ISSN : 0973-6204

PHARMBIT

INTERNATIONAL JOURNAL
INDEXED IN "CHEMICAL ABSTRACT"
Vol. XXX. No. 2, Jul - Dec, 2014

The Official publication of
PHARMACEUTICAL SOCIETY
DEPARTMENT OF PHARMACEUTICAL SCIENCES
BIRLA INSTITUTE OF TECHNOLOGY
MESRA, RANCHI, INDIA
www.bitmesra.ac.in

PHARMBIT, Jul – Dec 2014; XXX (2)

Amlodipine and Atenolol Combination Therapy in Hypertensive Diabetic: A Case Study

Mukul Chandra Gope

Department of Physiology, Rajendra Institute of Medical Sciences, Ranchi – 834009

Abstract

An old man of 72 years, suffering from type ll diabetes (NIDDM) as well as hypertension was on glyburide and β-blockers (atenolol) for last one year. He used to remain bed ridden for most of the time, due to extreme weakness. His blood glucose level, both 'fasting' and 'post-prandial' was high. His blood pressure was 146/92 mm of Hg. He has been advised by a physician to switch over to Insulin. Furthermore, due to the fear of complications of injectable insulin in such old age, he has been presented to the author. Amlodipine, combined with Atenolol (5 mg + 50 mg) was started, along with Glyburide continued. It was a great experience that, the patient became ambulant within 15 days. He visited my clinic, and explained, how gradually his weakness has gone and how better was he feeling himself.

Key words: Type ll Diabetes, Hypertension, β-blockers, Amlodipine combination

Introduction

Diabetes and hypertension often co-exist in old age. Moreover, treatment becomes complicated due to adverse effects of antihypertensives on insulin 'release' and/or 'sensitivity'. Most commonly, inhibition of insulin release by a β-blocker (e.g. 'propranolol') becomes evident due to lack of adequate control of the plasma glucose by oral hypo- glycemics. The β-adrenergic stimulation enhances both, insulin and glucagon secretion. Additionally, it also leads to glycogenolysis, gluconeogenesis and lipolysis. Alpha- adrenergic stimulation inhibits insulin secretion and may inhibit glucagon secretion along with enhanced liver glycogenolysis. In nondiabetics, β-blockers represent minimal risk of affecting glucose control. On the contrary, in insulin-dependent diabetics, β-blockers can prolong, enhance or alter the symptoms of hypoglycemia. Similarly, hyperglycemia appears to be a major risk encountered with β--blockers in noninsulin-dependent diabetics. Thus, β-blockers can potentially increase blood glucose concentrations by antagonizing the action of oral hypoglycemic drugs.

A number of clinical data indicate that, 30% of diabetics develop arterial hyper- tension. Moreover, 25% of patients with hypertension are diagnosed as diabetic on routine screening. Furthermore, rapid development of atherosclerosis is the principal cause of morbidity and mortality among diabetics.

Background and History

The β-blockers inhibit insulin release; hence in a patient with type ll diabetes, they often pose problems. Moreover, insulin release occurs through the voltage gated Ca^{++} channels of the beta-cells of ilets. Amlodipine blocks these channels to inhibit the release of insulin. At the same time, Amlodipine increases insulin sensitivity also, apart from exhibiting anti-hypertensive effect.

Cardioselective β-blockers seem to be best suited in diabetics. After insulin-induced hypoglycemia, rise in blood sugar level is less delayed and symptoms of hypoglycemia are less attenuated. Numerous studies have consistently demonstrated that certain classes of antihypertensive medications have differential effects on carbohydrate and lipid metabolism in humans. In general, higher doses of thiazide diuretics (i.e., ≥ 25 mg/day hydrochlorothiazide) and β-blockers, at any antihypertensive dose, worsen glycemic control, with β-blockers worsening insulin sensitivity. Conversely, ACE inhibitors, angiotensin II receptor blockers (ARB) and calcium channel blockers (CCBs) have neutral or beneficial effects on these variables. It is noteworthy, however, that not all drugs within the same class have similar effects on insulin sensitivity. This is exemplified by the effects of vasodilating β-blockers failing to worsen insulin resistance and consequently having neutral effects on glycemic control.

Furthermore, β-blockers used for the treatment of hypertension may be associated with increased risk for new-onset diabetes mellitus. A search by Medline, PubMed, and EMBASE was conducted for randomized controlled trials of patients taking β-blockers as first-line therapy for hypertension with data on new-onset DM and follow-up for > or =1 year.

Conclusion of the study was that, β-blockers are associated with an increased risk for new-onset DM, with no benefit for the end point of death or myocardial infarction and with a 15% increased risk for stroke compared with other agents. This risk was greater in patients with higher baseline body mass indexes and higher baseline fasting glucose levels and in studies in which, β-blockers were less efficacious antihypertensive agents compared with other treatments.

Atenolol (AT) is a recommended drug for control of hypertension in diabetic patients. Moreover, although treatment with Amlodipine in NIDDM rats causes a decrease in insulin release, however, glucose levels were found to be lowered significantly indicating that Amlodipine causes an increase in insulin sensitivity. Furthermore, various data indicate that Amlodipine increases insulin sensitivity in neonatal-STZ (streptozotocin) NIDDM rats. Hence, combination of 'Amlodipine and Atenolol' appear to be a suitable regime for the control of hypertension in NIDDM. Moreover, Amlodipine has been found to be renal protective on long term use. Additionally, in the absence of albuminuria and with a preserved GFR (>60 ml/min), a CCB can be contemplated as first-step therapy and seems to preserve GFR in an adequate manner. To add further, in the presence of a diminished GFR without albuminuria, a CCB can be used as first-step therapy. However, a high percentage of patients will require combination therapy, and addition of an ACE inhibitor or an ARB (Angiotensin II receptor blocker) will be adequate. Furthermore, Atenolol has been a proven cardioprotective for even those with coronary artery disease.

Results

The present case had been monitored regularly at one month of interval. The plasma glucose, both 'fasting' (90-106 mg/dL) and 'post-prandial' (120-132 mg/dL) were under control. The blood pressure also remained well regulated within the range of 128-136 mm of Hg for systolic and 78-82 mm of Hg for diastolic.

There was marked improvement of the overall condition of the present case. His weakness had gone. He was able to walk upto one kilometer at a stretch. His mentation also improved considerably due to unknown reasons.

Discussion

Both Amlodipine and Atenolol inhibit insulin release, but when given in combination to a hypertensive patient of NIDDM, it provides better blood pressure control, cardio- protection as well as glycemic control. Such effect is due to, 'increased insulin sensitivity' caused by Amlodipine, leading to better glycemic control despite 'inhibiting the insulin release' also. In other words, such combination may prove very successful for long term management of hypertensive diabetics since, Amlodipine preserves the insulin stores by inhibiting its secretion, whereas at the same time it increases insulin sensitivity also, and thereby, provides better glycemic control.

Nonetheless, the unexpected and rapid improvement of the present case definitely warrants further study on combination of 'Atenolol and Amlodipine' in hypertensive cases of NIDDM. The present case had been monitored for the next 2 years. The case remained stable in that duration, indicating very good prognosis.

Bibliography

1. Ling G, Liu AJ, Shen FM, Cai GJ, Liu JG, Su DF. Effects of combination therapy with atenolol and amlodipine on blood pressure control and stroke prevention in stroke-prone spontaneously hypertensive rats. Acta Pharmacologica Sinica 2007; 28(11): 1755-60.
2. Xu LP, Shen FM, Shu H, Miao CY, Jiang YY, Su DF. Synergism of atenolol and amlodipine on lowering and stabilizing blood pressure in spontaneously hypertensive rat. Fundamental & Clinical Pharmacology 2004; 18(1): 33-8.
3. Mills GA, Horn JR. Beta-blockers and glucose control. Drug Intelligence & Clinical Pharmacy. 1985; 19(4): 246-51.
4. Albert J, Ekoe JM. Control of blood sugar and beta blockers in the diabetic patient. Sem Hop. 1983; 59(42): 2939-43.
5. Cardiologist Cautions Some Older Beta-Blockers May Cause Diabetes - http://wexnermedical.osu.edu/mediaroom/pressreleaselisting/cardiologist-cautions-some-older-beta-blockers-may-cause-diabetes (Last visited on May 2014)
6. Jenny Hope, Beta-blockers 'increase diabetes risk by 50 per cent' http://www.dailymail.co.uk/health/article-403973/Beta-blockers-increase-diabetes-risk-50-cent

.html (Last visited on April 2014)

7. Sarafidis PA, Bakris GL. Antihypertensive Therapy and the Risk of New-Onset Diabetes. Diabetes Care 2006; 29(5): 1167-69.
8. Bell DSH. Diabetic Cardiomyopathy. Diabetes Care 2003; 26(10): 2949-51.
9. Bangalore S, Parkar S, Grossman E, Messerli FH. A meta-analysis of 94,492 patients with hypertension treated with beta blockers to determine the risk of new-onset diabetes mellitus. Database of Abstracts of Reviews of Effects (DARE): Quality-assessed Reviews. http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0025368/ (Last visited on May 2014)
10. KE Barrett, S Boitano, SM Barman, HL Brooks. Endocrine Functions of the Pancreas & Regulation of Carbohydrate Metabolism in Ganong's Review of Medical Physiology, Ed. 23rd. McGrawHill Medical, New York
11. Segura J, García-Donaire JA, Ruilope LM. Calcium Channel Blockers and Renal Protection: Insights from the Latest Clinical Trials. Journal of American Society of Nephrology. 2005; 16(1): S64-6.
12. Ma G, Allen TJ, Cooper ME, Cao Z. Calcium channel blockers, either amlodipine or mibefradil, ameliorate renal injury in experimental diabetes. Kidney International 2004; 66(3): 1090–98.
13. Gokhale MS, Shah DH, Hakim Z, Santani DD, Goyal RK. Effect of chronic treatment with amlodipine in non-insulin-dependent diabetic rats; Pharmacol Res. 1998 Jun; 37(6):455-9.
14. Fogari R, Zoppi A, Mugellini A, Corradi L, Lazzari P, Preti P, Derosa G. Comparative evaluation of effect of valsartan/amlodipine and atenolol/amlodipine combinations on atrial fibrillation recurrence in hypertensive patients with type 2 diabetes mellitus. Journal of Cardiovascular Pharmacology 2008; 51(3): 217-22.
15. Mehvar R. Effect of experimental diabetes mellitus on the pharmacokinetics of atenolol enantiomers in rats. Journal of Pharmaceutical Sciences 1991; 80(3): 207-11.

Calcium Silicate Based Microspheres of Salbutamol Sulphate for Gastro Retentive Floating Drug Delivery: Investigate *In Vitro* and *In Vivo* Description

Kedar Prasad Meena*, J.S. Dangi

SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur-495009

Abstract

The aim of present study was to develop gastroretentive multiple unit controlled-release drug delivery systems of Salbutamol, a fast and short-acting meglitinide analog used for long term treatment of asthma. It has a very short half-life (2-5 h), low bioavailability (48%) and poor absorption in the upper intestinal tract. So a controlled release system was designed to increase its residence time in the stomach. Floating microspheres were prepared by emulsion solvent diffusion technique consisting of calcium silicate (CaSi) as porous carrier; salbutamol (Sbt.), as an antiasthamatic agent; and Eudragit S-100 as polymer. The prepared formulations were characterized for micromeritic property, *in vitro* floating behavior, drug loading, and surface morphology, Interaction study by FTIR, DSC and XRD followed by pharmacokinetic study. The microspheres were found to be spherical in shape and porous. The release rate was determined in simulated gastrointestinal fluids at 37±1˚C. The formulation demonstrated favorable *in vitro* floating and release characteristics. The drug encapsulation efficiency was 84±1.6. The pharmacokinetic study of developed formulation compared with marketed formulation and reveals maintenance of plasma drug concentration, which is comparable that of marketed formulation. The designed system, combining excellent buoyant ability and suitable drug release pattern, could possibly be advantageous in terms of increased bioavailability of salbutamol.

Key words: Emulsion solvent diffusion**,** Asthma**,** Microspheres**,** Calcium silicate**,** Salbutamol

Introduction

Asthma is a common chronic inflammatory disease of the airways, characterized by hyper responsiveness to a variety of stimuli. It may be classified as mild intermittent or mild, moderate, or severe persistent [1]. Asthma affects 14 to 15 million persons in the United States. An estimated 4.8 million children have asthma, which makes it the most common chronic disease of childhood. With the increased understanding of the role inflammation in asthma and the addition of new pharmacologic agents, the management of this disease has improved. The drug β-agonists used in the treatment of asthma include albuterol (salbutamol), isoproterenol (isoprenaline), metaproterenol (orciprenaline), clenbuterol, formoterol, fenoterol and terbutaline. Other chiral antiasthmatic drugs include the 5-lipooxygenase inhibitor zileuton, and the anticholinergic agent ipratropium bromide.

Oral delivery of drugs is the most preferable route of drug delivery due to the ease of administration, patient compliance and flexibility in formulation, etc. From immediate release to site specific delivery, oral dosage forms have really progressed. However, it is a well-accepted fact that it is difficult to predict the real *in vivo* time of release with solid oral controlled release dosage forms. Thus, drug absorption in the gastrointestinal (GI) tract may be very short and highly variable in certain circumstances. One of the most feasible approaches for achieving a prolonged and predictable drug delivery profile in the GI tract is to control the gastric residence time (GRT). Dosage forms with a prolonged GRT, i.e. gastroretentive dosage forms (GRDFs), will provide us with new and important therapeutic options [2]. The controlled gastric retention of solid dosage forms may be achieved by the mechanisms of mucoadhesion [3] floatation [4] sedimentation [5] expansion [6,7] and modified shape systems [8,9] or by the simultaneous administration of pharmacological agents [10,11]. Floating drug delivery (FDD) is of particular interest for drugs as it (1) acts locally in the stomach (2) is primarily absorbed in the stomach (3) poorly soluble at an alkaline pH (4) has a narrow window of absorption (5) and are unstable in the intestinal or colonic environment. Floating drug delivery systems (FDDS) have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from the stomach. This results in an increased GRT and a better control of the fluctuations in plasma drug concentration [12, 13]. The aim of present study was to develop gastroretentive multiple unit controlled-release drug delivery systems of Salbutamol, a fast and short-acting meglitinide analog used for long term treatment of asthma. It has a very short half-life (2-5 h), low bioavailability (48%) and poor absorption in the upper intestinal tract. So a controlled release system was designed to increase its residence time in the stomach. Floating microspheres were prepared by emulsion solvent diffusion technique consisting of calcium silicate (CaSi) as porous carrier and calcium silicate abbreviated as (CaSi) in manuscript. Due to porous character of (CaSi), high amount may lead to reduced entrapment of drugs and unpredictable release thus optimized ratio of (CaSi) was necessary to get floating nature of microspheres and adequate entrapment and predictable release rate; salbutamol (Sbt.), as an antiasthamatic agent; and Eudragit S-100 as polymer. The prepared formulations were characterized for micromeritic property, *in vitro* floating behavior, and drug loading, and surface morphology, Interaction study by FTIR, DSC and XRD followed by pharmacokinetic study.

Materials and Method

Salbutamol sulphate (SS) was received as a gift sample from Zydus Cadila Healthcare Ltd, Changodhar, Ahmadabad, Gujarat, (India), highly porous calcium silicate (CaSi) was purchased from Sigma Aldrich. Eudragit S-100 was obtained as a gift sample from Degussa (GmbH, Ethanol and dichloromethane was obtained from (Ranbaxy Fine Chemicals, New Delhi) and other solvents were purchased from Himedia Chemical, India. All other chemicals used were of analytical grade.

Preparation of Drug Adsorbed CaSi

The porous carrier (CaSi) was dispersed into 10 ml of ethanolic solution of SS. This dispersion was ultrasonicated (Soniweld, Imeco Ultrasonics, India) to take up the drug solution inside the pores of porous carrier, while removing the air. The excess ethanolic solution was removed by filtration and dried in vacuum, which produced drug adsorbed porous carrier.

Preparation of SS Microspheres

The microspheres were prepared using an emulsion solvent diffusion technique with modification [14]. The drug adsorbed CaSi was added into the polymer solution (Eudragit S-100) in ethanol and dichloromethane sonicated using probe sonicator (Soniweld, Imeco Ultrasonics, India). The resulting suspension was poured into an aqueous solution of polyvinyl alcohol (0.75% w/v) at 40˚C. The emulsion or suspension was stirred at 500 rpm employing a vortexer for 3 h. The microparticles were separated by filtration, washed with water and dried at room temperature in desiccators for 24 h. The microspheres of SS without CaSi were also prepared using same method (NFM) for comparative study. Non floating microspheres were taken as control in present study as main aim of present study was to develop floating microspheres of salbutamol.

Characterization of Microspheres

Micromeritic Properties

The microspheres were characterized by their micromeritic properties, such as particle size, tapped density, compressibility index and flow properties. The particle size of microspheres was determined using an optical microscope fitted with a previously calibrated ocular microscope [15]. The tapping method was used to determine the tapped density and percent compressibility index [16] as follows:

$$Tapped\ Density = \frac{Mass\ of\ microsphere\ after\ tapping}{Volume\ of\ microsphere\ after\ tapping}$$

$$\%\ \text{Compressibility index} = \frac{1 - V}{V0}\ \text{x}\ 100$$

Where, Vo and V are the volume of the sample before and after tapping.

For the determination of angle of repose, θ of the microspheres, a glass funnel is held in place with a clamp on a ring support over a glass plate. The glass plate is placed on a stand. Approximately 100 g of particles is transferred into funnel keeping the orifice of the funnel blocked by the lower thumb. As the thumb is removed, the particles are emptied from funnel, and the angle of repose [17] is determined by above mentioned formula and calculated as tanθ = 2H/D, where 2H/D is the surface area of the free standing height of the microspheres heap that is formed on a graph paper after making the microspheres flow from the glass funnel.

Morphology

The morphology of microspheres as well as CaSi was studied by its SEM imaging. The sample for SEM was prepared by sticking the microspheres on a double sided adhesive tape stuck to an aluminum stub. Scanning was done using JEOL model JSM-6390LV, Japan at Central Instrumentation Facility of Birla Institute of Technology, Mesra, Ranchi, India. The stubs were then coated with gold to a thickness of 15 nm under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The pictures were taken at an excitation voltage of 20 Kv. The coated samples were then randomly scanned and photomicrographs were taken with an SEM. The quality of the microspheres (with respect to surface properties) and the nature and size of pores developed on the surface were studied.

Drug Content

The drug content of Eudragit S-100 microspheres was determined by dispersing 50 mg formulation (accurately weighed) in 10 ml ethanol followed by agitation with a magnetic stirrer for 12 h to dissolve the polymer and to extract the drug. After filtration through a 5 µm membrane filter (Millipore), the drug concentration in the ethanol phase was determined spectrophotometrically at 243 nm (UV-Visible spectrophotometer, Shimazdu-1800). Eudragit S-100 and the SS powder did not interfere under these conditions. Each determination was made in triplicate. The percentage drug entrapment and yield were calculated as follows:

% Drug entrapment = [Calculated drug conc. /Theoretical drug content] x 100

% Yield = [Total weight of floating microparticles/Total weight of drug and polymer x 100

Floating Behavior

100 mg of the floating microspheres were dispersed in solution (300 ml, pH 2.0 buffer, 37±1ºC) containing Tween 20 (0.02 w/v %) is hydrophilic surfactant with HLB value 16.7 used to prevent particle aggregation during characterization studies. Particle aggregation might lead to dose dumping and altered behavior. The mixture was stirred at 100 rpm in a magnetic stirrer. After 12 h, the layer of buoyant particles was pipetted and the floating particles were separated by filtration. Particles in the sinking particulate layer were separated by filtration. Both particles were dried at 40ºC overnight [17]. Each weight was measured and buoyancy was determined by the weight ratio of the floating particles to the total weight of microspheres.

$$\text{Buoyancy (\%)} = Wf / (Wf + Ws) \times 100$$

Where, Wf and Ws are the weights of the floating and settled microspheres, respectively. All the determinations were made in triplicate.

FTIR Study

IR Spectra of SS, SS adsorbed with CaSi, Eudragit S 100, and drug loaded formulations were taken with the help of a FTIR Spectrophotometer (Shimadzu-IR Affinity-1) scanned with wave

number range of 400-4000cm^{1}. The interaction between the components was observed.

Differential Scanning Colorimetry (DSC)

DSC studies of SS, SS adsorbed CaSi, Eudragit-S 100, and drug loaded formulations were carried out using a differential scanning calorimeter (Mettler DSC 30 instrument). All samples of about 7 mg were placed in an aluminum pans and sealed. The temperature range of probe was from 10^0C to 300^0C at the heating rate of 10^0C/minute under nitrogen atmosphere. Thermographs of all samples were recorded.

In vitro Release Studies

The drug release rate from floating microspheres was determined using USP XXIII basket type dissolution apparatus in three different media such as simulated gastric fluid, pH 2.0, simulated intestinal fluid pH 6.8 and Phosphate buffer of pH 7.4. A weighed amount of floating microspheres equivalent to 50 mg drug was filled into a capsule (# 3) and placed in the basket. All the three media (900 ml) containing tween 20 (0.02 w/v %) maintained at 37±1˚C at a rotation speed of 100 rpm. Perfect sink conditions prevailed during the drug release studies [14], 5 ml sample was withdrawn at 0 min, 30 min, 1 h, 2h, 3h, up to 24 h. Samples were passed through a 5 µm membrane filter (Millipore), and analysed spectrophotometrically at 276 nm (UV-Visible spectrophotometer, Shimazdu-1800) to determine the concentration of drug present in the dissolution medium. The initial volume of the dissolution fluid was maintained by adding 5 ml of fresh dissolution fluid after each withdrawal to maintain sink condition.

Pharmacokinetic studies

The *in vivo* study was performed using the protocol approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The experimental protocol was approved by Institutional Animals Ethical Committee of SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur, India, (994/a/GO/06/CPCSEA). The *in vivo* studies were conducted in healthy male albino rabbits weighing 2.3-2.4 kg. Rabbits were kept for one week in animal house to acclimatize them and were provided fixed standard diet. Twelve rabbits were divided into two groups of 6 each and were fasted for 24 h. One group was fed with a marketed Sbt equivalent to 100 mg of salbutamol at each 8 hour interval, while Sbt FMCaSi-D was fed to 2nd group of animals equivalent to 300 mg of Sbt. Water was given *ad libitum* during fasting and throughout the experiment. They swallowed the formulation without any difficulty. Blood samples (2 ml) were collected from the marginal ear vein into heparinized centrifuge tubes just before dosing and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h during the study. The blood samples withdrawn as above were transferred to a series of graduated centrifuge tubes containing 0.4 ml of 2.5% w/v sodium citrate solution. The samples were centrifuged at 2500 rpm for 5 min. The plasma was

transferred into another set of sample tubes and frozen until assayed. One µl dosed plasma sample was kept as blank. The sample was filtered through 0.25 µm membrane filter (Milli pore). The salbutamol concentration in blood samples was analyzed using the HPLC method as described earlier [19].

Statistics Analysis

Different *in vitro* drug release of SS from CaSi based microspheres (FM) and microspheres without CaSi (NFM) were statistically analysed by one way analysis of variance (ANOVA) with post test (Dunnett's multiple comparison tests). Statistically significant differences between *in vitro* drug releases of formulations were defined as $p < 0.05$ was statistically significant. Calculations were performed with the GraphPad-Instat Software Program (GraphPad-Instat Software Inc., San Diego).

Results

Formation of Microspheres

The floating microspheres were prepared by emulsion solvent diffusion technique [20] Solution or suspension of Eudragit S-100 and SS adsorbed CaSi in ethanol and dichloromethane was poured into an agitated aqueous solution of polyvinyl alcohol. It was found that a saturated solution of polymer produced smooth and high yield microspheres [18] for the optimization of formulation the parameters like Polymer: CaSi ratio due to porous character of (CaSi), high amount may lead to reduced entrapment of drugs and unpredictable release thus optimized ratio of (CaSi) was necessary to get floating nature of microsphere and adequate entrapment and predictable release rate, temperature and stirring rate are two parameters which affect the polymer physical properties and thus alter physio-chemical properties of prepared formulations. Thus these two parameters were taken into considerations to obtain homogenous and stable floating formulations taken into consideration, shown in Table 1.

Table- 1: Optimization parameters of floating microspheres

S.No.	Formulation code	Drug and polymer ratio (SS:ES-100)	Carrier and Polymer ratio (CaSi:Eudragit S-100)	Temp. (˚C)	Solvent ratio (Ethanol: DCM)	Stirring rate (rpm)
1	SF-1	1:5	1:10	24	1:1	230
2	SF-2	1:5	1:10	28	2:1	475
3	SF-3	1:5	1:10	42	3:1	700
4	SF-4	1:5	1:10	55	4:1	925
5	SF-5	1:5	1:10	60	5:1	1250

Micromeritic Properties

The particle size of a pharmaceutical substance is strictly maintained in order to get optimal biological activity. The size of microspheres was calculated by measuring 100 particles with help of ocular micrometer. The mean particle sizes were 144±15 µm for CaSi powder and 685±22, 698±17, 703±20, 707±16 and 709±19 µm for formulation containing CaSi in the range of 30-230 mg. The tapped density values ranged from 0.18-0.56g/cm^3, while their true densities ranged between 1.44 to 1.88 g/cm^3 of all the formulations, which may be due to the presence of low density CaSi particles in the microspheres. The compressibility index ranged between 24.4% to 33.1%. All formulations showed excellent flow ability as expressed in terms of angle of repose (<30˚), shown in Table 2. The better flow property indicates that the floating microspheres produced are non-aggregated.

Table- 2: Micromeritic properties of microparticles

Formulation	CaSi content (mg)	Angle of repose	Mean particle size (µm)	True density (g/cm^3)	Tapped density (g/cm^3)	Compressibility index (%)
CaSi	---	46.3±5	144±15	1.44±0.21	0.18±0.03	24.4±1.3
NFS	0	50.2±2	185±24	1.66±0.14	0.59±0.05	26.2±1.9
SF 1	30	36.6±3	685±22	1.48±0.13	0.46±0.11	31.1±0.4
SF 2	80	37.6±4	698±17	1.59±0.12	0.47±0.07	32.6±1.6
SF 3	130	34.9±2	703±20	1.69±0.19	0.56±0.10	32.2±1.4
SF 4	180	41.7±6	707±16	1.70±0.24	0.53±0.19	32.9±1.9
SF 5	230	40.6±4	709±19	1.88±0.36	0.56±0.03	33.1±1.3

Values are average of three readings ± standard deviation.

Morphology

SEM image of CaSi based Eudragit-S 100 microspheres, CaSi and CaSi adsorbed with SS clearly suggests that, CaSi based Eudragit-S 100 microspheres were predominantly spherical in appearance; however some were found to be elongated Figure 1 to 5. SEM photomicrograph of CaSi justifies the porous nature of CaSi. Further SEM image of drug adsorbed CaSi clearly suggests entrapment of drug inside the pores of CaSi, which make them float on the simulated GIT fluids.

Drug Entrapment

The % of drug entrapment is shown in Figure 6 which clearly justifies that with increase in temperature and stirring rate the entrapment efficiency increase up to a certain extent followed by decrease in particle size. With further increase in stirring rate there may be increase in aggregation of polymers so entrapment efficiency decreases.

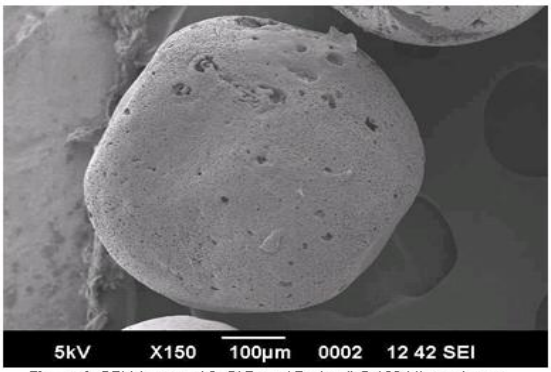


Figure 1: SEM Image of CaSi Based Eudragit-S 100 Microspheres

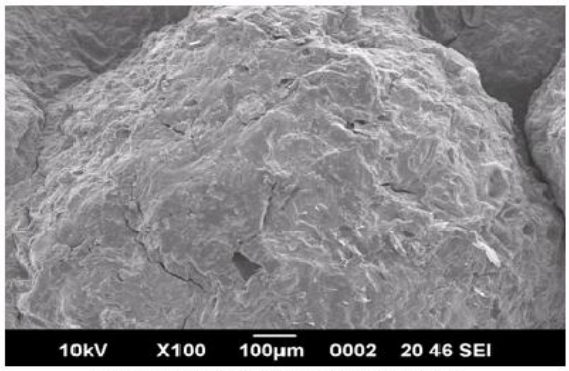


Figure 2: SEM Image of surface morphology SS FM

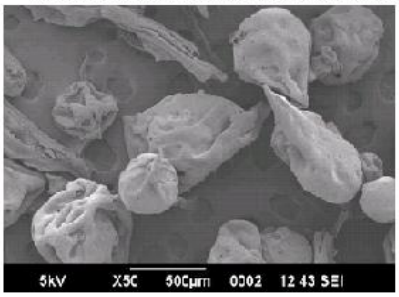


Figure 3: SEM Image of CaSi Adsorbed SS

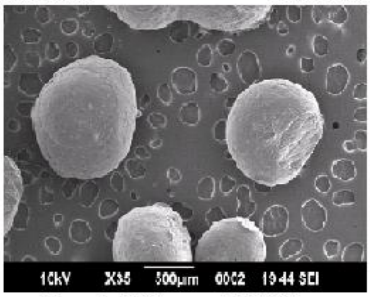


Figure 4: SEM Image of SS NFM

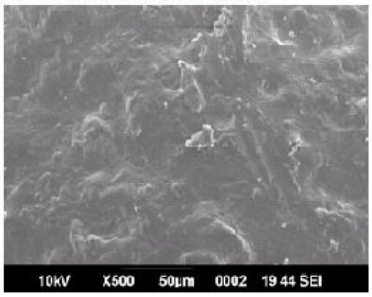
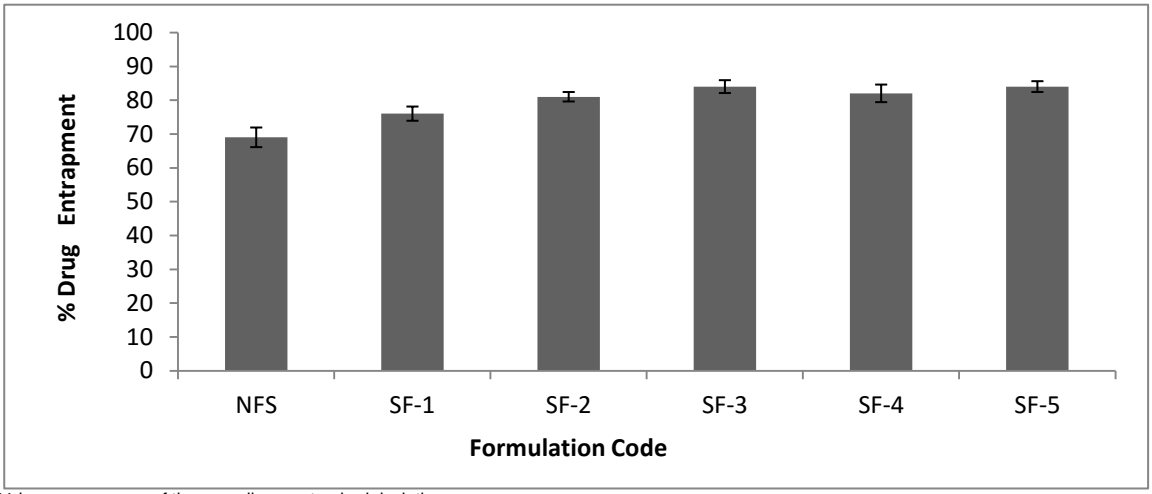


Figure 5: SEM Image of surface morphology SS NFM



Values are average of three readings ± standard deviation

Figure 6*:* Drug Entrapment of Different Floating and Non-floating Microspheres

Floating Behavior

The floating ability differed according to the formulation tested and the medium used. The microspheres were spread over the surface of SGF and the fraction of microspheres settled down as a function of time was quantified. All the CaSi based formulations showed good floating ability, shown in Table 3.

Table- 3: In vitro floating behavior of the prepared formulations

Time (h)	Buoyancy (%)				
	SF-1	SF-2	SF-3	SF-4	SF-5
0	100	100	100	100	100
1	100	100	100	100	100
2	96.76±1.23	98.64±1.01	100	100	98.56±1.15
3	95.65±1.02	97.77±1.12	98.30±1.12	98.54±1.42	97.32±1.37
4	89.98±1.45	94.85±1.32	97.62±1.21	97.60±1.03	95.56±1.34
5	87.56±1.32	93.66±1.21	95.29±1.57	96.88±1.34	94.34±1.27
6	86.22±1.21	91.65±1.31	90.66±1.42	96.56±1.62	93.56±1.52
7	74.45±1.57	88.77±1.56	89.78±1.23	96.34±1.62	90.56±1.57
8	68.44±1.63	87.66±1.32	87.72±1.82	95.37±1.52	87.39±1.62
9	66.45±1.12	86.78±1.67	86.75±1.42	94.88±1.48	86.22±1.01
10	65.67±1.72	85.67±1.72	83.76±1.27	93.12±1.44	84.55±1.62

Values are average of three readings ± standard deviation

More than 80% of the particles kept floating for at least 10 h. The good buoyancy behavior of the microspheres may be attributed to the hollow nature of the microspheres [18] and entrapment of CaSi of low true density [21]. Formulation SF-4 gave the best floating ability (>93%) in SGF. But by considering the entrapment efficiency, SF-4 shows satisfactory floating ability, which can be considered as optimized formulation. Tween 20 (0.02% w/v), added to SGF, counteracted the downward pulling at the liquid surface by lowering surface tension, because relatively high surface tension of simulated gastric fluid causes the highest decrease of surface area at the air fluid interface. It was also observed that the microspheres of larger size, showed the longer floating time. The high entrapment efficiency of SS is believed to be due to its poor aqueous solubility. When the loading was high, the proportion of larger particles formed was also high.

FTIR Study

FTIR spectra of SS, SS adsorbed with CaSi, Eudragit S 100, and drug loaded formulations were studied. The characteristic peaks of the individual components retained suggest that there is no interaction between the different components Figure7-10.

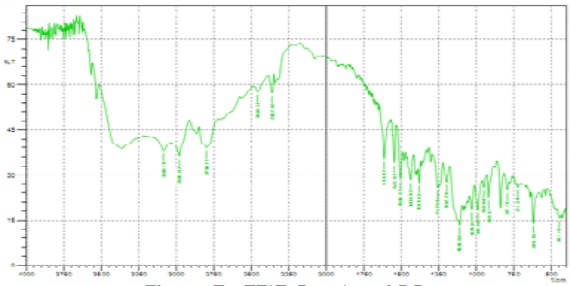


Figure 7: FTIR Spectra of SS

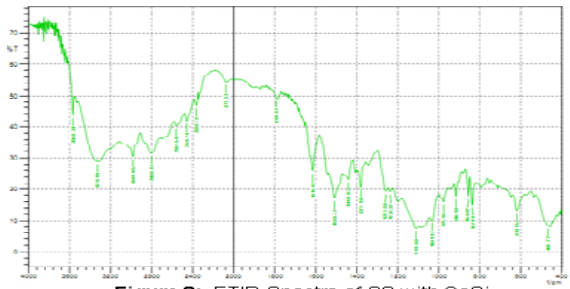


Figure 8: FTIR Spectra of SS with CaSi

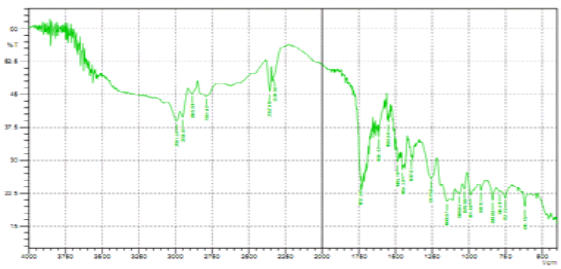


Figure 9: FTIR Spectra of SS with Eudragit S-100

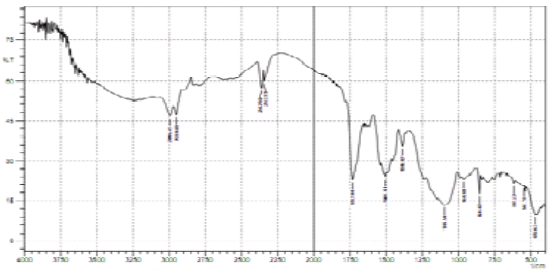


Figure 10: FTIR Spectra of developed formulation

Differential Scanning Colorimetry

In order to determine the physical state of drug i.e. amorphous or crystalline, before and after floating microsphere formulation, DSC examination was conducted for the pure drug, the polymer, CaSi and optimized formulation. Thermograms of the single component(s) and microspheres are shown in Figure 11 (a-d).

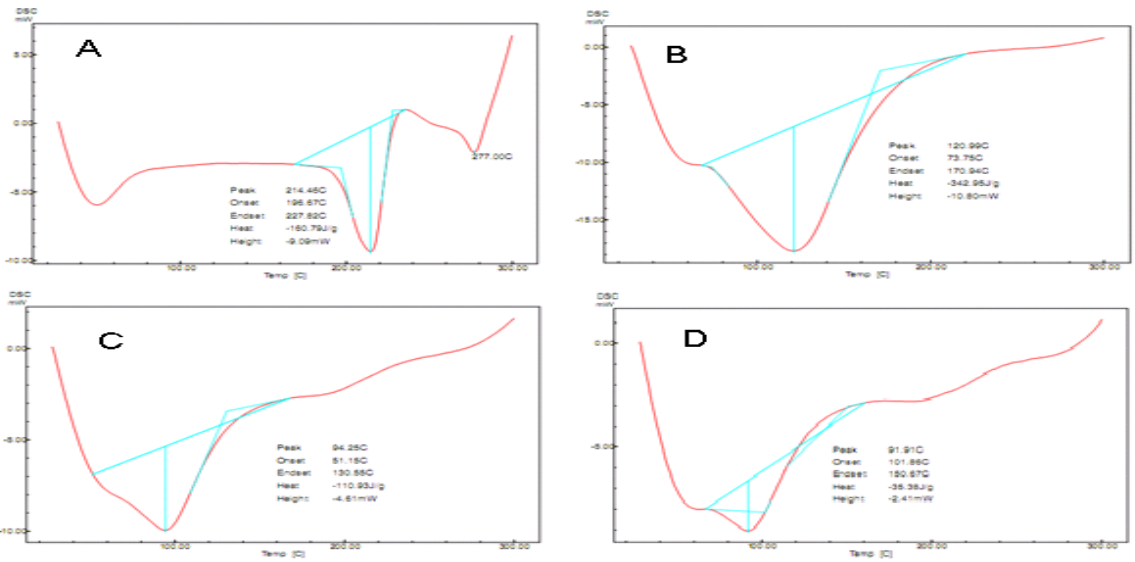


Figure 11: DSC thermogram of (a) Pure salbutamol sulphate (b) Salbutamol sulphate adsorbed CaSi (c) Eudragit S-100 (d) Salbutamol Sulphate Microspheres

A sharp melting transition of SS (pure) was observed at 218.5 ˚C. CaSi adsorbed with SS shows a broad peak at broad peak maximum at 120˚C. Eudragit S-100 showed a broad transition peak at 98˚C. DSC thermogram of optimized formulation showed broad peak nearer to 100˚C, which suggest that drug is partly dissolved in the polymer and partly in the amorphous form distributed throughout the system.

In vitro Drug Release Study

Release of SS from CaSi based microspheres was evaluated in SGF of pH 2.0, SIF of pH

6.8 and Phosphate buffer of pH 7.4. Drug release from Eudragit S-100 in SGF took place only through diffusion and drug release in PBS (pH 7.4) might have involved both mechanisms i.e. diffusion and erosion. The other reason for the slow dissolution rate of drug may also be attributed to low solubility of SS at acidic pH. There was no burst effect from any of these formulations.

Soppimath and Aminabhavi [15] had reported previously that solvent evaporation method used for the preparation poly (lactide) microspheres results in the initial rapid release of drug. Figure 12, 13 shows the cumulative % release of SS from microspheres containing varying amounts of drug adsorbed CaSi in pH 2.0, 6.8 and 7.4 at 37±1˚C. These figures clearly show that formulation without CaSi released the drug more rapidly as compared to formulations containing CaSi, which released the drug in a more controlled manner. The drug release from different formulations in pH 2.0, 6.8 and 7.4 followed the order: NFS>SF-1>SF-2>SF-3>SF-4>SF-5. The pattern also provides an idea about the effect of CaSi content on drug release, i.e., more the CaSi content, lesser was the drug release. When the data of formulation NFS (without CaSi) is compared with formulations containing CaSi by one-way ANOVA test, the *in vitro* release in SGF (pH 2.0) from SF-4 and SF-5 were found to be significant. The drug release in pH 6.8 was found to be insignificant (P>0.05) in all the formulations.

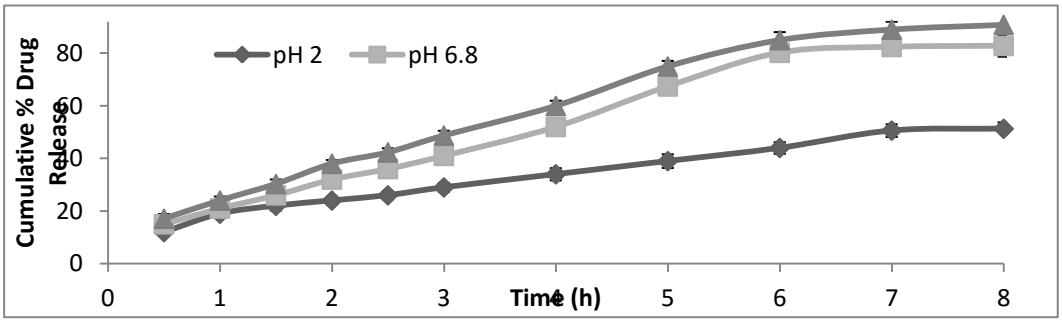


Figure 12: Cumulative % Drug Release from NFS (Non Floating Microspheres) of Developed Formulations in Different pH (2.0, 6.8, & 7.4) at 37±1˚C

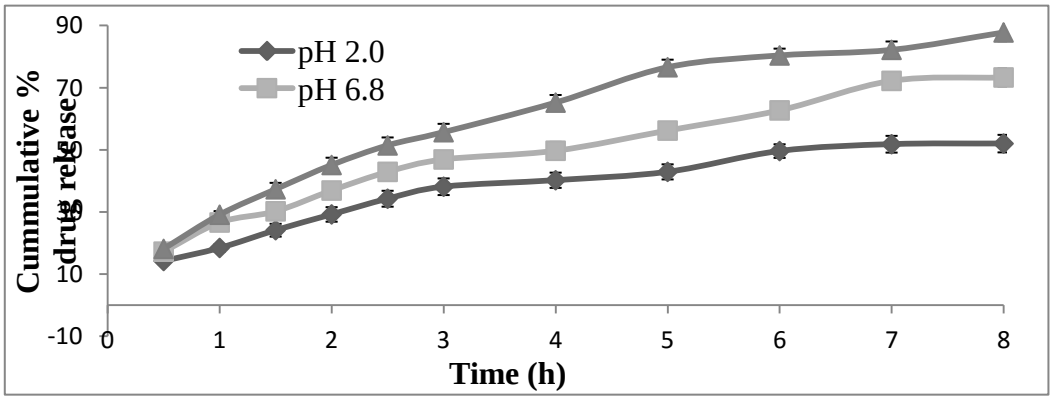


Figure 13: Cumulative % Release of SF-4 from the Optimized Floating Microspheres Developed in Different pH (2.0, 6.8, & 7.4) at 37±1˚C

Model Fitting of the Release Study

When plotted with Peppas-Korsmeyer's power law equation all the prepared formulations showed high r^2 value ($r^2 > 0.99$). Release exponent (n) value was determined from the slope of the straight line of this plot and was found to be within the range of 0.489 to 0.644. The fit of the release data was tested with the following kinetics model (a) zero order kinetics, (b) first order kinetics, (c) Peppas-Korsmeyer (d) square-root of time equation (Higuchi equation). The Higuchi equation suggests that drug release is controlled by the diffusion of drug through the pores and not through the swollen polymer. The diffusion exponent, n, specifies the mechanism of release, which depends on the release mechanism and the shape of the matrix tested. Exponent (n) for polymeric controlled delivery systems of spherical geometry has values of $n = 0.43$ are for Fickian diffusion; $0.43 < n > 0.85$ are an indication of both diffusion controlled drug release and swelling controlled drug release (anomalous transport or non-Fickian transport); and $n > 0.85$ indicate case-II transport which relate to polymer relaxation during gel swelling [22,23]. Therefore it is concluded that all the formulations followed a non-Fickian release mechanism i.e. release was governed by both diffusion and swelling of polymer as described by the Peppas-Korsmeyer model, shown in Table 4, 5 & 6. Formulation SF-4 was assigned as optimized formulation due to its better buoyancy behavior and controlled release as compared to other prepared formulations and it was selected for further stability and *in vivo* studies.

Pharmacokinetic studies

Blood samples were collected from the marginal ear vein into heparinized centrifuge tubes just before dosing and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12and 24 h during the study. Analysis of blood samples for the content of salbutamol was performed by the developed HPLC method. Salbutamol shows a good linear relationship between 20-150 ng. Standard chromatogram of salbutamol is shown in Figure 14. The plasma drug concentration of salbutamol is shown in Figure 15. This depicts that the developed formulation shows maintenance of plasma concentration of salbutamol for 24 hours, which is comparable to that of salbutamol sulphate in three divide dosage form.

Table 4: Comparison of Different Dissolution Kinetics Models for the Release of NFS and SS Floating Microspheres from Different Formulations in SGF, pH 2.0

Formulation	Zero order	First order	Higuchi-matrix	Peppas-Korsmeyer	
	r^2	r^2	r^2	r^2	N
NFS	0.936	0.929	0.992	0.989	0.509
SF-1	0.914	0.948	0.991	0.981	0.504
SF-2	0.893	0.976	0.990	0.989	0.536
SF-3	0.865	0.978	0.982	0.972	0.510
SF-4	0.874	0.973	0.986	0.983	0.495
SF-5	0.852	0.972	0.977	0.971	0.489

Table 5: Comparison of Different Dissolution Kinetics Models for the Release of NFS and SS Floating Microspheres from Different Formulations in SIF, pH 6.8

Formulation	Zero order	First order	Higuchi-matrix	Peppas-Korsmeyer	
	r^2	r^2	r^2	r^2	*N*
NFS	0.965	0.890	0.959	0.980	0.664
SF-1	0.963	0.926	0.980	0.994	0.644
SF-2	0.950	0.927	0.989	0.994	0.559
SF-3	0.928	0.959	0.994	0.995	0.563
SF-4	0.917	0.954	0.994	0.992	0.515
SF-5	0.917	0.954	0.994	0.992	0.515

Table 6: Comparison of Different Dissolution Kinetics Models for the Release of NFS and SS Floating Microspheres from Different Formulations in PBS, pH 7.4

Formulation	Zero order	First Order	Higuchi-matrix	Peppas-Korsmeyer	
	r^2	r^2	r^2	r^2	*N*
NFS	0.961	0.918	0.974	0.990	0.649
SF-1	0.943	0.949	0.985	0.995	0.637
SF-2	0.929	0.958	0.993	0.996	0.567
SF-3	0.925	0.967	0.996	0.996	0.567
SF-4	0.903	0.978	0.991	0.989	0.568
SF-5	0.909	0.975	0.992	0.990	0.574

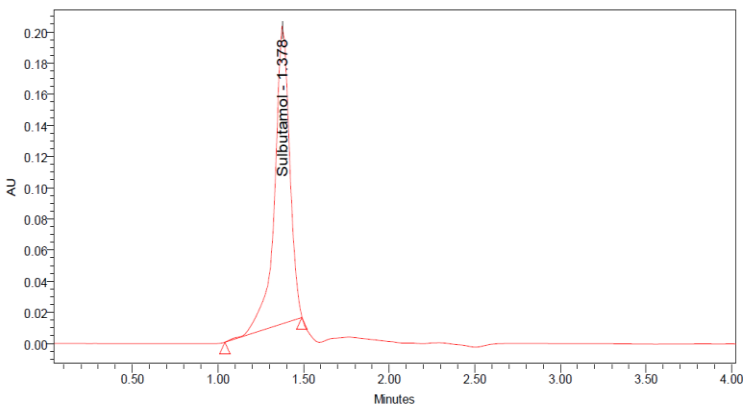


Figure 14: HPLC Chromatogram of Salbutamol

The data obtained in terms of plasma concentration of SS with respect to time as shown in Table 7 and Figure 15, was used for the calculation of various pharmacokinetic parameters with the help of sigma-plot software and excel Figure 15 clearly reveals that Peak plasma concentration (C_{max}) for SS Marketed formulation and developed microspheres was found to be 247.67±10.84

ng/ml and 177.54±8.62 ng/ml respectively. Time to reach peak plasma concentration (t_{max}) for SS marketed formulation and developed microspheres were found to be 2.13±0.83 and 8.12±1.76 h respectively. Further AUC for SS marketed formulation and developed microspheres were found to be 2131.85±169.16 ng/ h/ml and 2369.55±149.35 ng/ h/ml respectively.

Table 7: Pharmacokinetic study of SS marketed drug and optimized floating microspheres

S.No.	Pharmacokinetic parameters	Marketed Drug (SS)	Optimized Floating microspheres
1.	AUC_{0-24} (ng ml^{-1} h^{-1})	2131.85±169.16	2369.55±149.35
2.	C_{max} (ng ml^{-1})	247.67±10.84	177.54±8.62
3.	T_{max} (h)	2.13±0.83	8.12±1.76
4.	T (1/2) (h)	4.94±0.15	8.77±0.21
5.	K_{el} (h^{-1})	0.140±0.002	0.079±0.003
6.	Vd (liter)	0.0534±0.012	0.0854±0.011

Values are average of three readings ± standard deviation

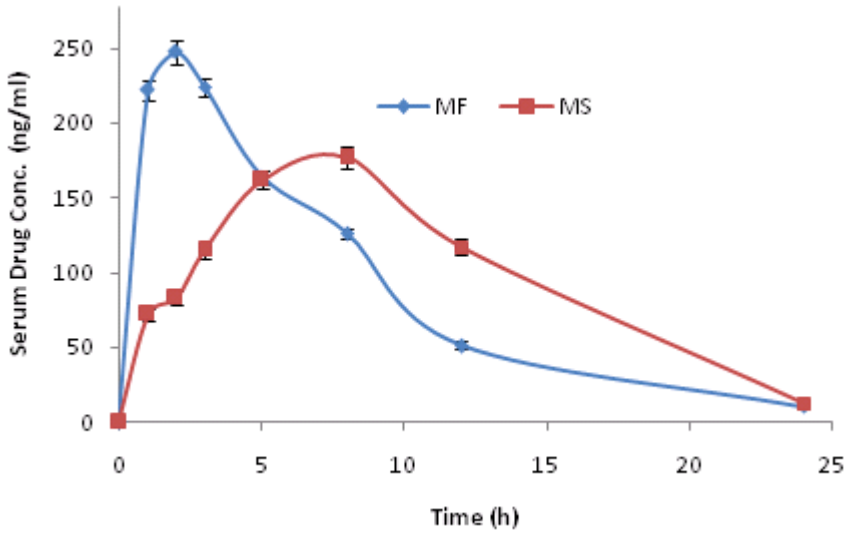


Figure 15: Mean SS concentration level after oral administration of Marketed formulation (MF) and Developed microspheres (MS) of SS.

Conclusions

Present study for the formulation and characterization of floating microsphere delivery of SS was performed. CaSi plays a major role in the floating of microspheres for desired release behavior and buoyancy. The performance of the developed formulations was evaluated with various parameters like drug entrapment, floating behavior and *in vitro* release study etc. The result of

this study clearly reveals that the developed system have excellent buoyant ability and suitable drug release pattern, this may be helpful for increased bioavailability of SS in a sustained manner. It can be concluded that the developed formulations have good advantage over conventional dosage form i.e. easy preparation method, excellent bouncy ability, good encapsulation efficiency and sustained drug release. The microspheres could be compressed into tablets, filled into capsules, or formulated into oral suspensions for reconstitute.

References

1. Korenma P, Charles DP. (1998) New strategies in the medical management of asthma. American Family Physician, 1998 Jul; 58(1):89-100, 109-12.
2. Arora S, Ali J, Ahuja A, Khar RK, Baboota S. (2005) Floating drug delivery systems: a review. AAPS Pharm Sci Tech ; 6: E372-E390.
3. Ponchel G and Irache JM. (1998) Specific and non-specific bioadhesive particulate system for oral delivery to the gastrointestinal tract. Adv Drug Del Rev; 34: 191-219.
4. Sing BN and Kim KH. (2000) Floating Drug Delivery Systems: An approach to oral controlled drug delivery via gastric retention. J. Control. Rel; 63: 235-259.
5. Rednick AB and Tucker SJ. Sustained release bolus for animal husbandry. US patent, 1970; April 22(3): 507 952.
6. Urguhart J and Theeuwes F. Drug delivery system comprising a reservoir containing a plurality of tiny pills. US patent, 1994; February 28(4): 434 153.
7. Mamajek RC and Moyer ES. Drug dispensing and method. US patent 4, 207, 890, 1980;June 17 through C.A.1984 K50391.
8. Fix JA, Cargill R,.Engle K. (1993) Controlled gastric emptying. III. Gastric residence time of a non-disintegrating geometric shape in human volunteers. Pharm Res; 10: 1087- 1089.
9. Kedzierewicz F, Thouvenot P, Lemut J, Etienne Hoffman M, Maincent P. (1999) Evaluation of peroral silicone dosage forms in humans by A gamma-scintigraphy. J Control Release; 8:195-205.
10. Groning R and. Heun G. (1984) Oral dosage forms with controlled gastrointestinal transit. Drug Dev Ind Pharm; 1:527-539.
11. Groning R and Heun G. (1989) Dosage forms with controlled gastrointestinal passage studies on the absorption of nitrofurantion. Int J Pharm; 56: 111- 116.
12. Deshpande AA, Shah NH, Rhodes CT, Malick W. (1997) Development of a novel controlled release system for gastric retention. Pharm Res; 14: 815-819.
13. Leung SH, Irons BK, Robinson JR. (1993) Polyanionic hydrogel as a gastric retention system. J Biomater Sci Polym Ed; 4: 483-492.
14. Kawashima Y, Niwa T, Hino T, Itoh Y. (1991) Preparation of multiple unit hollow microspheres (microballoons) with acrylic resin containing tranilast and their drug release characteristics (*in vitro*) and floating behavior (*in vivo*) J. Control. Release; 16: 279-290.

15. Soppimath KS and Aminabhavi TM. (2002) Ethylacetate as a dispersing solvent in the production of poly(DLlactide co-glycolide) microspheres: effect of process parameters and polymer type. J. Microencapsul; 19: 281-292.
16. Martin A. Physical Pharmacy, IVth ed., Lea Febiger, Philadelphia 1993; pp. 431–432.
17. Ritschel WA, Thompson GA, Lucker PW, Wetzelsberger K. (1980) Biopharmaceutic evaluation of etofylline clofibrate and its drug formulation. Arzneim.-Forsch/ Drug Res ; 30:2020–2023.
18. Sato Y, Kawashima Y, Takeuchi H, Yamamoto H. (2003) Physicochemical properties to determine the buoyancy of hollow microspheres (microballons) prepared by the emulsion solvent diffusion method. Eur. J. Pharm. Biopharm; 55: 297–304.
19. Rajeev G, Cynthia S, Lisa A, James S, Grant S, Bahram F, Thomas N. (1982) Transdermal drug delivery systems of albuterol; in vitro and in vivo studies. J. Pharm. Sci.;81: 996–1000.
20. Kawashima Y, Niwa T, Takeuchi H, Hino T, Itoh Y. (1992) Hollow microspheres for use as a floating controlled drug delivery system in the stomach. J. Pharm. Sci; 81: 135–140.
21. Streubel A, Siepmann J, Bodmeier R. (2003) Floating matrix tablet based on low density foam powder: effects of formulation and processing parameters on drug release. Eur J Pharm Sci; 18: 37-45.
22. Siepmann J, Peppas NA. (2001) Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv Drug Del Rev; 48: 139-157.
23. Ritger PL and Peppas NA. (1987) A simple equation for description of solute release II Fickian and anomalous from swellable devices.J. Control. Rel; 5: 37–42.
24. Ahmed TN, Abd EL-Gawad H, Abd El-Gawad H, Tawhida KG. (2010) Formulation and bioavailability of controlled release salbutamol sulphate tablets using natural additives.Drug Discoveries & Therapeutics; 4 (2): 85-92.
25. Chung BH, Shim CK. (1987) Dissolution of theophylline from sustained release dosage forms and correlation with saliva bioavailability parameters. J. Pharm. Sci; 76 (10): 784-787.
26. Shan-Yang L, Juei-Chyi Y. (1987) Bioavailability studies of Theophylline ethylcellulose microcapsules prepared by using ethyl-vinyl acetate copolymer as a coacervation inducing agent. J. Pharm. Sci; 76 (3): 219-223.

PHARMBIT, Jul – Dec 2014; XXX (2)

Formulation and evaluation of dipyridamole gastroretentive floating drug delivery system

P. N. Murthy*, Anjan K. Mahapatra, Diptanu Dey

Royal College of Pharmacy and Health Sciences, Berhampur-760002, India.

Abstract

The present study was aimed towards the development and evaluation of a floating- drug delivery system, which can provide gastric retention and sustained release of the drug dipyridamole there by targeting site specific drug release and absorption in the proximal upper GIT for local or systemic effects. The floating tablets were formulated using polymers HPMC K100M CR, guar gum, and xanthan gum along with other excipients. Dipyridamole is a BCS class II drug having low solubility and high permeability. It is soluble at low pH but insoluble in high pH. The above reasons are suitable for gastro retentive drug delivery system of dipyridamole. From FTIR & DSC Studies it was found that dipyridamole is compatible with all excipients used in the formulation and there is no significant inter action between the drug and the excipients used. Optimized formulation F9 containing polymer concentration of HPMC K100M CR and Sodium bicarbonate (25% + 19%) was considered as best formulation with respect to log time, in vitro drug release for 24 hrs and total floating time. Stability study was conducted on tablets of optimized batch at 40±2 0C for one month. Tablets were evaluated for drug release pattern, hardness, floating behavior and in vitro drug release. No significant changes were observed in any of the studied parameters after the study period.

Key words: Floating drug delivery, Gastric residence, Polymers, Release kinetics

Introduction

Floating drug delivery systems (FDDSs) were first described by Davis in 1968[1].These systems were used to prolong the gastric residence time of drug delivery systems. They remain buoyant in the stomach for prolonged period of time without affecting the gastric emptying rate of other contents[2, 3]. FDDSs have a bulk density less than gastric fluids and so remain buoyant in the stomach. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. FDDS are advantageous for drugs meant for local action in the stomach, also are advantageous in case of vigorous intestinal movements and in diarrhoea to keep the drug in floating condition in stomach to get a relatively better response. Acidic substances like dipyridamole cause irritation on the stomach wall when come in contact with it hence; floating formulations may be useful for the administration of dipyridamole and other similar drugs. The FDDS are advantageous for drugs absorbed in the stomach[4, 5]. Antiplatelet drugs are vital

components of normal haemostasis and key participants in atherothrombosis by virtue of their capacity to adhere to injured blood vessels and to accumulate at sites of injury. Currently available antiplatelet drugs interfere with some of the steps leading to platelet aggregation and have a measurable impact on the risk of arterial thrombosis that cannot be dissociated from an increased risk of bleeding[6]. The drugs used for antiplatelet action includes indobufen, triflusal, ticlopidine, clopidogrel, prasugrel, ticagrelor, cangrelora, elinogrela, cilostazol and dipyridamole works by the mechanism of Phosphodiesterase inhibition[7]. Dipyridamole is a pyrimidopyrimidine derivative with vasodilator and antiplatelet properties. Its mechanism of action as an antiplatelet agent has been a subject of controversy. Dipyridamole was synthesized half a century ago and introduced clinically in the early 1960s as a coronary vasodilator. Dipyridamole was shown to inhibit platelet adhesiveness in patients with coronary artery disease and to reduce thrombus formation in experimental models. These findings led Boehringer Ingelheim to develop dipyridamole as an antithrombotic agent. Although the clinical efficacy of dipyridamole, alone or in combination with aspirin, has been questioned on the basis of earlier randomized trials, the issue has been reopened by the reformulation of the drug to improve its relatively low bioavailability and the positive results with the new preparation of the European Stroke Prevention Study-2 (ESPS-2) on 6602patients with cerebrovascular disease. Unexpectedly, dipyridamole did not increase the bleeding complications in these patients, raising the possibility that other properties of the drug may have contributed to its beneficial effects on stroke prevention. Steve Prescott at the University of Utah has recently reported that dipyridamole inhibits inflammatory gene expression in human platelet monocyte interactions, which may be involved in atherosclerosis and in its thrombotic complications[8]. The structure of dipyridamole is given under Figure 1.

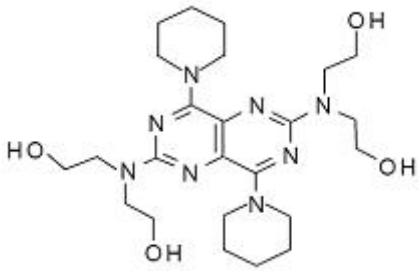


Figure 1. Structure of dipyridamole

The pharmacokinetics parameters for the drug show variable and slow absorption, bioavailability ranges from 27% to 59%, wide distribution into body tissues and small amounts cross the placental barrier, protein-binding ranges from 91 to 97% and metabolized by the liver. Elimination occurs via biliary excretion of glucuronide conjugates. Some dipyridamole and conjugates may undergo enterohepatic circulation and fecal excretion; a small amount is excreted in urine. Half-life varies from 10 to 12 hours. Dipyridamole is suitable for designing into FDDS as it shows solubility at low pH of gastric environment and absorption at stomach region. Few works by other researchers has been reported on dipyridamole but use of HPMC K 100 M CR, guar gum,

xanthan gum and comparative studies has not been reported.

Materials and Method

Materials

Dipyridamole was procured from Vergo Pharma Research Laboratories Pvt. Ltd. (Goa, India). HPMC (Methocel) K4M CR and K100M CR were obtained from Colorcon Asia Pvt. Ltd. (Mumbai, India) as gift samples. Xanthan gum, guar gum, microcrystalline cellulose, sodium bicarbonate, citric acid and talc were obtained from Loba Chemie Pvt. Ltd. (Mumbai, India). Crospovidone was obtained from (BASF, Germany) as gift sample, Magnesium stearate was obtained from (Thomas Baker, Mumbai). All other materials and chemicals were of analytical grade.

Methods

UV-Spectroscopy of dipyridamole[9]

The UV-Vis spectrophotometric analysis of dipyridamole was carried out by using methanol as co-solvent and 0.01N HCl as solvent. Standard plot dipyridamole was constructed by taking a series of standard concentrations from 5 - 40µg/ml. The absorption maxima λ_{max} was observed at 282 nm and used in the estimation of drug.

Fourier-Transform Infrared Spectroscopy (FT-IR studies)[10]

The FTIR spectra were obtained by using an FTIR spectrometer (Shimazdu, Japan). The samples were previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix, at 1:100 (Sample: KBr) ratio respectively. The KBr discs were prepared by compressing the powders at a pressure of 5 tons for 5 min in a hydraulic press. Scans were obtained at a resolution of 2 cm^{-1}, from 4000 to 400 cm^{-1}.

Differential scanning calorimetry[10]

Measurements were performed on a DSC- 6100 (Seiko Instruments, Japan) with a thermal analyzer. All accurately weighed samples (about 2 mg of dronedarone hydrochloride or its equivalent) were placed in sealed aluminum pans, before heating under nitrogen flow (20 mL/min) at a scanning rate of 10 ˚C min^{-1} from 50 to 300 ˚C. An empty aluminum pan was used as reference.

General description of the manufacturing of floating formulations of dipyridamole

The floating tablets of dipyridamole with dose 100 mg were prepared using direct compression method. All the ingredients were weighed accurately using electronic balance (Sartorius) as per the weights in the formula. These powders were passed through 40 mesh sieves separately and mixed thoroughly. Finally lubricant and glidiant were mixed prior to the compression and were compressed to tablets using multi station automated tablet compression machine (Rimek Tablet Mini Press- Ahmedabad), using 10 mm round flat faced punches. The formulas for different batches are given under **Table 1**.

Table 1: Formula of Different Batches

Ingredients	Dipyridamole	HPMC K-100 CR	HPMC K-4 CR	Xanthan gum	Guar gum	Sodium bicarbonate	Citric acid	Talc	Magnesium state	Crospovidone	Microcrystalline cellulose	Total weight (mg)
F1	100	75	-	-	-	75	-	-	5	145	-	400
F2	100	75	-	-	-	150	-	-	5	35	35	400
F3	100	75	-	-	-	225	-	-	5	35	35	400
F4	100	150	-	-	-	75	-	-	5	35	35	400
F5	100	225	-	-	-	75	-	-	5	35	35	400
F6	100	75	-	-	-	75	75	-	5	35	35	400
F7	100	75	-	-	-	100	50	-	5	35	35	400
F8	100	75	-	-	-	75	-	-	5	70	75	400
F9	100	100	-	-	-	75	10	-	5	-	110	400
F10	100	100	-	-	-	50	15	-	5	-	130	400
F11	100	100	-	-	-	25	20	-	5	-	150	400
F12	100	-	100	-	-	50	15	-	5	-	130	400
F13	100	-	-	100	-	50	15	-	5	-	130	400
F14	100	-	-	-	100	50	15	-	5	-	130	400
F15	100	80	-	-	-	10	10	4	2	-	194	400
F16	100	80	-	-	-	20	-	4	2	-	194	400
F17	100	120	-	-	-	15	15	4	2	-	144	400
F18	100	120	-	-	-	30	-	4	2	-	144	400
F19	100	160	-	-	-	5	5	4	2	-	144	400
F20	100	160	-	-	-	40	-	4	2	-	94	400

Buoyancy lag time and the duration of buoyancy[11]

The buoyancy lag time and the duration of buoyancy were determined in the USP dissolution apparatus II in an acidic environment. The time interval between the introduction of the tablet into the dissolution medium and its buoyancy to the top of dissolution medium was taken as buoyancy lag time and the duration of buoyancy was observed visually.

***In vitro* drug release studies[12]**

Dissolution studies of dipyridamole floating formulations were performed by using dissolution test apparatus type-I (Lab India, Mumbai) with the basket rotation speed of 100 rpm, using 900 mL of

0.01N HCl as dissolution media at 37±0.5 ˚C. At the specified time intervals, 5 mL samples were withdrawn by using difluoride (PVDF) membrane and then assayed for the dipyridamole content by measuring the absorbance at 282 nm using the UV-visible spectrophotometer and volume is adjusted by fresh medium maintained at 37 ˚C after each sampling to maintain its constant volume throughout the test. Dissolution studies were performed in triplicate (n=3), calculated mean values of cumulative drug release and data were used while plotting the release curves.

Drug release kinetics for floating drug delivery systems[13-15]

The kinetic studies in designing a pharmaceutical floating dosage form depends on a good understanding of the drug release mechanism and kinetics. As the qualitative and quantitative changes in a formulation design could change drug release and *in vivo* performance of a dosage form, it seems very essential to have a thorough insight into the mechanisms of drug release kinetics. Different of approaches used for kinetic investigations are: model-dependent methods comprising a variety of kinetic models expressing dissolution profiles and overall release of drug from the formulations. Zero-order, First-order, Higuchi, Korsmeyer-Peppas, Hixson-Crowell, Baker-Lonsdale, Weibull, and regression models are the commonly used models for clarifying the mechanism of drug release. Studying drug release kinetics is often useful in obtaining one or two physically meaningful parameters that are used for comparative purposes and relating the release parameter with important parameters such as dissolution and bioavailability. For example, the n value is generally used in the Korsmeyer- Peppas model to characterize different release mechanisms. This equation has two distinct physical realistic meanings in the two special cases of n = 0.5 and n = 1, indicating diffusion-controlled drug release for the former and erosion controlled drug release for the latter. More to the point, an n value between 0.5 and 1 could be regarded as an indicator for the superposition of both phenomena (anomalous transport). In the case of the Weibull model, according the exponent of time b is linearly related to the exponent n of the power law derived from the analysis of the first 60% of the release curves. The value of the exponent b is an indicator of the mechanism of transport of a drug through the polymer matrix. Estimations for b less or equal to 0.75 indicate Fickian diffusion, whereas a combined mechanism (Fickian diffusion and Case II transport) is associated with b values in the range $0.75 < b < 1$. For values of $b > 1$, drug transport follows a complex release mechanism. With this, the authors have tabulated some kinetic data to have an overview on release kinetics and presenting a rule in relation to drug release kinetics from floating dosage forms. Different ingredients in the tablets appeared to be the key factor responsible for the multiplicity of the models fitting the dissolution data and also the differences in drug release patterns. Also, different models in analyzing the drug release data in each study made it difficult to acquire a general rule in proposing a model for the best fit of dissolution data.

Results

UV-Spectroscopy of dipyridamole

The UV-Vis spectrophotometric analysis of dipyridamole was carried out by using methanol as co-solvent and 0.01N HCl as solvent. Standard plot dipyridamole was constructed by taking a series of standard concentrations from 5 - 40µg/ml. The absorption maxima λ_{max} was observed at 282 nm and used in the estimation of drug.

The post compression parameters, buoyancy lag time and floating time

The post compression parameters along with buoyancy lag time, total floating time and swelling index are given under **Table 2**.

Table 2: Post compression parameters

Formulation code	Hardness (kg/cm2)	Friability (%)	Weight variation (mg)	Drug content (%)	Buoyancy lag time (sec)	Total floating time (hrs)	Swelling index (%)
F1	5.5±0.30	0.47	400±0.76	97.40±0.12	180sec	>12hrs	87.90±0.24
F2	5.42±0.25	0.58	400±0.98	71.12±0.11	80sec	>12hrs	90.80±0.28
F3	5.33±0.10	0.53	400±0.91	98.02±0.13	-	-	-
F4	5.28±0.10	0.44	400±1.53	97.75±0.11	60sec	>10hrs	89.76±0.38
F5	5.26±0.15	0.47	400±1.75	75.38±0.12	75sec	>24hrs	96.90±0.45
F6	5.22±0.15	0.48	400±1.60	98.16±0.10	-	-	-
F7	5.31±0.10	0.52	400±0.61	99.15±0.17	-	-	-
F8	5.34±0.15	0.51	400±1.71	82.34±0.12	40sec	>12hrs	94.12±0..35
F9	5.38±0.15	0.49	400±1.60	99.92±0.10	25sec	>24hrs	96.28±0.32
F10	5.31±0.15	0.53	400±1.66	99.42±0.15	13sec	>24hrs	94.52±0.27
F11	5.29±0.20	0.41	400±0.82	99.22±0.14	12sec	>24hrs	99.60±0.33
F12	5.30±0.18	0.42	400±1.48	80.75±0.18	70sec	>12hrs	98.86±0.25
F13	5.26±0.11	0.52	400±0.90	97.40±0.12	-	-	-
F14	5.22±0.10	0.41	400±1.51	98.08±0.11	-	-	-
F15	5.31±0.12	0.46	400±1.72	99.02±0.13	5sec	>24hrs	94.58±0.44
F16	5.34±0.16	0.47	400±1.60	97.75±0.11	-	-	-
F17	5.38±0.13	0.51	400±0.61	98.20±0.12	37sec	>14hrs	94.12±0..35
F18	5.31±0.14	0.54	400±1.72	98.26±0.10	-	-	-
F19	5.29±0.21	0.48	400±1.61	98.08±0.10	-	-	-
F20	5.30±0.17	0.52	400±1.64	99.02±0.15	-	-	-

In-vitro drug release studies

The *in vitro* drug release of selected or optimized formulations i.e., F9, F10, F11, F15 are given under **Figure 2.**

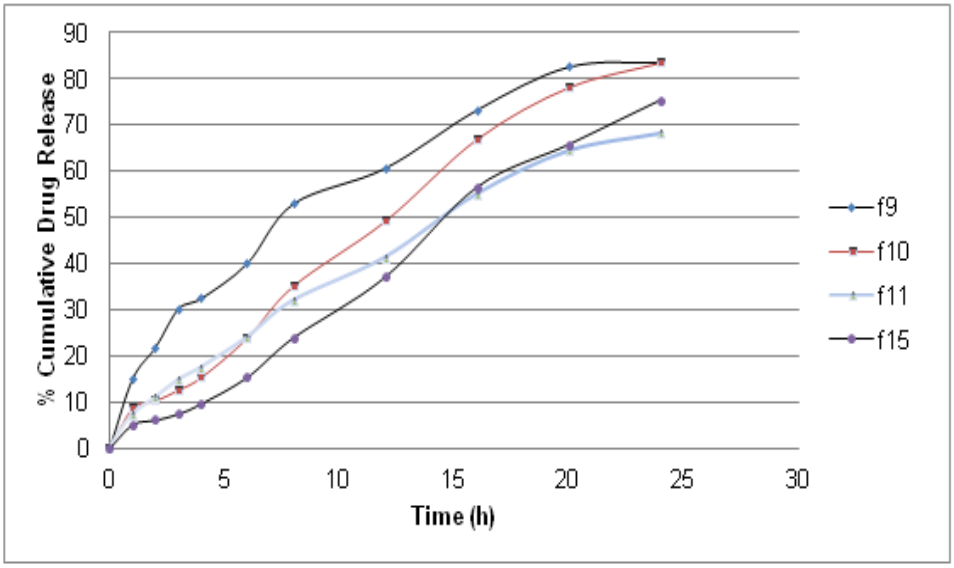


Figure 2. *In vitro* dissolution profile of F9, F10, F11, F15

FT-IR studies

The interaction between drug and the excipients often leads to identifiable changes in the IR spectra of the drug excipient mixture. Dipyridamole is having a characteristic peak at 3379.43 cm^{-1} for OH group. Peak at 3029 cm^{-1} is observed for aromatic C-H (sp^2) stretch. Peaks at 2921, 2852.84 cm^{-1} indicates stretch of C-H (sp^3) bond in drug structure. Peak at 1536.37is due to C=N stretching. Peak at 1442 cm^{-1} in the IR spectrum indicates C-C stretching. Peak at 1358.9 cm^{-1} is due to C-N stretching. Peak at 1085 and 1019 cm^{-1} is due to C-C-O symmetry and asymmetry. HPMC K 100 M CR shows peak at 3564.6 due to OH group. Peaks at 2980.1, 2881.7 and 2837.41 cm^{-1} indicates stretch of C-H (sp^3) bond in the structure of HPMC K100 M CR. Disappearance in peak at 2055 cm^{-1} and 1648 cm^{-1} is observed for HPMC K 100 M CR may be due to overlapping of OH broad peak. Peaks at 1112.01 and 1065 cm^{-1} is due to C-C-O symmetry and asymmetry. Presence of important characteristic peak for drug and HPMC mixture in the IR spectrum without any significant shift in the peaks indicates no significant interaction. The FTIR spectrogram of (A) Dipyridamole (B) HPMC K 100 M CR (C) Dipyridamole and HPMC K 100 M CR mixture were presented in **Figure 3.**

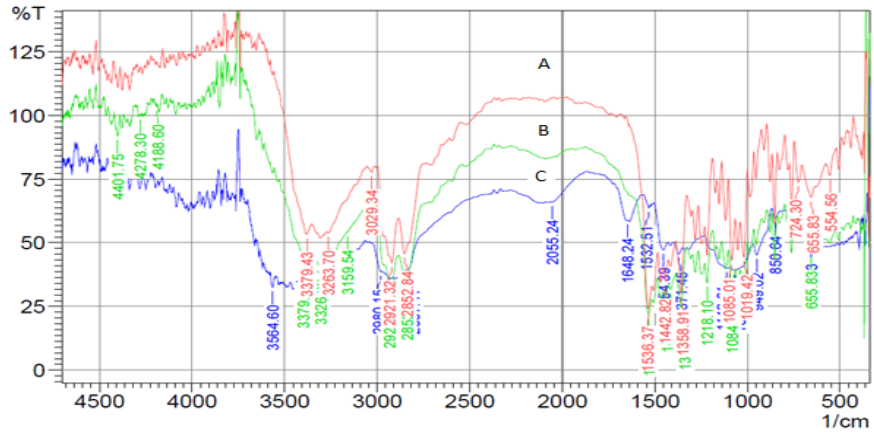


Figure 3. FT-IR Spectra of pure drug of (A) dipyridamole,(B) HPMC K100 M CR and (C) dipyridamole + HPMC K100 M CR.

DSC studies

The compatibility of dipyridamole in formulations had evaluated through DSC analysis. The thermogram was obtained at a heating rate of 10 °C/min and was run from 30 to 300 ºC. It was evident from the DSC profile that dipyridamole exhibited a sharp endothermic peak associated with crystal melting at a temperature of 165.8 °C. This further indicates that the drug was pure dipyridamole. From the DSC analysis, pure drug and formulation mixture was observed that there was no significant interaction between drug and polymers used in the formulation of floating tablets. DSC profile also results in a decrease in the intensity of the peak indicating a partial physical transformation of the drug from crystalline form to amorphous form. The DSC thermogram of pure drug and formulation mixture was presented in **Figure 4**.

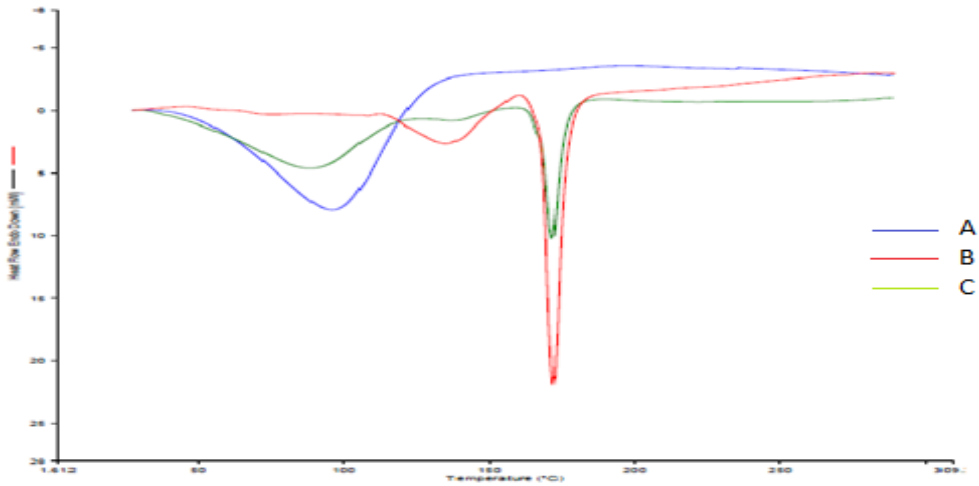


Figure 4. DSC thermogram pure drug of (A) HPMC K100 M CR, (B) dipyridamole and (C) dipyridamole + HPMC K100 M CR.

Kinetic release studies

The mechanism of drug release for all formulations were determined by finding the coefficient of determination (r^2) by applying kinetic model equations (zero order, first order, Higuchi model, Korsmeyers - Peppas model and Hixon-Crowell cubic root model). Further it was found that mechanism of drug release following more towards Higuchi equation i.e., diffusion type of drug release. To confirm the diffusion mechanism, the data were fitted into Korsmeyer-Peppas equation. The 'n' value of Korsmeyer-Peppas model of all formulations obtained in between 0.5-0.895. So the probable mechanism of drug release pattern follows non Fickian diffusion or anomalous diffusion. This deviation may be due to increased drug diffusivity from the matrix by solvent induced relaxation of the polymers.

Table 3: Correlation coefficient values of different mathematical models from F9, F10, F11, F15

Sl. No.	Formulations	Zero order Plot		First order plot		Higuchi model		Korsmeyer Peppas plot			Hixon Crowel plot	
		r^2	K_0	r^2	K_1	r^2	K_H	r^2	n	K	r^2	K
1	**F9**	0.99	3.29	0.97	-0.03	0.90	16.77	0.96	0.95	3.37	0.98	-0.07
2	**F10**	0.99	3.64	0.98	-0.03	0.94	19.00	0.9	0.8	6.31	0.99	-0.09
3	**F11**	0.98	2.86	0.99	-0.02	0.9	15.18	0.99	0.74	6.59	0.99	-0.06
4	**F15**	0.92	3.29	0.9	-0.03	0.99	18.23	0.99	0.55	15.38	0.98	-0.08

DISSCUSION

In the current investigation floating matrix tablets were prepared and evaluated with the aim to sustain the release and enhance the residence time of dipyridamole in stomach region. The main objective of this work was to formulate oral dipyridamole gastro floating tablets to improve the release of drug in the acidic pH. Suitable categories of polymers in combination with $NaHCO_3$ and citric acid were used in the formulation to achieve controlled release as well as to make the formulation buoyant. In the present study, in order to enhance the residential time of dipyridamole, floating tablets of dipyridamole were prepared using several hydrophilic release retardants.

It was found that dipyridamole is compatible with all excipients used in the formulation and

there is no significant interaction between the drug and the excipients used. Optimized formulation F9 containing polymer concentration of HPMC K100M CR and Sodium bicarbonate (25% + 19%) was considered as best formulation with respect to *in vitro* drug release for 24 hrs and total floating time. This result showed that the combination of the polymer optimized the amount of drug release with less lag time and prolonged floating time. Floating lag time of all four formulations showed within 1 min and total floating time was more than 24 hrs. The *in vitro* release profile indicated that Batch (F9) was the most promising formulation as the extent of drug release from this formulation was high as compare to other formulations, which are suitable for sustained release drug delivery system. The *in vitro* drug release studies in stomach pH (acidic) conditions was carried out in 0.01 N HCL (pH≈ 2) buffer for 24 hrs. Formulation Batch F9 shows 99.92% release in 24 hrs, so we concluded that rate of drug release increases in the acidic environment of stomach. Formulation F9 show desirable swelling index. Release kinetic data of all the formulation showed that F9, F10, F11, F15 formulation follows zero order release i.e., release is independent of drug concentration and obeys non Fickian type as n vale is more than 0.5 under K-P model. Stability study was conducted on tablets of Batch F9 at 40 ± 2 °C for one month. Tablets were evaluated for drug release pattern, hardness, floating behavior and *in vitro* drug release. No significant changes were observed in any of the studied parameters during the study period.

CONCLUSIONS

Floating tablets of dipyridamole can be formulated using HPMC K 100 M CR, sodium bi carbonate and citric acid at appropriate concentrations and considered to be promising ingredients for formulating FDDS. Maximum absorption of drugs at gastric pH and drugs showing absorption window at gastric region are suitable candidates to be formulated into floating drug delivery systems (FDDS) or gastro retentive drug delivery systems. Optimized formulation F9 was considered as best formulation with respect to *in vitro* drug release in 24 hrs and total floating time with less lag time (< 10seconds). From the kinetic model fitting study the optimized formulation shows non- Fickian diffusion as the n value is more than 0.5 (i.e., 0.954). Formulation of floating drug delivery systems (FDDS) or gastro retentive drug delivery systems (GRDDS) is a novel approach for drugs showing absorption at gastric pH or drugs showing absorption window at gastric region to enhance their absorption and bioavailability, hence therapeutic efficacy.

References

1. Chugh, I., Nimrata, S., Rana, A.C. and Gupta, S. (2012) Oral sustain release drug delivery systems: An overview. Int Res J Pharm.; 3(5); 57-68.
2. Shweta, A., Javed, A., Ahuja, A., Roop, K. and Sanjula, B. (2005) Floating drug delivery system: A review. AAPS PharmSciTech.; 6 (3); E375-78.
3. Praveen, N., Sheefali, M. and Deepika, S. (2010) Floating systems: A novel approach towards gastro retentive drug delivery systems. Int J Pharm Pharm Sci.; 3(2):2-7.

4. Shaik, F., Vikram, A. and Dhanunjaya, M.A. (2012) Review on floating drug delivery system. Asian J. Res. Pharm. Sci.; 2(2); 46-52.
5. Thakur, S.D., Singh, G., Rehni, A.K., Sood, S. and Arora, S.(2011) Gastroretentive: a controlled release drug delivery system. Asian journal ;4(1); 5-13
6. Patrono,C., Andreotti,F., Arnesen, H., Badimon,L., Baigent, C., Collet,J.P., De Caterina, R., Gulba,D., Huber,K., Husted,S., Kristensen, S.D., Morais,J., Neumann,F.J., Rasmussen,L.H., Siegbahn, A., Steg,P.G., Storey,R.F., deWerf, F.V. and Verheugt, F. (2011) Antiplatelet agents for the treatment and prevention of atherothrombosis. European Heart Journal; 1-13.
7. Eikelboom,J.W., Hirsh,J., Frederick, A., Trevor, P. and Jeffrey, I.(2012) Antithrombotic therapy and prevention of thrombosis, 9th ed: american college of chest physicians evidence-based clinical practice guidelines. CHEST; 141(2); e89S–e119S.
8. Born,G. and Patrono, C.(2006) Antiplatelet drugs. British Journal of Pharmacology; 147; S241–S251.
9. Shivprasad, B. (2008) Development and evaluation of floating-mucoadhesive Dipyridamole tablet. International journal of pharmamedix india; 233-257.
10. Panda, S., Pattnaik, S., Maharana, L. and Girish, B. (2013) Formulation and evalution of zidovudine loaded olibanum resin microcapsules: exploring the use of natural resins as biodegradable polymeric materials for controlled release. Asian J Pharm Clin.; 6(3); 191-96.
11. Chavanpatil, M., Jain,P., Chaudhari,S., Shear, R. and Vavia,P. (2005) Development of sustained release gastroretentive drug delivery system for ofloxacin: *In vitro* and i*n vivo* evaluation. International journal of pharmaceutics; 304; 178-184.
12. Thakkar,S., Hardik, R. and Swamy, V.B.N. (2011) Design and Evaluation of Gastroretentive Floating Tablets of Dipyridamole. Asian Journal; (1).
13. Mahapatra, A.K., Murthy, P.N., Samoju, S. and Mohapatra, A.K. (2014) Tiny Technology Proves Big: A Challenge at Pharmaceutical Sciences, Medicine and Engineering Interface Critical Reviews in Therapeutic Drug Carrier Systems; 31(1); 1-47.
14. Mahapatra, A.K., Murthy, P.N., Swain, R.P., Sravani, Y. and Sagar, G. (2013) Sustained Release Products: A Review on Formulation Technologies and Regulatory Aspects. Research J. Pharm. and Tech; 6(12); 1415-1425.
15. Khosro, A., Sanaz, H. and Yousef, J. (2011) Drug release kinetics and physicochemical characteristics of floating drug delivery systems. Expert Opin; 8(7); 891-903.

A Smart Bioretardant from *Piper betle* L. as a Co-processing Agent used in Chlorpromazine Bio-nano Gel for Brain Specificity via Nasal Mucosa

Deepika Raina*, N.V.Satheesh Madhav

Novel Research Lab, Dehradun Institute of Technology, Bhagwantpura, Dehradun - 248009

Abstract

Blood Brain Barrier (BBB) is highly selective and stringent barrier in human body due to tight junctions between the endothelial cells. It restricts the passage /entry of the foreign molecule on to the brain. Chlorpromazine is a phenothiazine derivative used as anti-psychotic, anti-emetic, & tranquilizer having oral bioavailability of 30-50%. Our work aimed to isolate the biopolymer from the natural edible source and incorporate the drug in to the nanoparticles which were prepared by solvent evaporation method to target to the brain. Nanoparticulate drug delivery is prognosticating as it delivers the drug in close locality of the target tissue. Twenty formulations were prepared ten from the bio-polymer and ten from the synthetic polymer. The formulation prepared by the biopolymer using *piper betle* as co-processing agent (FP2 formulation 1:0.5) displays an R^2 value of 0.9671. According to the release kinetics the best fit model was found to be First Order with Fickian diffusion as the mechanism of drug release. For the synthetic polymer Pullulan gum FPG4(1:5) were the best formulations and their R^2 value were 0.9324 and best fit model were peppas korsmeyer and higuchi matrix and mechanism of release were Anomalous Transport obtained using BITS software. From the above data it can be concluded that the formulation FP2 (1:0.5) prepared from the piper betle biopolymer showed the good *in-vitro* response and hence found to be the best formulation. Appreciable amount of the drug reached to the brain overcoming the BBB which was explored by the pharmacodynamic study bypassing the hepatic metabolism hence increasing the bioavailability.

Key words: *Piper betel,* Phenothiazine, Bioavilability, Nanogels, Chlorpromazine

Introduction

Chlorpromazine [3-(2-chloro-10H-phenothiazin-10-yl)propyl]dimethylamine is a first generation antipsychotic drug. It prevents hallucinations and illusions[(1)] and is also used as anti-psychotic,anti-emetic, & tranquilizer. Like the other drugs in this class, chlorpromazine's antipsychotic actions are believed to be because of long-term versification by the brain to blocking dopamine receptors. Chlorpromazine has various other effects and therapeutic uses like in treatment of singultus, treatment of dementia praecox. Chlorpromazine accomplishes at all levels of the central nervous system- chiefly at subcortical levels-as well as on several organ systems. Chlorpromazine has impregnable antiadrenergic and feebler peripheral anticholinergic activity; ganglionic blocking action is comparatively slender. It also possesses slight antihistaminic and

antiserotonin activity. The limitation with the treatment of chlorpromazine is it requires chronic medication[2] Chlorpromazine induces life threaten which includes seizures, blood bysceaseas, neurolepic, maligant syndrome, temperature disorders, ventricular arrhythmias, jaundice, tradivk dyskinesia which are responsible for discontinuation of chlorpromazine.

Psychosis is a state of hallucination where a person believes/interpret things differently from the reality and is a state of delusion. It interrupts in the perception, thinking emotion and behavior responses. According to the recent study it was figured that 3 in 100 individuals have leastwise one incident of psychosis at some point. Treatment of Psychosis includes the combination of antipsychotic drugs, psychological therapy, and social support.[3]

The betel (*Piper betle*) is the leaf of a vine belonging to the Piperaceae family. The leaf bears Water (85-90%), Proteins (3-3.5%), Carbohydrates (0.5-6.1%), Minerals (2.3-3.3%), Fat (0.4-1%), Fibre (2.3%), Essential oil (0.08-0.2%), Tannin (0.1-1.3%), Alkaloid (arakene). It also contains different vitamins, minerals. It shows various therapeutic activities like antimicrobial activity, gastro protective activity, antioxidant activity, anti-diabetic activity, radio protective activity[4]. The drug is targeted directly to the brain via Trans nasal route bypassing the BBB. The drug is transported via olfactory system or trigeminal nerves into the CNS.[5]

The current research work aimed to target chlorpromazine to brain via trans nasal mucosal route and was achieved by suitably designing bio nano particles loaded with nanosized chlorpromazine using biopolymer as stabilizer cum retardant .Our experimental results reveled that the bio nano-particles displayed significant pharmacodynamic activity upon intra nasal administration to the experimental animals indicates that as appreciable quantity of chlorpromazine reached to the D_2 receptor site may be via olfactory or Trigeminal nerve pathway.

Materials and Method

The drug Chlorpromazine was obtained as a gift sample from GlaxoSmithKline, India. Piper betle was procured from the local market. All other reagents used were of highest purity and analytical grade. Double distilled water was used throughout the experimental work.

Bio-material extraction

The bio-material was isolated from the leaves of piper betle by addition of optimized quantity of non solvent (acetone) by non solvent precipitation method to the aqueous extract and subjected for refrigeration for 24 Hrs. It was recovered by centrifugation at 3500 rpm and dried for the use.
(Indian patent application no. 2373/DEL/2014).

Formulation of Nanoparticles

The polymeric nanoparticles (PNPs) are prepared from biodegradeble polymers in size between 10-1000 nm where the drug is dissolved, trammeled, enclosed or seized to a nanoparticle array.[6]

The solvent evaporation method was used to prepare the Nano-particles of chlorpromazine. In this method the biopolymer/ standard polymer was accurately weighed in different ratios and treated with glycerine (100 µl), glycerine is used as a wetting agent, and then to this slurry distilled water (5 ml) was added and transferred into mechanical stirrer. The drug (10mg) solution was prepared separately with methanol (5 ml). The drug solution was added to the polymeric solution under stirring 4,500 RPM until the formation of nanoparticles for about half an hr. The beaker containing the sample was subjected for 5 cycles of sonication for 3 min. The sample was micro-centrifuged at 5000RPM for 10 mins, and was dried at room temperature for 24 hrs.

Table 1. Formulation of CPZ bio-nanoparticles loaded with *Piper betle* (FP) biopolymer

Formulations	FP1 (1:1)	FP2 (1:0.5)	FP3 (1:10)	FP4 (1:5)	FP5 (1:2)	FP6 (1:3)	FP7 (1:20)	FP8 (1:15)	FP9 (1:7)	FP10 (1:9)
Drug:polymer ratio	1:1	1:0.5	1:10	1:5	1:2	1:3	1:20	1:15	1:7	1:9
Chlorpromazine(mg)	10	10	10	10	10	10	10	10	10	10
***Piper betle* Bio-polymer (mg)**	1	0.5	100	50	20	30	200	150	70	90
Glycerin (µl)	100	100	100	100	100	100	100	100	100	100
Distilled water(ml)	5	5	5	5	5	5	5	5	5	5
Buffer (ml) pH 5.5	5	5	5	5	5	5	5	5	5	5

Table 2. Formulation of CPZ bio-nanoparticles loaded with *pullulan gum (pg)* biopolymer

Formulations	FPg1	FPg2	FPg3	FPg4	FPg5	FPg6	FPg7	FPg8	FPg9	FPg10
Drug:polymer ratio	1:1	1:0.5	1:100	1:5	1:2	1:3	1:200	1:150	1:7	1:9
chlorpromazine(mg)	10	10	10	10	10	10	10	10	10	10
***Pullulan gum*(mg)**	1	0.5	100	50	20	30	200	150	70	90
Glycerin (µl)	100	100	100	100	100	100	100	100	100	100
Distilled water(ml)	5	5	5	5	5	5	5	5	5	5
Buffer (ml)pH 5.5	5	5	5	5	5	5	5	5	5	5

Evaluation

Drug excipient interaction studies

Drug excipient interaction studies showed that *piper betle* biopolymer and CPZ used in varied

ratios, when scanned between 200-800nm by UV Spectroscopy, the maximum absorbance was recorded to be at 258nm. The results showed that there was no significant change in maximum wavelength and also the absorbance's which reveal that bio-polymer is compatible because the source of the polymer is edible, biodegradable and non toxic in nature. Hence the drug was considered to have no interactions with the polymer.

Drug content

Drug content was determined by weighing an equivalent amount (10 mg) of nanoparticles and suspended into 10 ml of acetone; it was kept over for orbital shaking for 24hrs. The 1ml solution was taken and diluted with acetone up to 10 ml, the resultant solution was filtered through .45mm Whatman filter paper. This solution was assayed for drug content by U.V spectroscopy at λmax 258nm.

Entrapment efficacy

To determine the entrapment efficacy in nanoparticles, a weighed amount (10 mg) of nanoparticles was suspended into 10 ml of acetone, it was left over for orbital shaking for 24hrs. The 1ml solution was taken and diluted with methanol up to 10 ml; the resultant solution was filtered through .45mm Whatman filter paper. This solution was assayed for drug content by U.V spectroscopy at λmax 258nm.

Entrapment efficacy was calculated according to the following formula:

$$\text{Entrapment efficacy} = \frac{\text{Amount of drug in nanoparticles} \times 100}{\text{Drug added in nanoparticles}}$$

Nano size range determination by U.V spectroscopic method

It is a novel primilary screening method for nano-size range particles by U.V spectroscopy. Transmittance is based on the concept of Tindal effect, which specify that when light of specified wavelength passes through the media containing particles less than or greater than the specified particle range, the % blocked represents particles beyond the size range whereas the % transmittance is considered that the particles lies above the size range at particular range.[7]

Invitro drug release

All the formulations were evaluated for its invitro release by static and dynamic method using modified M.S appratusand Franz diffusion cell.

***M.S diffusion apparatus*:** In-vitro drug release studies were performed using M.S diffusion apparatus at 37±2 °C. A small piece of egg membrane (2.5 cm) was cut and mounted on the receptor compartment Donor compartment was fixed and temperature of diffusion medium was maintained at 37±1 °C and weighed amount of nanoparticle equivalent to 10mg were placed in the egg membrane in the terminal end of the donor compartment, 7.4 buffer were placed in the

receptor compartment maintained at 37 °C. Sample were completely withdrawn and replaced at appropriate time intervals. The amount of drug release was assessed by measuring the absorbance at 258nm by using double beam U.V Spectrophotometer. The mechanism of chlorpromazine released from the nanoparticles was studied by fitting the dissolution data in different kinetic models by BITS software.

***Franz diffusion*:** A weight amount of nanoparticles equivalent to 10mg were placed in the egg membrane in the terminal end of the donor compartment, 7ml of 7.4 buffers were placed in the receptor compartment maintained at 37^0C under mild agitation using a magnetic stirrer. 2ml sample were withdrawn at appropriate time intervals and diluted to 10 ml, 2ml of buffer in the receptor compartment was immediately replaced. The amount of drug release was assessed by measuring the absorbance at 258nm by using double beam U.V Spectrophotometer.

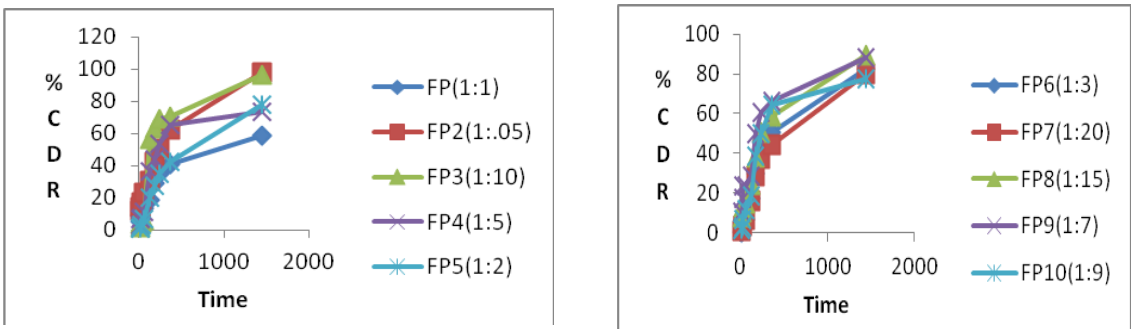


Fig 1 *In-vitro* drug release of chlorpromazine bio-nanoparticles prepared by using paan biopolymer

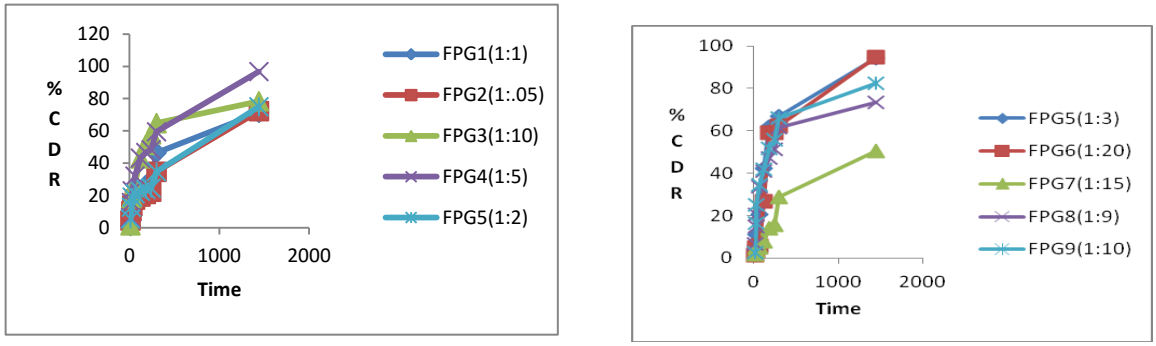
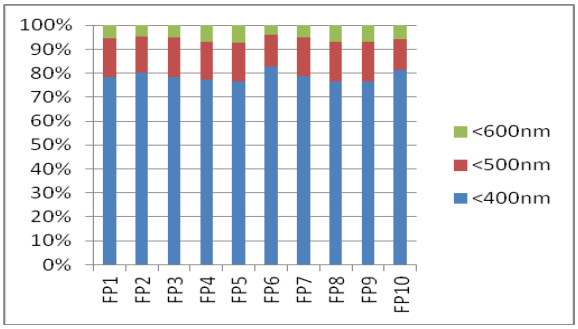


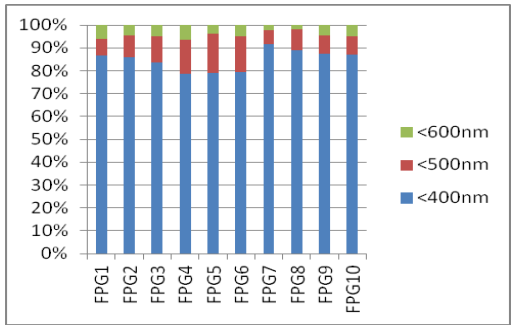
Fig 2 *In-vitro* drug release of chlorpromazine bio-nanoparticles prepared by using Pullulan gum

Percentage range screening of particle of piper betle by U.V method

Stock solution of 1mg/ml was prepared using buffer solution of 7.4 pH for each formulation and from this stock solution 1ml solution was taken and diluted up to 10 ml having concentration of 100µg/ml.the above solution were subjected to the U.V analysis for the screening of % particle size range

Principle: When a light passes through a medium or solution which doesn't contain solute between 200-400 nm size range 100% light gets transmitted. If a solvent contains any solute particles ranging 200-800nm then the particles may absorb a light and proportionally shows absorbance by reducing the %T 0.05 by reducing / blocking 10% of absorbance and 90% of transmittance similarly 1% absorbance with 10 % T and 90% blockage.[7]





Stability studies

The stability studies were done according to the ICH guidelines and showed no physical changes, related to the color, odor, taste etc. The drug content, entrapment efficacy and *in-vitro* release was found to be the same, no significant change was observed. So it can be concluded that the formulation me nanoparticles of CPZ is found to be stable.

Results

Physicochemical properties of the isolated biomaterial

The biomaterial (piper betle) extracted was brown in color, with a characteristic odor, slightly bitter in taste, gritty in texture, color changing point ranging from (200-210 0C) and freely soluble in water, sparingly soluble in acetone, and insoluble in chloroform and dimethyl formamide**.** The biopolymers *piper betle* showed the presence of carbohydrates (Benedict and Molish test) and proteins, were devoid of and starch.

Drug excipient interaction studies

The results showed that there was no significant change in maximum wavelength and also the absorbances which reveal that bio-polymer is compatible because the source of the polymer is edible, biodegradable and non toxic in nature. Hence the drug was considered to have no interactions with the polymer.

Entrapment Efficiency

The drug content was found to be in the range of 0.87±0.02 - 0.68±0.01 for which when compared with the standard polymer was found that the drug content range of the formulations prepared from the biopolymer *piper betle* was significantly greater than that of the standard polymer.

The entrapment efficacy was found to be in the range of 90.1±0.016-88.7±0.019 for *piper betle.*

***In-vitro* drug release by Franz Apparatus (Dynamic Method) at pH 7.4**

All the formulations were subjected to drug release M.S and franz both static and dynamic method. Data was plotted as % of drug in y-axis and time in x-axis then t50% and t80% were calculated. All the release data based on t50% &t-80% data including dispersibility, and particle size the formulation were arranged in decreasing order as mentioned. The drug release pattern for formulations FP1-FP10 was found to be in the order of FP2 > FP3 > FP8 > FP9 > FP6 > FP7 > FP5 > FP10 > FP4 > FP1 showing percentage drug release from 58 to 97 % at the end of 30 hr.

The release kinetics was depicted by BITS software and the best fit model for FP2 (1:0.5) formulation was found to be First Order with Fickian diffusion as the mechanism of drug release. The FP2 (1:0.5) formulation displays an R^2 value of 0.9671. According to the release kinetics the best fit model was found to be First Order with Fickian diffusion as the mechanism of drug release. For the synthetic polymer Pullulan gum FPG4(1:5) were the best formulations and their R^2 value were 0.9324 and 0.9447 best fit model were peppas korsmeyer and higuchi matrix and mechanism of release were Anomalous Transport. From the above data it can be concluded that the formulation FP2 (1:0.5) prepared from the piper betle biopolymer showed the good *in-vitro* response and hence found to be the best formulation

Discussion

Nanoparticles (NPs) could be stimulating prognosis for transnasal drug delivery as they have higher surface area to cover highly vascularised nasal absorptive area furnishing a larger concentration gradient. NPs are used as a sustained drug delivery system. NPs , interacts with mucus to prolong the residence time of drug carrier at the drug absorption sites and protected the entrapped drug from enzymatic degradation until they are absorbed. Therefore, the bioavailability of drug is improved. In the present research work biopolymer were isolated from piper betle. The biomaterials were non-toxic, biodegradable, and edible in nature, which ensured its safe and effective use in the preparation of bionanoparticles

The current research explores the optimized method for designing chlorpromazine loaded bionano gel using biopolymer as retardant cum stabilizer . A biopolymer was isolated from *piper betle* leaf by optimized economic process using addition of anti solvent like acetone to the aqueous extract in order to recover the biopolymer. The biopolymer was characterized for its functional group which indicates that the biopolymer posses inbuilt mucoadhesive property. The biopolymer was devoid of drug excepient due to absorbance of non-reacting functional group considering the compatible nature of the biopolymer. It was used as retardant cum stabilizer for formulating chlorpromazine loaded nanoparticles. Ten different formulations were made of chlorpromazine by varying the concentration of Biopolymer to study the impact of biopolymer on stabilizer and

retarding nature nature.The research results reveled promising encouraging invitro and invivo results of the formulated dosage forms over period of 30 hrs.these formulation were evaluated for pharmacokinetic and pharmacodynamic study in experimental animals . the formulation was administered to observe the pharmacodynamic property. The animals showed significant therapeutic response like drowsiness at 10 times reduced dose level. This activity exerted by the formulation may be due to inter and intra neuronal pathway upon reaching at olfactory bulb and trigeminal nerve endings. It clearly indicated the appreciable amount of the drug reaches to te brain receptor site in order to illicit the pharmacological properties in experimental animals The researchers who are brain targeting the molecules via nasal route can use this biomaterial for formulating micro and nano formulations for nose to brain targeting

References

1. http://www.drugbank.ca/drugs/db00477 (Last accessed on 23/3/15)
2. http://www.drugs.com/pro/chlorpromazine.html (Last accessed on 23/3/15)
3. www.nhs.uk/conditions/Psychosis/Pages/Introduction.aspx (Last accessed on 22/5/15)
4. D. Pradhan, K. A. Suri, Dr. D. K. Pradhan, P. Biswasroy. Golden Heart of the Nature: *Piper betle* L. Journal of Pharmacognosy and Phytochemistry 2013; 1(6): 147-167.
5. A. Mistry, S. Stolnik, L. Illum. Nanoparticles for direct nose-to-brain delivery of drugs. International Journal of Pharmaceutics 2009; 379(1):146-57.
6. B V N Nagavarma, H. K.S.Yadav, A. Ayaz, L.S. Vasudha, H.G. Shivakumar. Different techniques for preparation of polymeric nanoparticles - A review. Asian Journal of Pharmaceutical and Clinical Research 2012; 5(3):16-23
7. H Varley, Practical Clinical Biochemistry. 6th ed. New Delhi: CBS Publishers and Distributers; 2006.