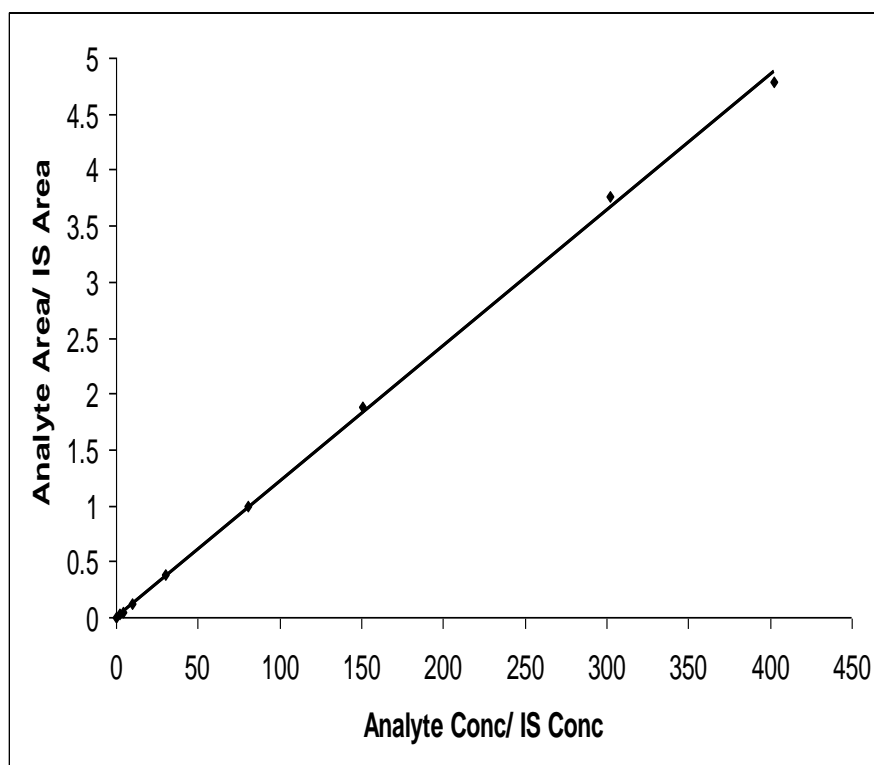
C. Calibration curve standards:

I. Standard MPS solution preparation stock dilutions:

Standard MPS solution from stock dilutions was made with 60% methanol of ranging from 40.240ng/mL to 8047.32ng/mL.

II. Calibration curve standards - Spiking of plasma:

MPS concentrations ranging from 2.01ng/mL to 402.366ng/mL were created with plasma and labelled as MSC1- MSC8. The calibration curve standards were made for each validation run (17). As shown in **Figure 1**.

Figure 1: Calibration Curve of MPS in Plasma

III. Preparation of Sample:

Step-1: Allow the blanks, subject samples, and calibration curve standards to defrost after removing them from the deep freezer. To make sure that the contents are completely mixed, vortex the freeze-thawed samples. In a ria vial, add 0.5mL of plasma sample and 50μL of Propranolol HCl (1μg/mL). Add 50 μL of a 60

percent methanol in water solution to the plasma blank and pre-dose (0.0 hr)[25]. To ensure complete mixing of the contents, the thawed samples were vortexed.

Step-2: Approx. 3mL of ethyl-acetate solution was added and centrifuged at 4000rpm for 10min at 20°C. Fill another ria vial with the supernatant (organic layer). At 45°C, use a stream of nitrogen gas to evaporate the organic layer. After rehydrating the residue with 0.25 mL of reconstitution solution, vortex it. Place the samples in the auto-injector vials and turn it on. A total of 20 µL of sample were injected into the LC-MS/MS apparatus. As shown in **Table 2**.

Table 2: Analytical data of Plasma with MPS Standard Solution

Sr. No.	Samples	Conc. (ng/mL)	Peak area	Peak Area (IS)	Area ratio	Calculated Conc. (ng/mL)	Accuracy (%)
01	Aqueous-mixture	N/A	125661	116069	1.08	87.168	--
02	Plasma	0	0	0	0	--	--
03	Blank+IS-TD	0	0	85038	0	--	--
04	MSC 1	2.012	2196	90727	0.02	1.997	99.23
05	MSC 2	4.024	4798	95821	0.05	4.077	101.33
06	MSC 3	10.060	11343	91483	0.12	10.026	99.66
07	MSC 4	30.177	35748	93281	0.38	30.887	102.35
08	MSC 5	80.472	94468	93587	1.01	81.275	101.00
09	MSC 6	150.887	175097	92885	1.89	151.741	100.57
10	MSC 7	301.775	299210	79548	3.76	302.725	100.31
11	MSC 8	402.366	430049	90027	4.78	384.441	95.55

Processing of Data: Applied Bio-systems was used to create the chromatograms, Canada's Analyst program (version 1.4.2), as shown in **Figure 2**. The conc. of the unknown samples must be calculated from the equation using regression analysis of spiked plasma calibration standard with $1/x^2$ as weighting factor (26).

y = mx+c;

Where,

y = The ratio of ISTD peak area vs MPS peak area
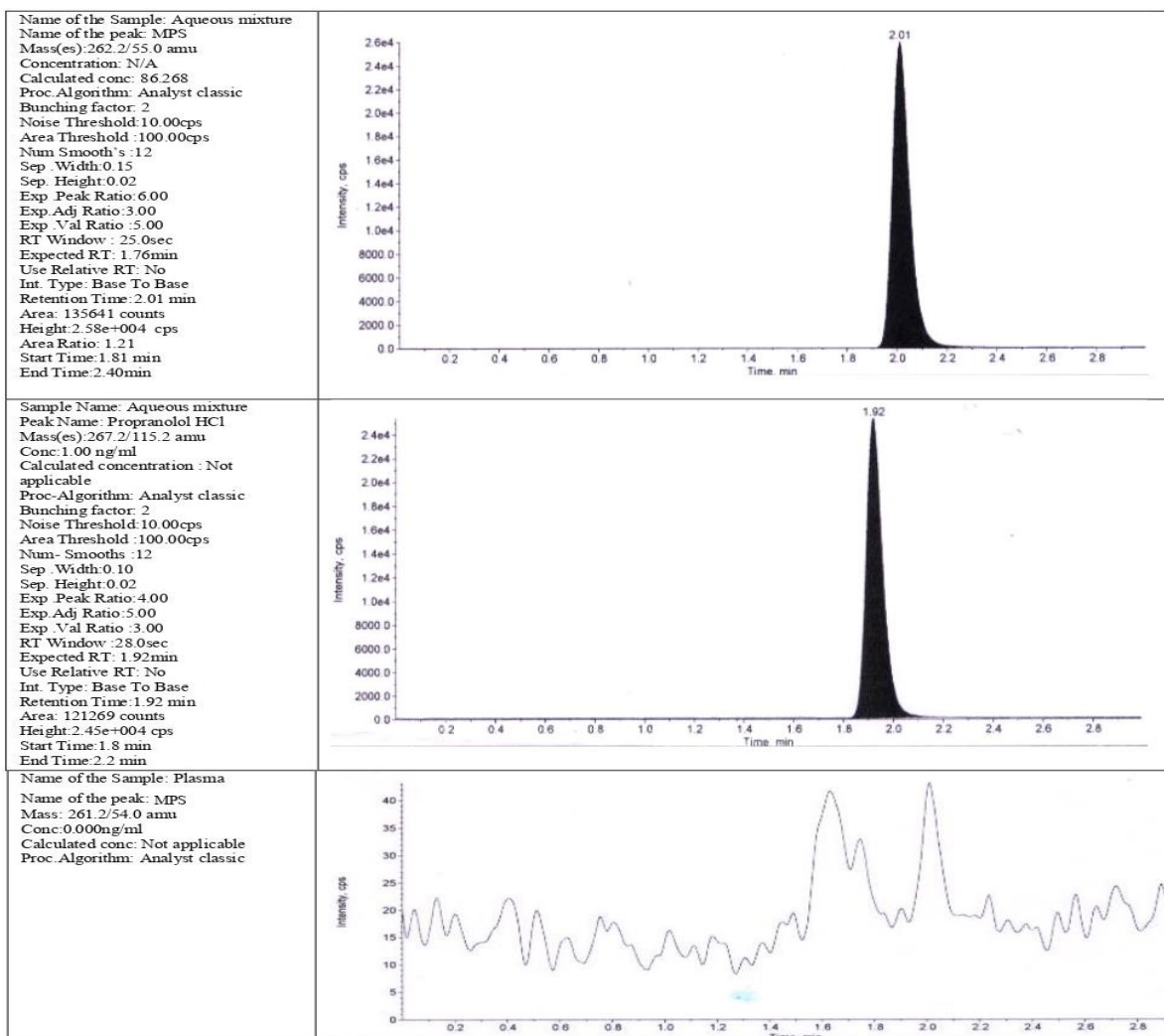
x= Conc. of MPS

m=calibration curve slope

c= intercept value of y-axis

Linear regression-analysis equation is y= 0.0124x–0.000604.

Figure 2: Standard MPS Solution Stock Dilutions with Plasma Chromatograms

Pharmacokinetic Parameters Determination:

Several pharmacokinetic-parameters like Cmax, Tmax, rate of absorption constant (Ka), elimination rate constant (Kel), $AUC_{(0-t)}$, $AUC_{(0-inf)}$, mean-residence time (MRT) (30) and biological half-life ($t_{1/2}$) were examined by non-compartmental pharmacokinetics data analysis software (27) PK Solutions 2.0™ (Summit Research Services, Montrose, CO, USA) (28) and Cmax, MRT, Ka, Ke, and AUC_0-α values were statistically evaluated for normal distribution results using a paired sample t-test. The selected threshold of significance was 0.001 (29).

RESULTS AND DISCUSSION

A capsule with a water soluble cap and a water insoluble body is known as a pulsin-cap dosage form. A hydrogel plug was used to seal the microparticles within the capsule body. Following ingesting, the water-soluble cap dissolves in gastric juice, causing the exposed hydrogel-plug to expand. The inflated plug will be evacuated and the encapsulated medication formulation will be released into the colon after intake at a predetermined time. It will disintegrate before being taken into the bloodstream. In this study, a 12-hour formaldehyde treatment hardened the capsule bodies, which were then sealed with an

unhardened cap. Microparticles were made using the counter ion induced gelation/aggregation approach with sodium citrate (SC), sodium sulphate (SS), and sodium tripolyphosphate (STPP). Sodium citrate-based microparticles were brown in color, with a regular shape and a rough surface, whereas sodium sulphate-based microparticles were light brown in color, with a flat surface, and STPP-based particles were white in color (30). Because chitosan has a pKa of around 6.5, it only shows positive charge at low pH and forms ionic complexes as aggregates with opposite charge anion. The pH of the medium had a significant impact on the swelling of chitosan microparticles due to the ionization of both poly anions (SC, SS, and STPP) with chitosan (20). Tightly cross-linked chitosan matrix does not expand as much as loosely cross-linked chitosan matrix (lower water absorption). Because poly anions were tightly linked with chitosan in sodium citrate and sodium sulphate cross linking solutions, swelling was minimal, whereas in sodium tri polyphosphate, the chitosan network is loose and has a large hydrodynamic free volume to accommodate more solvent molecules, causing chitosan-STPP matrix swelling(17). The formulations indicated that the microparticles were spherical and appeared as distinct particles or aggregates under microscopic examination.

The flow characteristics of all the preparations were excellent. Microparticles varied in size from 613.2 to 662.53 µm. The % drug concentration and entrapment effectiveness of these microparticles were also measured. **Table 3** summarizes the findings. The entrapment effectiveness of the approach was also discovered to be rather high. Among the three polyanionic solutions, STPP microparticles conained maximum drug content whereas SS microparticles (*STPP > SC > SS*) was observed the least. The percentage entrapment efficiency for the drug was more in STPP.

Table 3: MPS microparticle evaluation data

Composition code	(θ) Angle of Repose	(g/ mL) Bulk-Density	(g/ mL) True-density	% CI	HR	Avg. Particle Size(µm)	Percentage Drug-Content	Percentage Entrapment-Efficiency
MS1	25.93±0.022	0.277±0.013	0.312±0.012	11.22±0.023	1.137±0.012	613.23	48.91	97.82
MS2	25.60±0.031	0.350±0.012	0.408±0.011	14.21±0.022	1.161±0.014	632.46	39.86	99.66
MS3	25.42±0.052	0.320±0.020	0.370±0.009	11.89±0.009	1.134±0.017	662.53	32.89	98.76
MS4	26.85±0.024	0.319±0.005	0.362±0.021	11.87±0.017	1.130±0.024	589.12	49.13	98.26
MS5	27.01±0.035	0.351±0.009	0.393±0.019	10.68±0.014	1.119±0.014	602.17	39.67	99.17
MS6	25.76±0.05	0.255±0.025	0.291±0.005	12.37±0.024	1.142±0.014	623.16	32.58	97.83

MS7	27.64±0.014	0.514±0.021	0.611±0.009	15.87±0.014	1.188±0.021	562.19	48.27	94.84
MS8	26.93±0.022	0.519±0.015	0.614±0.012	15.49±0.016	1.183±0.018	581.26	39.11	97.39
MS9	26.10±0.034	0.521±0.018	0.616±0.008	15.42±0.020	1.182±0.012	602.67	32.42	97.82

HR: Hausner ratio, BD: Bulk-Density, TD: True-density

The weight uniformity, hardness, lag-time and thickness of the Hydrogel-Plugs were all measured. **Table 4** summarizes the findings. At the conclusion of the fifth hour, the formulations fitted with the different hydrogel plugs MSH1, MSH2, MSH3, MSH4 demonstrated 0.82%, 8.25 %, 20.55 % and 22.34% drug release respectively. It was discovered that a 100mg hydrogel plug with a hardness of 4.7 kg.cm^{-2} (Methocel K100M: Lactochem with a 1.00: 1.00 ratio) was satisfactory. The microparticles are released into colonic fluid when the medication is delayed into small intestine fluid and the clog in colonic fluid is thrown out. This suggests that the lag duration is influenced by the plug composition (31).

Table 4: Physico-chemical characterization of fabricated hydrogel plugs and various polymers

Hydrogel-Plug Code	Composition (ratio)	UOD (mg)	T (mm)	H (kg.cm^{-2})	LT (Hrs)	Percentage Drug Release
MSH 1	Methocel K100M :Lactochem	100±1.4	3.16	4.7	5.0	0.82%,
MSH 2	Carbopol 934 : Lactochem	100±1.1	3.29	4.2	4.5	8.25%,
MSH 3	CMC Sodium : Lactochem	100±1.5	3.24	3.8	4.0	20.55%
MSH 4	Methocel A4CP : Lactochem	100±1.3	3.56	3.4	3.0	22.34%

UOD: Uniformity of dosage form, T: Thickness, H: Hardness, LT: Lag time

The enteric coating of the cellulose acetate phthalate was unbroken in 2 hours in pH 1.2, solubilized in intestinal pH, and the soluble cap of the capsule dissolved in pH 7.4 according to in-vitro release studies (32). The medicine was released through the swelling microparticles when the exposed polymer plug absorbed the surrounding fluid and expanded. The plug developed a soft mass after being completely wet, which was readily expelled from the capsule body, releasing the microparticles into simulated intestinal fluid (pH 6.8 phosphate buffer). In-vitro release studies of the device revealed that there was no drug release in simulated gastric fluid (acidic pH 1.2) or simulated intestinal fluid for 2 hours in all formulations (pH 7.4 phosphate buffer). The burst effect was seen in colonic media (pH 6.8 phosphate buffer) (33).

The maintaining efficacy of in-vitro drug release profiles in colonic medium was shown to be extremely good[34]. Pulsin-caps loaded with MPS microparticles prepared with chitosan and sodium tripolyphosphate in 1.00: 0.50, 1.00: 0.75, and 1.00: 1.00 ratios demonstrated sustained drug release for 10 hours (5th hr to 15th hr), 11 hours (5th hr to 16th hr), and 12 hours (5th hr to 17 hr), respectively, as shown in **Figure 3**. Pulsin caps

formulated with chitosan and sodium sulphate in 1.0: 0.5, 1.0: 0.75, and 1.0: 1.0 ratios showed extended drug release for 9.5 hours (5th hr to 14.5 hr), 10.5 hours (5th hr to 15.5th hr), and 11.5 hours (5th hr to 16.5 hr), respectively as shown in **Figure 4**.

Pulsin caps loaded with MPS microparticles prepared with chitosan and sodium citrate in 1.0: 0.5, 1.0: 0.75, and 1.0: 1.0 ratios demonstrated extended drug release for 9.5 hrs (5th hr to 14.5th hr), 10.5 hrs (5th hr to 15.5th hr), and 11.5 hrs (5th hr to 16.5th hr), respectively, as shown in **Figure 5.**

Figure 3: Comparative *In-vitro* drug release profiles of Metoprolol Succinate microparticles formulated with chitosan by using Sodium TPP in different ratios

(-♦-)MS1 (-■-   )MS2   (-×-)MS3
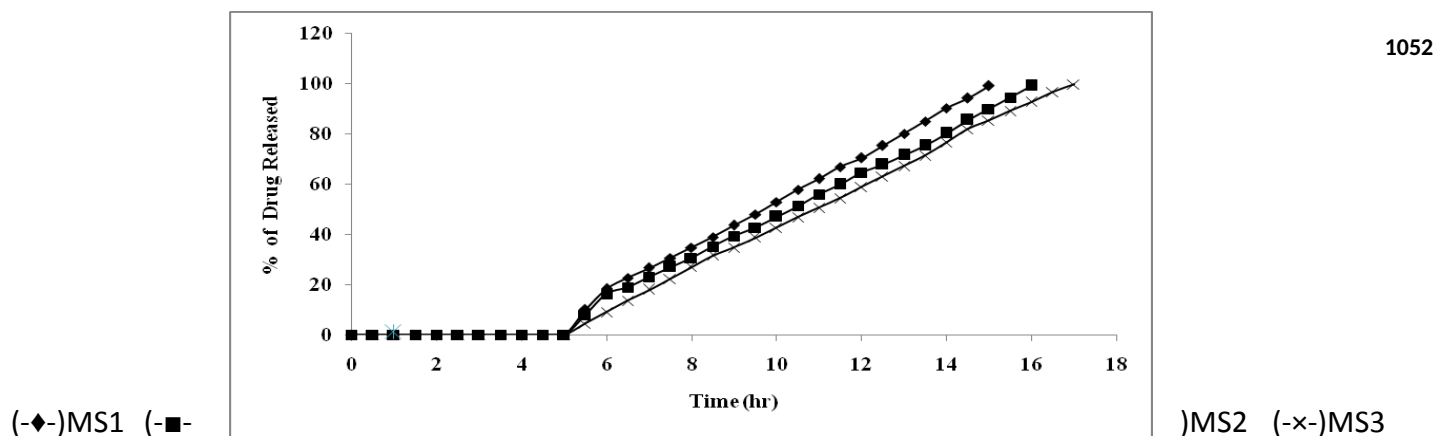
Figure 4: Comparative *In-vitro* drug release profiles of Metoprolol Succinate microparticles formulated with chitosan by using Sodium Sulphate in different ratios
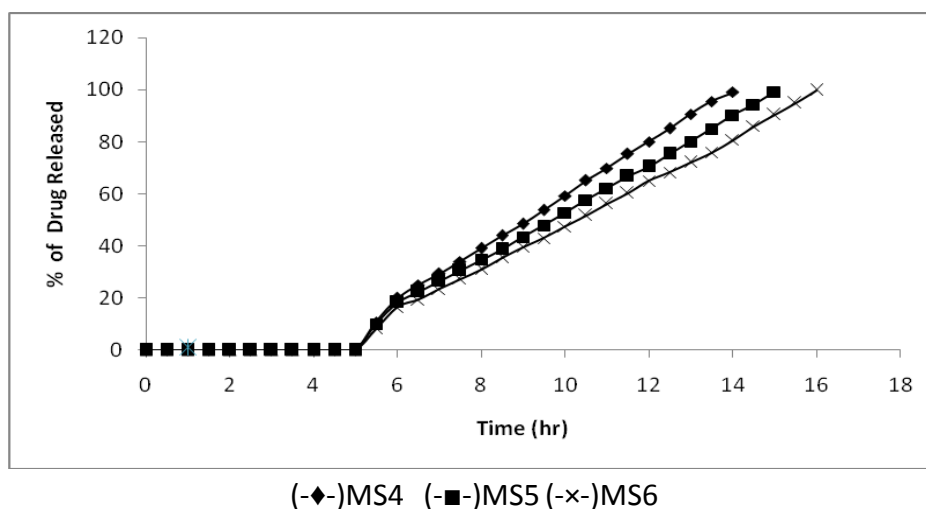

(-♦-)MS4   (-■-)MS5 (-×-)MS6

Figure 5: Comparative *In-vitro* drug release profiles of Metoprolol Succinate microparticles formulated with chitosan by using Sodium citrate in different ratios
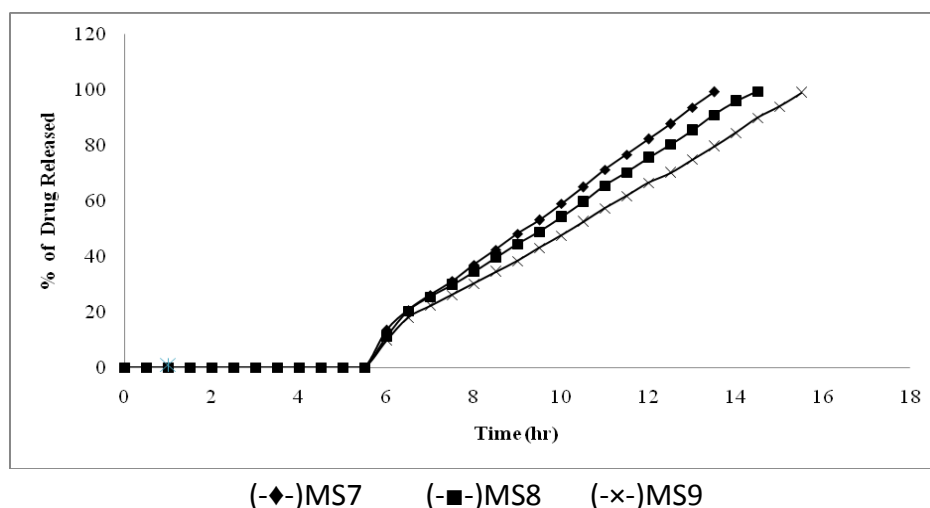
(-♦-)MS7   (-■-)MS8   (-×-)MS9

Table 5 displays the corresponding (R^2) values for dissolution kinetics data. The values show that the drug release is governed by zero order kinetics and the Peppas-Korsmeyer model of drug release (8). The release exponent (n) values ranged from 0.7552 to 0.9731, showing a non-fickian diffusion process. **Figure 6** depicts a SEM picture of the improved formulation. The FT-IR analysis of MPS pure medication **(Figure 7)** revealed distinctive peaks at wave numbers of 3148.88 cm^{-1}, 1555.69 cm^{-1}, 1047.85 cm^{-1}, 1238.65 cm^{-1}, and 1111.20 cm^{-1}, indicating the presence of MPS. N-C stretching vibration, C=C Ring-symmetric stretching, carbonyl-stretching C-O-C asymmetric-bending, and carbonyl-stretching C-O-C symmetric-bending[35]. The FT-IR analysis of optimized formulation was shown in **Figure 8.** Figures showed similar peaks from optimized formulations. Drug loaded microparticles did not show any change or shift of characteristic peaks. This indicates that no substantial drug polymer interaction occurred, indicating that the medication was stable in the improved formulation.

Table 5: *In-vitro* release kinetics data for MPS microparticles

Composition code		MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8	MS9
Values	**Korsmeyer–Peppas**	0.9964	0.9965	0.9992	0.9967	0.9963	0.9997	0.9956	0.9971	0.9964
	First order	0.8294	0.8122	0.7342	0.8357	0.7893	0.7802	0.8211	0.8206	0.8353
	Diffusion (Higuchi)	0.9524	0.9411	0.9342	0.9342	0.9456	0.9265	0.9561	0.9573	0.9506
	Zero order	0.9927	0.9952	0.9997	0.9921	0.9968	0.9997	0.9907	0.9895	0.9925
	K_o (mg.hr^{-1})	5.12	4.57	4.24	5.44	4.82	4.37	6.43	5.81	5.06
	T_{50} (hr)	4.89	5.48	5.88	4.59	5.18	5.79	3.9	4.3	4.93
	T_{90} (hr)	8.79	9.88	10.58	8.39	9.28	10.28	7.0	7.7	8.88
	"n"	0.7552	0.7975	0.9731	0.7593	0.8017	0.9696	0.7385	0.7508	0.7643

Figure 6: Scanning electron microscope of MPS microparticles formulated with chitosan by using Sodium TPP in 1:1 ratio
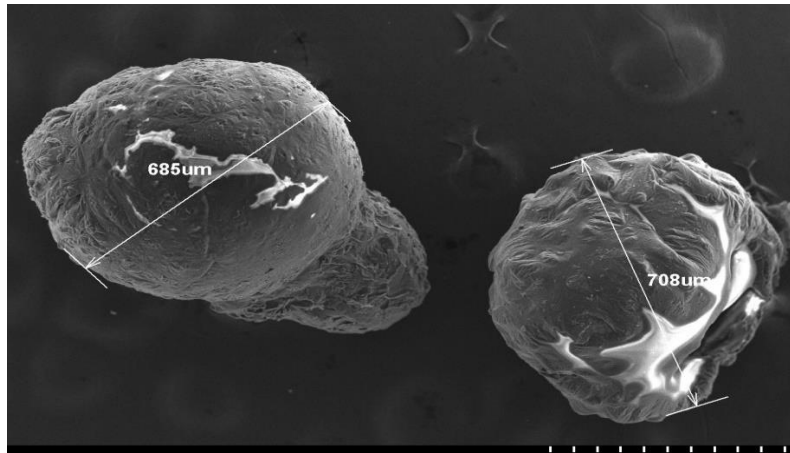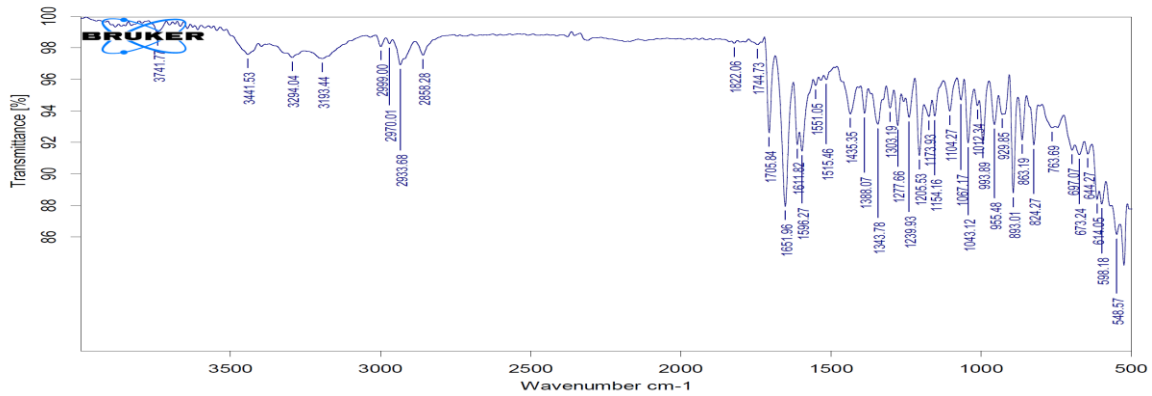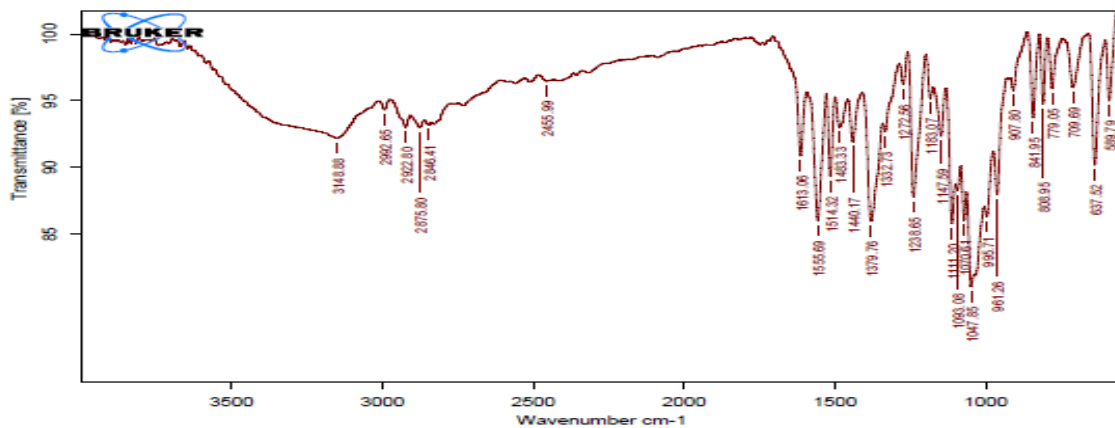
Figure 7: FTIR spectral analysis: Pure MPS

Figure 8: FTIR spectral analysis of Optimized formulation

The in-vivo studies were carried out. Pharmacokinetic factors such as Ka, Kel, t $_{1/2}$, AUC, and MRT were calculated. Plasma drug concentrations of MPS after marketed SR product and pulsatile formulations at different time beings were evaluated and were shown in **Table 6 & 7** and in **Figure 9 & 10**.

Table 6: Plasma drug concentration after Oral administration of MPS (Marketed SR Product)

| Time in hrs.	Plasma conc. of MPS Marketed Sustained Release product (ng / mL)
0.0	0
0.5	4.23±0.04
1.0	8.79±0.03
2.0	17.34±0.06
4.0	31.89±0.05
6.0	48.96±0.02
8.0	42.76±0.01
10.0	26.67±0.04
12.0	19.78±0.05
16.0	10.85±0.08
20.0	2.76±0.02
24.0	0.327±0.01
48.0	0.028±0.07

Table 7: Plasma drug concentration after Oral administration of MPS (Optimized formulation)

| Time in hrs.	Plasma drug concentration of MPS Optimized formulation (ng / mL)
0	0
2	0
4	0
6	12.43 ±1.12
8	27.13±1.23
10	44.19±1.38
12	52.11±1.81
14	48.23±1.29
16	42.56±1.23
18	37.29±1.68
20	31.45±1.56
24	25.54±1.43
28	22.01±1.26
32	16.01±1.12

Figure 9: Time Vs Plasma drug Concentration curve of MPS after Oral administration (Marketed SR product)
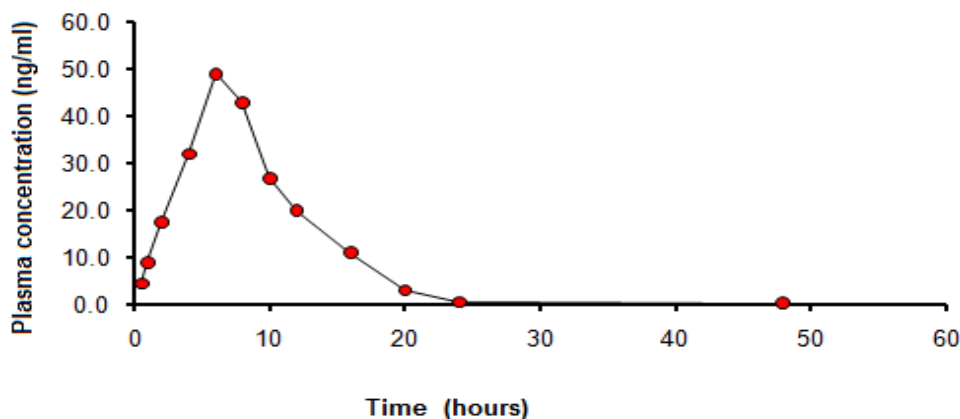
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Figure 10: Time Vs Plasma drug Concentration Curve of MPS after Oral administration (Optimized formulation)
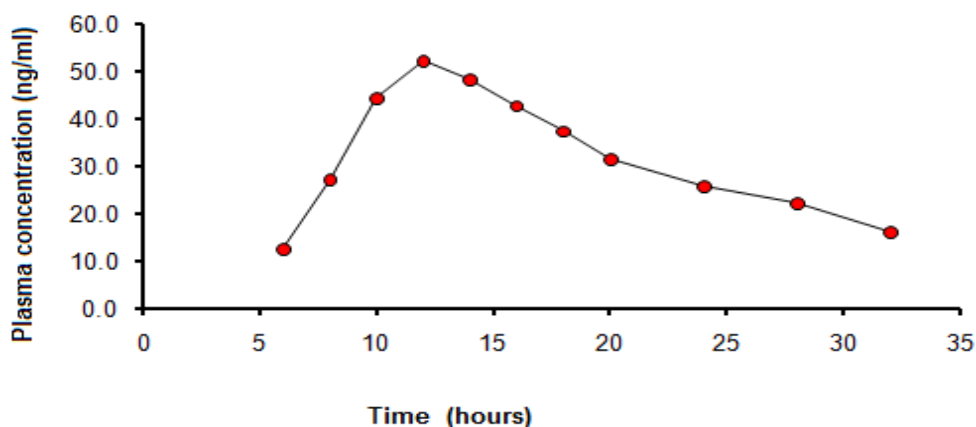
From the results of oral administration of Marketed SR product of MPS C_{max} was 48.9 ± 0.33ng/mL at 6 hrs (t_{max}) while pulsatile formulation showed the higher plasma concentration of 51.8 ± 0.42 ng/mL at 12 hrs (t_{max}) post an initial Lag-time 5 hours. Administration orally of marketed SR MPS resulted in a quite variable and low AUC of 356.2 ± 0.67 ng.hr/ mL, while the pulsatile formulations gave rise to AUC of 951.7 ± 1.58 ng.hr/mL. The pulsatile formulations mean residence time administration (23.2 ± 1.09 hours) was more than oral administration (14.9 ± 0.15 hours). The results were shown in **Table 8**.

Table 8: Pharmacokinetic data after Oral administration of MPS in Marketed SR product and Optimized formulation

Pharmacokinetic parameter	Marketed SR Product	Optimized formulation	't' value
C_{max}(ng/ml)	48.9±0.33	51.8±0.42	6.68***

MRT(h)	14.9 ± 0.15	23.2 ± 1.09	23.51***
$t_{1/2}$(h)	6.76 ± 0.017	8.84 ± 0.014	5. 69***
K_{el}(h^{-1})	0.10 ± 0.002	0.07 ± 0.002	3.73***
K_a(h^{-1})	0.41 ± 0.07	0.19 ± 0.27	6.45***
$AUC_{0-\infty}$ (ng h/ml)	356.2 ± 0.67	951.7 ± 1.58	56.60***

H_o: No change in the pharmacokinetic parameters of oral ingestion of Marketed SR product and Optimized formulations of MPS.

Degree-of freedom is 3.985 (at the 0.001).

Result: As the calculated value of 't' is higher than the deliberated 't' value, H_o is not accepted. Therefore the significant difference between the pharmacokinetic data of Marketed SR oral-administration and Optimized formulations of Metoprolol succinate.

*$p<0.05$,** $p<0.01$, *** $p<0.001$

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CONCLUSION: It was concluded that all the formulations of Pulsatile-capsules of MPS microparticles formulated with chitosan & sodium tripolyphosphate shown extended drug release for 12hrs. The results demonstrated the system's ability to postpone medication delivery for an extended period of time while targeting the colon. For a hypertension chronomodulated therapy, lag time of 5hrs and extended-release for 12hrs was satisfactory. It is suggested to take the dosage form at bed-time so that the contents are released during the early hours when hyper-tension is predominant (36).

LIST OF ABBREVIATIONS:

MPS: Metoprolol Succinate

HCl: Hydrochloride

FTIR: Fourier Transform Infrared Spectroscopy

BP: Blood pressure

IAEC stands for Institutional Animal Ethical Committee.

Na CMC: Sodium carboxymethyl cellulose

CAP: Cellulose Acetate Phthalate

CI: Compressibility Index

USP: United States Pharmacopeia

UV: Ultra violet

KBr: Potassium bromide

HPLC stands for high performance liquid chromatography

SEM: Scanning electron microscopy

LC-MS stands for liquid chromatography – mass spectroscopy

H_o: Null hypothesis

CPCSEA stands for Committee for the Control and Supervision of Animal Experiments

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