Ind. J. Pharm. Edu. Res., 2024; 58(1s):s316-s324.
https://www.ijper.org

Original Article

Preparation, *in vitro* and *in vivo* Characteristics of Floating *in situ* Gel of Carvedilol Using Semi Synthetic and Natural Polymers to Enhance the Oral Bioavailability

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ABSTRACT

Background/Aim: The purpose of this study was to design and prepare floating *in situ* gel to sustain Carvedilol (CVD) release and enhance oral bioavailability. Various floating *in situ* gel formulations of the CVD were prepared by ionic gelation method. **Materials and Methods:** A systematic approach in the design of the formulations was adopted, were using Hydroxypropyl Methyl Cellulose (HPMC K4M), Hydroxypropyl Methyl Cellulose (HPMC 100LV), Sodium alginate, *Mimosa pudica* seed mucilage and *Limonia acidissima* gum in various concentrations along with gas generating agent (sodium bicarbonate) were investigated for its physicochemical properties (*in vitro* floating behavior, drug release profile, etc.). Subsequently, a final optimization step is involved based on the physicochemical properties to achieve the desired effect. **Results:** Based on the study, the formulation with HPMC K4M, HPMC 100LV, Sodium alginate, and *Mimosa pudica* seed mucilage (F17) showed good floating properties (60 sec floating lag time) with drug release of 96.98 ± 2.1% for 12 hr and the drug release mechanism was found to be zero order (R^2 = 0.894). *In vivo* X-ray studies of F17 in albino rabbits showed a good floating ability up to 8 hr. Bioavailability of optimized and control (CARLOC) was found to be 41.95 ± 0.8892 µg.hr/mL and 26.36 ± 1.1603 µg.hr/mL respectively. The accelerated stability study was performed with optimized formulation and it was observed stable during the study. **Conclusion:** It was concluded that the floating *in situ* gel of Carvedilol developed with natural polymer is suitable for GRDDS to enhance oral bioavailability.

Keywords: GRDDS, *Mimosa pudica* seed mucilage, *Limonia acidissima* gum, *In situ* gel, Carvedilol, HPMC K4M.

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Received: 14-06-2022;
Revised: 18-03-2023;
Accepted: 14-08-2023.

INTRODUCTION

Gastroretentive Drug Delivery System (GRDDS) offers several pharmacokinetic and pharmacodynamic advantages over conventional dosage forms such as maintenance of constant therapeutic levels for prolonged period of time in the stomach thereby minimize the fluctuations in plasma drug concentration that improves bioavailability of drugs with narrow therapeutic index. GRDDS formulations might lower the risk of treatment failure, improve patient compliance by reducing frequency of dose, and administration of total dose.[1,2]

However, GRDDS dosage forms were not only developed to sustain drug release for a specific period of time, but also to prolonging the residence time of the dosage form in the specific site (stomach). The presence of a dosage form in the stomach was an important, especially for drugs that are degraded or metabolized in the small intestine or drugs had local action in the stomach, further for drugs with poor solubility in the small intestine and those with site-specific absorption. Limitations, stomach specific approach might increase the overall gastrointestinal absorption.[3-5]

Approaches to increase the Gastric Residence Time (GRT) of drug delivery systems include floating systems, low-density systems, high-density systems, bioadhesive systems, swelling systems, unfoldable and expandable systems, magnetic systems, and raft forming, biodegradable hydrogel systems. When the bulk density of dosage form is less than that of gastric fluids (1.003 g/cm^3), GRDDS remains floating in the stomach for a longer period of time without reducing the gastric emptying rate.[6-8]

While the system floats on the gastric contents, the drug is released slowly in sustained manner, resulting in an increased

DOI: 10.5530/ijper.58.1s.34

GRT and better control of fluctuations in plasma drug levels. Floating *in situ* gel offers the advantages of (i) increased contact time in the stomach, (ii) more effective absorption and bioavailability of drugs with absorption window in the proximal part of small intestine, and (iii) reduced frequency of dose administered.[9-16]

The main objective of the present study to develop the formulation of Carvedilol floating *in situ* gel and to study the effect of formulation variables on drug release using various concentrations of natural and semi synthetic polymers.

MATERIALS AND METHODS

Materials

Carvedilol was procured from RR Life Sciences, Chennai, India. HPMC K4M, HPMC 100LV, sodium alginate, trisodium citrate, sodium bicarbonate, sodium saccharin was procured from School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu. *Mimosa pudica* seeds and *Limonia acidissima* gum were collected from pachamalai hills, Topsengattupatti, Thuraiyur, India. All the chemicals and reagents used were of analytical grade.

Extraction of *Mimosa pudica* mucilage from seeds

100 g of *Mimosa pudica* seeds were soaked in a beaker containing 500 mL of distilled water for 48 hr, and before the filtration, soaked seeds boiled for few minutes, so that mucilage completely released into distilled water. The aqueous layer was filtered and added equal volume of absolute alcohol.[17,18] Mucilage were precipitated out and dried in hot air oven at temperature < 60°C. The powdered mucilage was pulverized and sieved in sieve # 80. The percentage yield was 13.4%.

Extraction of *Limonia acidissima* Gum

100 g of gum was collected from *Limonia acidissima* trees (injured trunk site). It was dried in an oven at 60°C, milled and passed through sieve #80. Dried gum powder was stirred in distilled water for 6-8 hr at room temperature. This procedure was repeated four more times. In the collected supernatant add equal quantities of acetone with continuous stirring. The precipitated powder was dried at 50-60°C under vacuum. The dried powder was pulverized to remove the clumpy mass.[19] Percentage yield was 87%.

Pre-formulation studies of Carvedilol

The preformulation studies are the initial step for the development of any formulation. It was defined as determination of physical and chemical properties of drug substance alone and combined with the excipients. The main objective of the study was to generate the information that useful to the formulator to develop stable preparations.

Determination of melting point

According to the USP, Carvedilol melting point was determined by using the capillary tube method. A sufficient quantity introduced into the capillary tube to give a short column of 4-6 mm in height. The tube was introduced in the electrical melting point apparatus. The melting point was recorded, which is the temperature at which the last solid particle of Carvedilol in the tube passed into the liquid phase.[20,21]

Determination of solubility

It was practically insoluble with water and soluble in methanol and dimethyl sulfoxide. Sparingly soluble with 95% ethanol and isopropanol.[22,23]

Determination of Carvedilol by Ultraviolet (UV)-visible spectrophotometric method

The drug solution concentration (100 µg/mL) was prepared with 2 mL of methanol and 98 mL of phosphate buffer pH 6.8 and the absorption was measured by Shimadzu UV-1601 UV/visible double beam spectrophotometer. The λ_{max} of Carvedilol was found to be 284 nm.

Drug-excipients compatibility studies

DSC studies of Carvedilol pure drug were compared with the physical mixture of HPMC K4M, HPMC 100LV, *Mimosa pudica* seed mucilage and *Limonia acidissima* gum. The presence of peaks at the expected range confirms that the materials taken for the study are genuine.

Preparation of calibration curve of Carvedilol

Preparation of primary stock solution

100 mg of Carvedilol was dissolved in 10 mL of methanol and 90 mL of phosphate buffer pH 6.8 and transferred in to 100 mL volumetric flask containing 1000 µg/mL concentration of drug.

Preparation of secondary stock solution

1 mL of primary stock solution was taken in 10 mL volumetric flask and made up to the mark with methanol and phosphate buffer pH 6.8 containing 100 µg/mL concentration of drug.

From this, aliquots were prepared and diluted up to the mark to produce 5, 10, 15, 20, 25, 30 µg/mL concentrations and the absorbance was measured at 284 nm using a spectrophotometer.

Preparation of Carvedilol oral suspension

In a beaker, sodium alginate was dissolved in deionized water and adds the trisodium citrate under continuous stirring on a magnetic stirrer at 70°C. Add the remaining polymer and sodium bicarbonate after cooling to below 40°C. In another beaker, the drug was dissolved in methanol and mixed with above polymeric solution under continuous stirring. Finally, a sufficient quantity of

methylparaben, propylparaben (9:1), sweetening agent (saccharin sodium) and flavoring agent (peppermint water) were added.[24-26] It was shown in the Tables 1 and 2.

Formation of *in situ* gel from Carvedilol suspension by the ionic-cross linking method

The formulated suspension containing ion-sensitive polysaccharides, it undergoes a phase transition in the stomach due to interaction with a glucuronic acid blockage in chains of *Mimosa pudica* gum, *Limonia acidissima* gum, and alginic acid undergoes gelation in the presence of divalent cations.[27-29]

Post formulation studies of Carvedilol *in situ* gel system

In situ gels were formulated, evaluated and characterized for following parameters.

Physical Appearance

The naked eye was used to check the clarity of all the prepared *in situ* gel using the black and white background.[30,31]

Determination of pH

The pH was measured by using calibrated digital pH. Accurately 50 mL of suspension was transferred into a 100 mL beaker. The pH meter was dipped into the suspension up to the mark to identify the pH. All the measurements of pH were made in triplicate.[32,33]

In vitro floating study

The floating study was carried out in 500 mL of 0.1N HCl (pH 1.2) in a beaker. Accurately measured 10 mL of suspension was added above buffer. Time period required to formation of gel on the surface of suspension (floating lag time), and the time period of gel retained (total floating time) was measured.[34,35]

In vitro gelation

The gelling capacity of formulation was evaluated by visual method. The prepared *in situ* suspension (1mL) transfer into the test tube containing 5 mL gelation solution and 0.1N HCl (pH 1.2). Gelling capacity was graded based on the gelation time and time for which formed gel retained.[36,37]

+ Gelation after few minutes dispersed rapidly

++ Gelation immediate remains for few hours

+++ Gelation immediate remains for an extended period

Measurement of Rheological properties

The viscosity of *in situ* gelling system was determined with a Brookfield viscometer using 20 mL aliquot of the sample. Measurements were performed by using spindle 16 (low viscous), and the temperature was maintained at 25 ± 1°C.[38]

Determination of drug content

1 mL of suspension (equivalent to 6.25 mg of Carvedilol) was added to 100 mL of methanolic water solution (1:1). From this, 1 mL was transferred into 10 mL standard flask and made up with methanolic water solution up to the mark. The UV absorbance of the sample was determined UV spectrophotometrically at 284 nm.[38]

In vitro drug release studies

The release rate of Carvedilol was determined by using USP apparatus 2 and the paddle speed was 50 rpm. 900 mL of dissolution media (0.1 N HCl) at 37°C ± 2°C was used to study the dissolution behaviors of drug. At a pre-determined time, 5 mL of the sample were withdrawn up to 12 hr. At the same time 5 mL of buffer was replaced to maintain a constant volume. The sample was filtered using a Whatman filter paper and analyzed by using UV spectrophotometer at 284 nm.[39,40]

In vivo radiographic study

The Institutional Animal Ethics Committee of Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram and Chennai-117 approved the protocol for *in vivo* study (IAEC approval number: XXII/VELS/PCOL/05/2000/CPCSEA/IAEC/11-2-2019. *In vivo* study of optimized formulation was performed with New Zealand male albino rabbit by an X-ray imaging method.[41,42] Suspension containing an acceptable limit of Barium sulfate (< 5%) given to an albino rabbit using an animal pet piller with 30 mL of water. The rabbit was placed in a supine posture to check the gel position in the gastric region by X-ray machine at the pre-determined time intervals. During the experiment, the animal was fasted overnight with free access to water. An X-ray image was made before the administration of suspension to ensure that the absence of any contrast media in the stomach. Gastric radiography was carried out at 0, 1, 2, 4, and 8 hr using an X-ray machine.

Pharmacokinetic study

After giving a one-week washout period, the same male albino rabbit was used for pharmacokinetic study. The rabbits were fasted overnight before the dose administration. Animals were held in rabbit restrainers during blood sampling, and they were conscious throughout the experiments.

Sample Collection and Analysis: After the administration of optimized formulation, a blood sample (1 mL) was collected at periodic time intervals (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hr) from the marginal ear vein of the rabbit.[43,44] It was transferred to the Eppendorf tube containing 10 µL of 5.0% (w/v) EDTA solution to avoid clotting and samples were centrifuged at 5,000 rpm to separate the plasma from RBC. This procedure was repeated with two more times after a given resting period. At the time of analysis, 0.5 mL plasma sample and the 0.5 mL of

acetonitrile was mixed and vortexed for efficient mixing for 2 min and centrifuged at 3,000 rpm for 10 min. After centrifugation, 0.2 mL of supernatant added with 0.5 mL of mobile phase. Precisely, 100 µL was injected into the HPLC column and measured at 284 nm. This procedure was repeated two more times to know the mean values and the peak areas were used for quantitative analysis.[45,46] Data treatment and statistics: Standard non-compartmental pharmacokinetic parameter was calculated by using the software (WinNonlin/Phoenix software).

Stability study

Accelerated stability of optimized formulation was performed at controlled temperature ((40 ± 2°C) and humidity (75% RH) for three months. Samples were removed periodically and evaluated for their pH, viscosity, drug content, *in vitro* gelling properties, floating lag time, total floating time, and *in vitro* drug release.

RESULTS AND DISCUSSION

Drug-Excipients Compatibility Studies

The DSC studies of Carvedilol pure drug were compared with the physical mixture of CVD, HPMC K4M, HPMC 100LV, *Mimosa pudica* seed mucilage and *Limonia acidissima* gum. It was observed that there were no appearances or disappearances of any characteristic peaks. The presence of peaks at the expected range confirms that the materials taken for the study are genuine.

Preformulation studies of Carvedilol

Melting point

The melting point of Carvedilol was determined, and it was found to be in the range 114-116°C, which was complied with I. P. standards.

Solubility

Solubility of the drug was determined, and it was found to be Carvedilol was practically insoluble in water, soluble in methylene chloride, freely soluble in dimethyl sulfoxide, sparingly soluble in isopropanol and ethanol and slightly soluble in ethyl ester.

Determination of λ_{max} of Carvedilol by UV visible spectrophotometric method

The absorption was measured by Shimadzu UV-1601 UV/Vis double beam spectrophotometer. The λ_{max} of Carvedilol was found to be 284 nm.

Standard calibration curve of carvedilol

The standard calibration curve of Carvedilol was determined by plotting concentration versus absorbance (nm) at 284 nm, and it was observed in the range 5-30 (µg/mL).

Differential Scanning Calorimetry study

Differential Scanning Calorimetric study of pure drug and the physical mixture were performed. Since the absorption peaks of the pure drug were detected even after studying with physical mixtures. It was concluded no drug-polymer interaction was observed during the thermal study.

In vitro evaluation of Carvedilol floating *in situ* gel

Determination of pH

The pH of Carvedilol suspension was determined, and it was observed that 6.8 to 7.2 and it is shown in Tables 3 and 4.

Determination of drug content

All batches of drug content of Carvedilol oral suspension were determined and observed in the range of 95-99%. It was observed uniform drug content in all batches.[39,40] and it is shown in Tables 3 and 4.

Formation of *in situ* gel from Carvedilol suspension by the ionic-cross linking method

Ion-sensitive polysaccharides in formulated suspension were undergoes a phase transition in the presence of various ions (Ca^{2+}, Mg^{2+}, and Na^{+}) in the stomach. It was observed that gel formed immediately and remains prolonged period of time.

In vitro floating studies of Carvedilol floating *in situ* gel

Floating behaviors of all batches were determined. A formulation with HPMC grades has shown a good floating behavior due to their low density. It was observed that formulation containing natural polymers do not show any floating tendency, but they enhance the floating by their swelling properties. Combined semi synthetic and natural polymers shown satisfactory floating lag time and total floating time in the range of 60-120 sec, and >12 hr respectively. Based on the study, it was observed that formulation with HPMC shown satisfactory floating behaviors.[36,37] and it is shown in Tables 3 and 4.

In vitro Gelation study of Carvedilol floating *in situ* gel

The gelling tendency of all formulations was determined, and it was observed that that formulation containing synthetic and natural polymer shown a good gelling tendency[34,35] and it is shown in Tables 3 and 4.

Rheological studies of Carvedilol floating *in situ* gel

The viscosity of all formulations was determined by using the brook field viscometer. It was observed that all batches have satisfactory flow properties and are viscous enough due to added natural and synthetic polymers.[45,46]

In vitro drug release studies of Carvedilol floating *in situ* gel

The *in vitro* drug release study was performed with 900 mL of 0.1N HCl and it was observed that all batches showed satisfactory drug release, and Formulation (F17) was shown a good drug release (97%) and drug released sustained manner for >12 hr due to added synthetic and natural polymer[45,46] and it is shown in Tables 5-7 and Figures 1-3.

Drug release kinetics of Carvedilol floating *in situ* gel

Drug release behaviors of all formulations were determined, and it was observed that all the formulations followed a diffusion-controlled mechanism.[40] Optimized formulation F17 showed zero-order drug release and non-Fickian diffusion mechanism.

In vivo radiographic studies of Carvedilol floating *in situ* gel

The radiographic studies were performed with optimized formulation (F17), in which the drug was replaced with the same amount of Barium sulfate while all other ingredients were kept constant. The suspension was given orally to the rabbit using animal pet piller; radiographic images were taken at different intervals of 0, 1, 2, 4, and 8 hr. The images are shown in Figure 4.

It was observed that formed *in situ* gel were visible in the stomach after its oral administration. Dense images were seen at 1 hr, but, as time passed, the images became lighter due to distribution and scattering of gel within the GI region. The radiographic images indicated that the formed *in situ* gel had retained successfully in the stomach >8 hr.[43,44]

Bioavailability studies of Carvedilol floating *in situ* gel

In vivo studies were performed with the same albino rabbit which was utilized in X-ray study. After given a sufficient washout period, study was performed with optimized test Formulation (F17) and the Conventional Tablet (CARLOC) orally administered to animal to quantify the plasma drug concentration. The different pharmacokinetic parameters were determined by applying the non-compartment model (WinNonlin software) and the values were shown in Table 8. The plasma concentration-time profile of CARLOC and F17 were represented in Figure 5.

T_{max} and C_{max} of the CARLOC and the optimized Formulation (F17) after its oral administration were found to be 1hr and 5.463

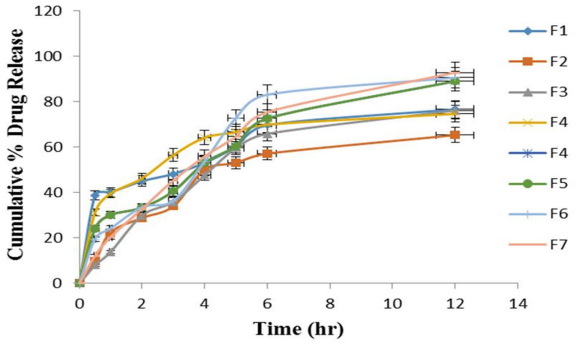

Figure 1: Cumulative % drug release of Carvedilol *in situ* gel (F1-F7).

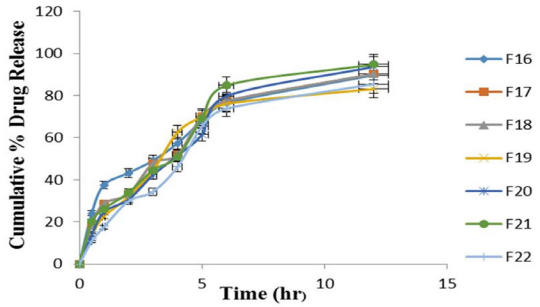

Figure 3: Cumulative % drug release of Carvedilol *in situ* gel (F16-F22).

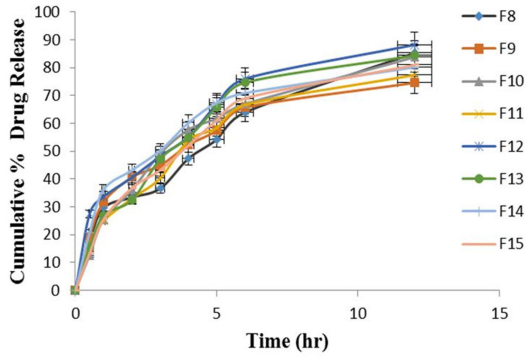

Figure 2: Cumulative % drug release of Carvedilol *in situ* gel (F8-F15).

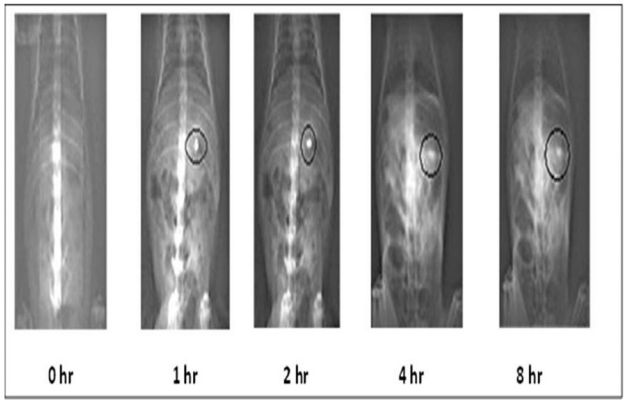

Figure 4: X-ray images of optimized Carvedilol *in situ* gel (F17) before and after the administration.

Table 1: Composition of Carvedilol oral suspension (F1-F11).

Ingredients (% w/v)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Carvedilol (6.25mg/5mL)	125	125	125	125	125	125	125	125	125	125	125
Sodium alginate (%)	1	1	1	1	1	1	1	1	1	1	1
Sodium bicarbonate (%)	1	1	1	1	1	1	1	1	1	1	1
Trisodium citrate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Mimosa pudica gum	0.25	0.5	0	0	0	0	0	0	0.125	0.25	0
Limonia acidissima gum	0	0	0.25	0.5	0	0	0	0	0	0	0.125
HPMCK4M	0	0	0	0	0.25	0.5	0	0	0.125	0.25	0.125
HPMC100LV	0	0	0	0	0	0	0.25	0.5	0	0	0
Methyl and propylparaben	9.0:1.0	9.0:1.0	9.0:1.0	9.0:1.0	9.0:1.0	9.0:1.0	9.0:1.0	9.0:1.0	9.0:1.0	9.0:1.0	9.0:1.0
Sodium saccharin (mg)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Peppermint water	1	1	1	1	1	1	1	1	1	1	1
Distilled water (mL)	100	100	100	100	100	100	100	100	100	100	100

Table 2: Composition of Carvedilol oral suspension (F12-F22).

Ingredients (%w/v)	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22
Carvedilol (6.25mg/5mL)	125	125	125	125	125	125	125	125	125	125	125
Sodium alginate (%)	1	1	1	1	1	1	1	1	1	1	1
Sodium bicarbonate (%)	1	1	1	1	1	1	1	1	1	1	1
Trisodium citrate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Mimosa pudica gum	0	0.125	0.25	0	0	0.08	0.166	0	0	0.0625	0.125
Limonia acidissima gum	0.25	0	0	0.125	0.25	0	0	0.08	0.166	0.0633	0.125
HPMCK4M	0.25	0	0	0	0	0.08	0.166	0.08	0.166	0.063	0.125
HPMC100LV	0	0.125	0.25	0.125	0.25	0.08	0.166	0.08	0.166	0.063	0.125
Methyl and propyl paraben	9.0:1.0	9.0:1.0	9.0:1.0	9.0:01	9.0:1.0	9.0:1.0	9.0:1.0	9.0:1.0	9.0:1.0	9.0:1.0	9.0:1.0
Sodium saccharin (mg)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Peppermint water	1	1	1	1	1	1	1	1	1	1	1
Distilled water (mL)	100	100	100	100	100	100	100	100	100	100	100

Table 3: Determination of pH, Drug content, FLT, TFT, Gelling capacity of all formulations (F1-F11).

F.C[a]	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
pH*	7.1± 02	6.8 ± 0.3	7.1± 0.1	7.2 ± 0.2	7.2 ± 0.1	6.8 ± 0.4	6.7 ± 0.3	7.2 ± 0.3	6.8 ± 0.3	7.2 ± 0.4	7.2 ± 0.3
D.C*	95 ± 0.1	99.6 ± 0.08	96.8 ± 0.02	96 ± 0.32	96.8 ± 0.2	99.8 ± 0.1	94.5 ± 0.1	97.3 ± 0.7	96.4 ± 0.1	96.4 ± 0.5	96 ± 0.7
FL[b] (Sec)	Not Floating	Not Floating	Not Floating	Not Floating	Not Floating	Not Floating	90	60	120	100	90
TFT[c] (Hrs)	-	-	-	-	-	-	>12	>12	>12	>12	>12
GC[d]	+	+	+	+	+++	+++	+++	+++	+++	+++	+++

Table 4: Determination of pH, Drug content, FLT, TFT, Gelling capacity of all formulations (F12-F22).

F.C[a]	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22
pH*	7.2 ± 0.2	6.8 ± 0.3	7.2 ± 0.1	7.2 ± 0.3	7.2 ± 0.2	6.8 ± 0.3	7.1 ± 0.8	7.2 ± 0.6	7.1 ± 0.3	7.2 ± 0.2	7.2 ± 0.1
D.C*	94.4 ± 0.5	96.4 ± 0.4	98.4 ± 0.4	97.8 ± 0.5	97.2 ± 0.8	98.6 ± 0.5	98 ± 0.64	94 ± 0.12	98.4 ± 0.6	95.4 ± 0.8	96.9 ± 0.9
FLT[b] (Sec)	60	120	100	120	100	60	100	90	109	120	110
TFT[c] (Hrs)	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12
GC[d]	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++

[a]FC indicates Formulation code, [b]FLT indicates Floating lag time, [c]TFT indicates Total floating time, [d]GC indicates Gelling capacity, *DC indicates Drug content, + indicates gelation in few mins, ++ indicates gelation immediately, +++ indicates gelation immediate and remains for extended period. *Indicates each formulation was in analyzed triplicate (n = 3).

Table 5: Cumulative % drug release of Carvedilol *in situ* gel (F1-F8).

TIME (Hrs)	F1	F2	F3	F4	F5	F6	F7	F8
0.5	38.71 ± 0.55	29.7 ± 0.05	27.99 ± 0.67	31.25 ± 1.04	24.19 ± 0.52	19.56 ± 0.58	12.20 ± 0.46	17.9 ± 0.23
1	40.21 ± 0.81	32.54 ± 0.43	33.91 ± 1.02	39.59 ± 0.44	30.19 ± 0.85	24.27 ± 0.56	20.45 ± 0.75	29.63 ± 0.45
2	45.03 ± 0.56	48.84 ± 0.91	49.92 ± 0.35	46.24 ± 1.18	33.37 ± 085	33.54 ± 0.25	32.85 ± 0.79	33.39 ± 2.65
3	48.24 ± 0.11	53.98 ± 0.56	55.93 ± 2.89	56.51 ± 1.52	40.61 ± 0.29	36.38 ± 0.29	45.38 ± 0.49	36.61 ± 2.89
4	53.13 ± 0.19	58.09 ± 0.34	67.71 ± 0.66	64.21 ± 1.4	53.11 ± 0.26	53.79 ± 0.12	55.99 ± 0.84	47.37 ± 1.04
5	70.28 ± 0.14	63.01 ± 0.58	79.61 ± 1.98	76.63 ± 0.99	60.54 ± 0.52	62.87 ± 2.1	64.67 ± 0.57	54.18 ± 0.89
6	97.71 ± 0.24	91.16 ± 1.73	95.9 ± 0.99	89.94 ± 1.22	72.64 ± 0.21	73.12 ± 2.8	75.47 ± 0.12	63.96 ± 1.56
12	-	-	-	-	82.64 ± 0.21	79.12 ± 2.8	85.47 ± 0.12	81.307 ± 0.58

Table 6: Cumulative % drug release of Carvedilol *in situ* gel (F9-F16).

TIME (Hrs)	F9	F10	F11	F12	F13	F14	F15	F16
0.5	19.29 ± 0.47	12.68 ± 0.56	16.30 ± 0.42	27.51 ± 0.69	15.49 ± 0.42	20.83 ± 2.4	13.34 ± 0.78	24.04 ± 2.1
1	31.82 ± 1.05	25.49 ± 0.47	25 ± 1.25	33.68 ± 0.42	27.01 ± 0.52	36.19 ± 0.89	25.61 ± 0.86	37.46 ± 0.54
2	40.63 ± 2.45	36.12 ± 0.54	33.02 ± 0.46	40.57 ± 0.58	32.44 ± 0.72	43.181 ± 0.45	36.79 ± 0.87	43.26 ± 0.68
3	44.89 ± 0.54	49.16 ± 0.59	40.33 ± 0.75	48.17 ± 0.12	47.23 ± 0.58	50.23 ± 0.17	43.22 ± 0.47	49.11 ± 0.24
4	52.27 ± 1.78	57.72 ± 0.478	53.92 ± 1.5	55.2 ± 0.12	54.92 ± 0.48	60.19 ± 0.25	52.14 ± 0.78	57.35 ± 0.78
5	57.41 ± 1.04	61.75 ± 0.12	58.33 ± 1.9	66.87 ± 2.8	65.79 ± 0.85	67.41 ± 0.36	61.15 ± 0.49	67.99 ± 0.39
6	65.68 ± 0.04	66.92 ± 0.88	66.66 ± 2.78	76.04 ± 2.8	74.7 ± 0.89	70.9 ± 0.41	69.03 ± 0.42	76.4 ± 0.74
12	78.6 ± 0.99	83.81 ± 3.24	77.39 ± 2.45	88.23 ± 0.47	84.38 ± 0.59	80.19 ± 0.84	80.98 ± 1.7	89.7 ± 0.26

± 0.8296 µg/mL and 3hr and 3.45 ± 0.0714 µg/mL respectively. The lowest C_{max} and highest T_{max} of F17 compared to CARLOC were observed, and it was suggested that the release of drug from the floating *in situ* gel was prolonged and sustained, these increases the duration of drug in systemic circulation for >12 hr. The AUC and MRT of F17 were observed 41.95 ± 0.8892 µg.hr/mL and 8.56 ± 0.8689 hr. 1-Fold increased bioavailability and MRT of the F17 were observed than that of CARLOC 26.36 ± 1.1603 µg.hr/mL and 3.71 ± 1.2578 hr.[43,44] Thus, we may infer that formulated GRDDS (Carvedilol floating *in situ* gel), extends the bioavailability and reduces the frequency of administration.

Stability study of Carvedilol suspension

Accelerated stability study of optimized Formulation (F17) performed under controlled environment condition (40°C ± 2°C / 75% ± 5% RH) as per ICH guidelines. The samples were withdrawn at intervals of one, two, and three months and analyzed for their pH, flow properties, gelling capacity, floating lag time, and total floating lag time, drug content and *in vitro* drug release.

Table 7: Cumulative % drug release of Carvedilol *in situ* gel (F17-F22).

TIME (Hrs)	F17	F18	F19	F20	F21	F22
0.5	18.45 ± 0.81	18.45 ± 2.1	13.89 ± 0.14	13.86 ±0.47	20.04 ± 2.2	10.85 ± 2.4
1	28.76 ± 0.17	28.76 ± 1.8	22.57 ± 0.26	24.71 ± 20	26.18 ± 2.1	17.6 ± 2.8
2	33.2 ± 0.42	33.20 ± 2.0	31.88 ± 0.85	30.62 ± 2.8	33.86 ± 0.65	29.84 ± 2.1
3	47.82 ± 0.14	47.82 ± 0.5	44.48 ± 0.47	42.26 ± 2.7	44.58 ± 0.84	34.36 ± 3.1
4	51.86 ± 0.74	51.86 ± 0.21	62.54 ± 0.54	51.5 ± 0.14	50.96 ± 0.57	46.16 ± 0.47
5	69.22 ± 0.29	70.22 ± 2.5	70.1 ± 0.3	61.46 ± 0.56	69.23 ± 0.47	65.31 ± 0.26
6	81.45 ± 0.45	77.45 ± 2.7	76.13 ± 0.74	79.7 ± 0.14	84.79 ± 0.21	73.80 ± 0.76
12	96.98 ± 2.1	92.288 ± 2.9	83.16 ± 0.18	93.67 ± 0.16	94.77 ± 0.47	85.48 ± 0.42

Table 8: Pharmacokinetic study of optimized formulation F17 and Control (CARLOC) Tablet.

Parameter	Control (CARLOC) Tablet	Carvedilol *in situ* gel (F17)
C_{max} (mcg/mL)	5.4631 ± 0.8296	3.45 ± 0.0714
T_{max}	1	3
AUC_{0-12} (mcg.h/mL)	26.36 ± 1.1603	41.95 ± 0.8892
$T_{½}$	2.57 ± 0.2494	5.93 ± 0.8313
MRT	3.71 ± 1.2578	8.56 ± 0.8689

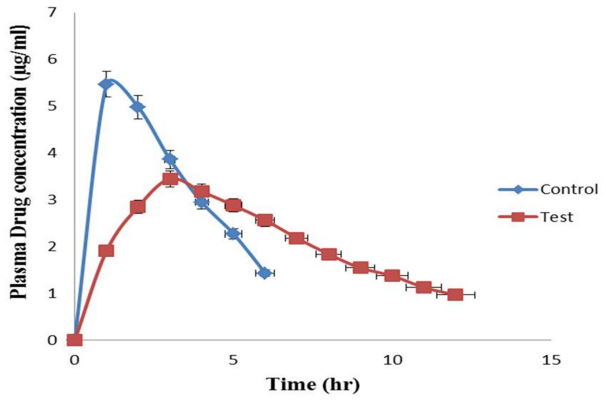

Figure 5: Pharmacokinetic study of optimized Carvedilol *in situ* gel (F17) and control (CARLOC) tablet.

Based on the study, it was observed that there were no significant changes in the optimized Carvedilol suspension (F17) at the end of the three-months.[45]

CONCLUSION

Sustained release gastroretentive floating *in situ* gel of Carvedilol were successfully prepared with HPMC K4M, HPMC 100LV, *Mimosa pudica* seed mucilage and *Limonia acidissima* gum. It was observed that acceptable floating lag time (60 sec) and total floating time (> 12 hr) for Formulation (F17). Based on results, the release mechanism of optimized Formulation F17 follows non-Fickian diffusion and offers optimum drug release and good floatancy. X-ray imaging of F17 in albino rabbits showed the good floatability in gastric region up to 8 hr and the results of *in vitro* and *in vivo* bioavailability studies proved that the optimized Formulation F17 showed enhanced bioavailability. Finally, it was concluded that the developed Carvedilol floating *in situ* gel could be successfully applied to enhance the bioavailability. However, further *in vivo* study with healthy volunteers is necessary to confirm the enhanced bioavailability and therapeutic efficacy.

ACKNOWLEDGEMENT

The authors acknowledge the School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Chennai, Tamil Nadu, India and Sriman Suresh Dada Jain College of Pharmacy, Nashik, Maharashtra for their support and encouragement to carry out the research work.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

ABBREVIATIONS

AUC: Area under the curve; **Ca^{2+}:** Calcium ions; **CVD:** Carvedilol; **EDTA:** Ethylene diamine tetra acetic acid; **FLT:** Floating lag time; **GRT:** Gastric residence time; **GRDDS:** Gastro retentive drug delivery system; **GC:** Gelling capacity; **HPLC:** High performance liquid chromatography; **HPMC:** Hydroxy propyl methylcellulose; **IAEC:** Institutional animal ethical committee; **Mg^{2+}:** Magnesium ions; **MRT:** Mean residence time; **RBC:** Red blood cell; **Na+:** Sodium ions; **TFT:** Total floating time; **UV:** Ultra violet.

SUMMARY

In this work *mimosa pudica* seed mucilage and *limonia acidissima* gum floating *in situ* gel containing carvedilol were formulated using ionic gelation method and characterized for DSC, *in vitro*

floating behavior and drug release. The optimized formulation F17 showed good floating properties (60 sec floating lag time) with drug release of 96.98% for 12 hr and the drug release mechanism was found to be zero order. The *in vivo* X-ray studies of F17 in albino rabbits showed a good floating ability (8 hr) and the bioavailability was found to be 41 µg.hr/mL The floating *in situ* gel of Carvedilol with natural polymers was able to sustain the release and enhance the oral bioavailability.

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Cite this article: Ananthakumar R, Kumar SS, Chitra K. Preparation, *in vitro* and *in vivo* Characteristics of Floating *in situ* Gel of Carvedilol Using Semi Synthetic and Natural Polymers to Enhance the Oral Bioavailability. Indian J of Pharmaceutical Education and Research. 2024;58(1s):s316-s324.