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FORMULATION DEVELOPMENT AND *IN-VIVO* RADIOGRAPHIC STUDIES OF DILTIAZEM HYDROCHLORIDE LOADED FLOATING CAPSULES PREPARED BY MODIFIED PULSINCAP TECHNOLOGY

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ABSTRACT: Oral extended release formulation with site-specific drug delivery formulations has been of greater importance in the field of drug delivery system to achieve adequate therapeutic results. The concept of novel gastro-retentive drug delivery system aroused due to site specificity behavior of the drug concerning drug absorption and solubility. The objective of the research work is to develop and evaluate gastro-retentive diltiazem hydrochloride modified pulisincap floating capsules. Diltiazem hydrochloride modified pulsincap floating capsules were formulated by filling the drug-polymer and diluents mixture in cross-linked hard gelatin capsules. Total of 15 formulations was developed and evaluated for pre capsule filling and post capsule filling parameters. Before development of formulation with different polymers, the cross-linked hard gelatin capsules were filled with only pure drug to carry out the diffusion studies of the drug through the cross-linked hard gelatin capsules among the developed, formulation containing carbopol 934p, *i.e*. F12 formulation was considered to be optimized based on its *in-vitro* drug release data and have shown the *in-vitro* drug release of 97.64 ± 3.45% at the end of 12th h, *in-vitro* drug release data were fitted into different kinetic models, optimized formulation has shown the zero order drug release with a mechanism of non-fickian diffusion, radiographic studies confirmed that optimized formulation with $BaSO_4$ administered to the healthy volunteer with food have shown more gastric residence time than that of the optimized formulation with $BaSO_4$ administered to the healthy volunteer in fasting condition.

INTRODUCTION: Diltiazem HCl is an active pharmaceutical compound that absorbs maximum specifically from the upper part of the gastrointestinal tract (GI) and poorly absorbed from the lower part of the GI tract, and it also shows the pH-dependent solubility, *i.e*., solubility is good at lower pH (stomach), solubility decreases with an increase in pH (*i.e.*, lower part of the GI tract), these factors affect the extent of absorption of drug from the conventional oral controlled dosage forms, as the dosage from crosses the upper part of gastrointestinal tract, unabsorbed drug causes impaired therapeutic effect, increases frequency of dose administration and contributes to more side effects.

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Therefore there is a need for developing a gastro-retentive dosage form that ensures the control of the delivery of diltiazem HCl within the solubility and absorption window. There is a developing requirement for the advancement of a pharmaceutical formulation that could enhance formulation performance and defeat the deficiencies of new and existing medication moieties, such as their site-specific solubility and membrane permeability [1]. Conventional oral controlled dosage forms suffer from mainly two adversities, short gastric retention time and unpredictable gastric emptying time.

A relatively brief GI transit time of most pharmaceutical formulation impedes the formulation of single daily dosage forms. Altering the gastric emptying can overwhelm these problems. Therefore it is desirable to develop the formulation that retains in the stomach for a maximum time which is desirable for some drugs that show maximum solubility or absorption from the upper part of gastrointestinal tract [2, 3]. Apart, due to the enhanced gastric residence time, these can also be formulated such that they can be either sustain or control the release of the drug from the formulations. This results in increasing the bioavailability as well as decreasing the frequency of the administration of formulation to the patient.

Several approaches [4] have been attempted and developed in the development of the gastro-retentive systems [5] they are mucoadhesive delivery systems that adhere to mucosal surface of the stomach, swelling and expanding delivery systems, these formulations rapidly increase in size greater than the pyloric sphincter so they can be retained in the stomach until the dosage form size was reduced to the size of pyloric sphincter and floating delivery systems, this formulation floats on gastric fluids. Diltiazem HCl is active pharmaceutical compound that absorbs maximum specifically from upper part of gastrointestinal [6] tracts and poorly absorbed from lower part of GI tract, and it also shows the pH-dependent solubility, *i.e.*, solubility is good at lower pH (stomach), solubility decreases with increase in pH (*i.e.*, lower part of GI tract), and due short half life[7] these factors affects the extent of absorption from the dosage form as the dosage from crosses the upper part of gastrointestinal tract.

The unabsorbed drug causes impaired therapeutic effect increases the frequency of dose administration and contributes to more side effects. Therefore there is a need for developing a gastro-retentive dosage form that ensures the control of delivery/retention of diltiazem HCl containing dosage form within the solubility and absorption window. In this research, an attempt was made and succeeded in developing a novel extended-release floating capsules of diltiazem hydrochloride with modified pulsincap technology, for this the solubility of capsules was reduced by treating the capsules with cross-linking agent formaldehyde.

Capsules used in the present research work were 00 sized. The polymer (HPMC K4M, HPMC K15M, HPMC K100M, carbopol 934P, sodium carboxymethyl cellulose), diluents (microcrystalline cellulose), glidant (talc), lubricant (magnesium stearate) and drug mixture was subjected to different pre-formulation evaluation, mixture of drug-polymer, glidant and lubricants was filled into 00 sized crosslinked hard gelatin capsules and these were subjected to post-formulation evaluation test, selection of optimized formulation was done based on *in-vitro* dissolution results and kinetic data obtained from dissolution data. Optimized formulation was screened for an *in-vivo* gastric resident time by radiographic studies.

MATERIALS: Diltiazem HCl obtained as a gift sample from obtained from Cipla Ltd., Mumbai, India; hydroxypropyl methylcellulose K4M, hydroxypropyl methylcellulose K15M, hydroxypropyl methylcellulose K100M, carbopol 934P obtained as a gift sample From Euro Drugs, Hyderabad.

METHODOLOGY:

Pre-formulation Studies: The powder blend of all formulations was evaluated [8, 9] for bulk density, tapped density, compressibility index, Hausner's ratio and angle of repose.

Drug-Excipient Compatibility Study:

A) Fourier Transform Infrared Spectroscopy (FT-IR) Studies: FT-IR studies were performed for drug and the optimized formulation using FT-IR (Bruker., India). The samples were analyzed between wave numbers 4000 and 400 cm^{-1}. FT-IR

spectral analysis of diltiazem HCl and excipients were carried out to investigate the changes in chemical composition of the drug after combining it with the excipients[10.] The pure drug, the mixture was correctly mixed and placed under Bruker FT-IR scanned in wavelength. The spectra were analyzed and interpreted.

B) Differential Scanning Calorimetry (DSC): Screening of pure drug and optimized formulation for confirming the compatibility was done by performing DSC using Universal DSC instrument of Universal V4. 5A TA Instrument, with Indium or zinc standards, was used to calibrate the DSC temperature [11] and enthalpy scale. The sample of granules was hermetically sealed in an aluminum pan and heated at a constant rate of 10 °C/min, over a temperature range of 0 °C to 300 °C. The inert atmosphere was maintained by purging nitrogen gas at the flow rate of 50 ml/min

Scanning Electron Microscopy (SEM): Morphological difference between the regular hard gelatin capsules and the cross-linked (formalin treated) capsules were studied by scanning electron microscope (SEM). Capsules were examined by JEM-1200 EX II electron microscope [12] (Jeol, Tokyo, Japan) equipped with an EM-ASID 11 Scanning Image Observation Device using secondary electron imaging.

Preparation of Crossed-linked Hard Gelatin Capsules: Hard gelatin capsules were taken, and their body was separated from the Cap and placed in Buchner funnel. 25 ml of (37%) [13] formaldehyde was taken in a conical flask. Capsules were kept in Buchner funnel was inserted into a conical flask and sealed with aluminum foil shown in the **Fig. 1**.

The body of the capsule was made to react with vapors of cross-linking agent (Formaldehyde). These treated capsules were kept in a hot air oven for 1-3 h, and temperature of 50 °C was maintained during this period, this facilitates the unreacted formaldehyde will react with the amino acids in gelatin capsules, and they were dried to facilitate the removal of any residual formaldehyde [14]. Thus processed capsules are safer for administration.

FIG. 1: TREATING OF CAPSULES WITH CROSS-LINKING AGENT

Optimization of Exposure Time of Hard Gelatin Capsules to Crosslinking Agent: Hard gelatin capsules are water soluble. When the hard gelatin capsules come in contact with water they dissolve in less than an hour. The objective behind the formulating the HBS system by using capsules is due to, the ability of capsule to float in water or gastric fluid. However, gelatin capsules are unable to extend the release of the drug due to its solubility in water or gastric fluid and dump the content of the capsules in the stomach these are unable to extend the release of the drug similar to conventional formulations. The development of any GRDDS systems is to restrict the dosage form to the stomach along with the extending the release of drug to reduce the frequency of formulation administration. Restricting the dosage form to the stomach is mainly because of drugs which show the site-dependent absorption, solubility, stability, action, which affect the bioavailability of the drug in turn which reduced the therapy efficiency.

Here the hurdle is the solubility of capsules, though the polymer filled in the capsule can swell and form a porous plug which controls the drug release and due to its porous nature it floats in the gastric fluid. However, due to the high solubility of gelatin, capsules are unable to provide enough time for the polymer to swell which in turn affect the gastric residence time of formulation and extending

the drug release. This is due to the dispersion of the polymer-drug in the gastric fluid. Which subsequently moves to the lower part of the GIT without being absorbed or may expose to conditions in which drug may be metabolized or degraded due to higher pH or colonic enzymes in the lower part of GIT.

FIG. 2: IMAGES ARE SHOWING THE RETAINING CAPABILITY OF HARD GELATIN CAPSULES IN 0.1N HCl. 1) Empty untreated hard gelatin capsule at 0 min, 2) Empty untreated hard gelatin capsule at 30 min, 3) untreated hard gelatin capsule with carbopol 934P at 0 min, 4) untreated hard gelatin capsule with carbopol 934P at 45 min, 5) Five hours treated empty hard gelatin capsule at 0 min, 20) five hours treated empty hard gelatin capsule at 250 min, 6) five hours treated gelatin capsule with carbopol 934P at 0 min, 7) five hours treated gelatin capsule with carbopol 934P at 480 min, 8) five hours treated gelatin capsule with HPMC K100M at 480 min.

This problem can be overcome by providing enough time for the polymer to swell and float in the gastric fluids and also to extend the drug release by polymer swelling. This can be facilitated by decreasing the solubility of capsules by cross-linking the amino acids present in the skeleton of the capsule. It can be done by exposing the empty capsules to the cross-linking agents for the appropriate time. The time of exposure is optimized by exposing the capsules to the cross-linking agents for different hours ranging from 1 h to 6 h and checking the effect of exposure time of cross-linking agent in decreasing the solubility of capsule shell shown in **Fig. 2**.

From research, it was found that capsules exposed to cross-linking agents for 5 h or more than 5 h showed predominantly reduced the solubility of the capsules and giving enough time for the polymer to swells and extend the time of residence in the stomach as well as drug release. Capsules filled with pure drug and without and polymer released the maximum amount of the drug (*in-vitro* drug release) less than the 5 h, to extend the release of the drug, different concentrations of polymers with different viscosity was used to develop the modified pulisincap floating formulations. Cross liked gelatin capsules were less solubilized than that of the normal gelatin capsule; this is due to the cross-linking of amino groups [15] presents the hard gelatin capsules by formalin. The intensity of the test sample was found to be less than that of standard, it indicates the free formaldehyde was less than the 0.002 PPM, the free formaldehyde concentration was very less due to the cross-linked hard gelatin capsules were kept in hot air oven at 50 °C for 3 h with which the unreacted free formaldehyde was removed this was due this is safer for human consumption.

Preparation of Floating Capsules of Diltiazem HCl: Required amounts of ingredients were passed through a 60-mesh sieve. Drug and excipients are weighed individually. Powders were transferred to motor and triturated with a pestle to form a uniform powder mixture. Powder blend containing the equivalent weight of 90 mg of drug was weighed and filled into the body of 00 sized cross-linked capsules and cap was fixed formulation composition of modified pulsincap floating capsules of diltiazem HCl was shown in **Table 1**.

TABLE 1: FORMULATION OF DILTIAZEM HCl FLOATING CAPSULES

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Diltiazem HCl	90	90	90	90	90	90	90	90	90	90	90	90	90	90	90
sodium CMC	90	135	180	-	-	-	-	-	-	-	-	-	-	-	-
HPMC (K_4M)	-	-	-	180	135	90	-	-	-	-	-	-	-	-	-
HPMC ($K_{15}M$)	-	-	-	-	-	-	180	135	90	-	-	-	-	-	-
Carbopol 934P	-	-	-	-	-	-	-	-	-	120	90	60	-	-	-
HPMC ($K_{100}M$)	-	-	-	-	-	-	-	-	-	-	-	-	180	135	90
MCC	205	160	115	205	160	115	205	160	115	175	205	235	205	160	115
Talc	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Magnesium Stearate	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Total weight	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400

Preparation of Calibration Curve of Diltiazem Hydrochloride: Standard graph of diltiazem hydrochloride was constructed [16] using 0.1N HCl. Various concentrations 4 to 16 µg/ml were prepared. The absorbance of prepared concentrations was measured at 237 nm by adjusting to zero with a blank sample. A graph was plotted by taking concentration on x-axis and absorbance on the y-axis, and the best fit line was drawn, and regression value and equation was calculated.

Evaluation of Floating Capsules of Diltiazem HCl: The following evaluation [17, 18] tests are performed for developed formulations:

a) *In-vitro* Buoyancy Studies: The *in-vitro* buoyancy was determined by floating lag time, as per the method described by the tablets were placed in a 250 ml beaker containing 0.1N hydrochloric acid [19]. The time required for the capsule to rise to the surface and to float determined as floating lag time. The duration of time for which the dosage form consistently remained on the surface of the medium was determined as the total floating time.

b) Weight Variation test for Capsules: To study weight variation content of 20 capsules of each capsule were weighed) separately using an electronic balance and the test was performed

according to the official method [20]. The average weight was noted and the standard deviation calculated. 20 Capsules were selected at random. One capsule was weighed. The capsule was opened, and the contents were removed as completely as possible. The emptied shells were weighed. The net weights of its contents were determined, that was by subtracting the weight of the shells from the weight of the intact capsule. The procedure was repeated with other 19 capsules:

$$\% \text{ Weight variation} = (W_A - W_I) \times 100 / W_A$$

c) Drug Content (Assay): The drug content of the floating was determined according to in-house standards, and it meets the requirements if the amount of the active ingredient in each of the ten tested capsules lies within the range of 90% to 110% of the standard amount. Content of ten capsules was weighed and taken into a Mortar and triturated to get a uniform mixture. An accurately weighed portion of the powder equivalent to about 100 mg of diltiazem HCl. was transferred to a 100 ml volumetric flask containing 70 ml of 0.1N HCl. It was shaken by mechanical means for 1h. Then it was filtered through a Whatman filter paper (No.1) and diluted to 100 ml with 0.1N HCl. From this resulted solution 1 ml was taken, diluted to 50 ml with 0.1N HCl and absorbance was measured [21] against blank at 237 nm.

d) Water Uptake Studies (Determination of Swelling Index): Water uptake studies of individual polymers and combinations were carried out using eight-stage USP type 1 (basket) Dissolution test apparatus at 50 rpm, and 0.1N HCl was used as the medium, and the temperature was maintained at 37 ± 0.5 °C. Weight of individual capsule was taken before the water uptake study (W_1). The capsule was kept in a basket; the weight of the capsule was taken at a different time interval (W_2). Percent hydration (Swelling index) was calculated [22] from the following formula:

$$\text{Swelling index} = W_2 - W_1 / W_1 \times 100$$

Where, W_1 is the initial weight of tablet & W_2 is the weight of the hydrated tablet

The same capsules were subjected for testing the expansion in length and in diameter with the help of Vernier Caliper at same time intervals as that of the swelling index after taking from the dissolution apparatus.

e) *In-vitro* Drug Release Studies: The *in-vitro* drug release study was performed for all the capsules using USP Type - II dissolution apparatus under the following conditions. The medium used was 900 ml of 0.1N HCl, temperature maintained drugging the *in-vitro* dissolution studies was 37 ± 0.5 ºC, the speed of the basket was set at 50 rotations per min. Sampling was done at different time intervals as follows 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 h and sample volume of 5 ml was collected and replaced by at pre-determined time intervals [23] samples (5 ml) were collected and replaced with the same volume of fresh preheated (37 ± 0.5 ºC) 0.1N HCl to maintain sink conditions. The samples withdrawn were filtered through Whatman filter paper (No.1), and drug content in each sample was analyzed by UV-visible spectrophotometer at λ_{max} 237 nm.

f) Drug Release Kinetics: The data obtained from the *in-vitro* dissolution studies were subjected to various kinetic models to estimate and explain the drug release kinetics from the extended release diltiazem HCl capsules. In this research, the drug release data were subjected to different kinetics [24, 25] like zero-order, first-order, Higuchi, and Korsmeyer-Peppas kinetic models. The best suit data was estimated by using the values of correlation coefficient (R^2).

Zero-Order: $F = K_0 t$: where, F represents the fraction of drug released at time t, and K_0 is the zero-order release constant.

First-Order: $\ln (1-F) = -K_1 t$: where, F indicates the fraction of drug released at time point t, and K_1 is the first-order release constant.

Higuchi Model: $F = K_H t_{1/2}$; where, F indicates the fraction of drug released at time point t, and whereas K_H is the Higuchi constant.

Korsmeyer–Peppas Model: $F = K_P t^n$: Where, F indicates the fraction of drug released at time point t, K_P represents the rate constant and n is the release exponent, suggestive of the drug release mechanism. An "n" value is less than or equal to 0.5 it represents the Fickian release mechanism, and the value of n ranging between 0.5 and 1

represents non-Fickian release mechanism. When the n value is greater than or equal to 1, it represents case-II transport, and this is due to the polymer dissolution and polymeric chain expansion or relaxation.

g) Qualitative test for determining the Free Formaldehyde: The crossed linked hard gelatin capsules were tested for the presence of free formaldehyde [26, 27]. The standard sample solution of formaldehyde (0.002 w/v) was prepared then the color intensity of standard was compared with the test, if the intensity of the test was less than that of standard, it indicates the presences of free formaldehyde was less than 0.002 w/v. Test sample solution was prepared by cross-linked gelatin capsules were taken in 25 in number, caps and bodies were separated and cut into very small pieces and kept in a beaker containing 25 ml of distilled water and stirred for an hour with magnetic stirrer this done to solubilize the free formaldehyde if any was present in the crosslinked hard gelatin capsules.

Then the solution was filtered and transferred into 50 ml volumetric flask and made up to 50 ml with distilled water then from the above volumetric flask 1ml was transferred into 10 ml volumetric flask and made up to with distilled water, from the 10 ml volumetric flask 1 ml was transferred into the clean test tube and 4 ml of distilled water was added and it was made up to 10 ml with the acetylacetone reagent then the test tube was heated in a water bath at temperature of 40 °C and it was allowed to stand for 40 min. It was compared with the standard formaldehyde solution containing 0.002 w/v formaldehyde which was prepared processed. In the same procedure as the test solution.

h) *In-vivo* (Radiographic) Studies: To make the floating capsule opaque in Radiographic studies $BaSO_4$ was added to the formulation by replacing the 40% of drug (*i.e.,* 40 percent of the drug was removed from the total dose of the formulation, and $BaSO_4$ replaced that) and was filled into the capsule. Floating capsules with $BaSO_4$ were evaluated were for total *in-vitro* floating time.

Study Protocol: The *in-vivo* radiographic studies were carried out by administering a capsule with $BaSO_4$ to human volunteers and monitoring them through a radiological method [28]. Three healthy male subjects (mean age 24 years: mean weight 55 ± 10 kg) capsule was administered after taking informed consent. The study was conducted in human volunteers with permission from Institutional Human Ethics Committee and their Approval no: REF. NO: ICE /RVSIMS/2017/09.

Fasted State: The human volunteer has fasted overnight then capsule was administered with 150 ml water then after volunteers were not allowed to eat anything.

Fed State: After a regular meal, capsules were administered to volunteers immediately with 250 ml of water, then after volunteers were not allowed to eat. In both fasting and fed study volunteers were asked to take a glass of water, for every hour. During the Radiographic studies, volunteers were informed to be in the upright posture. (Sitting or standing). X-ray was taken at predetermined time intervals.

TABLE 2: FORMULATION CONTAINING 40% OF $BaSO_4$

Ingredients	Weight (Milligrams)
Diltiazem HCl	54
$BaSO_4$	36
Carbopol 934p	60
MCC	235
Talc	10
Magnesium Stearate	5
Total weight	400

RESULTS AND DISCUSSION:

Pre-formulation Evaluation (Pre-Filling Evaluation): Powder blend (Pre-filling Evaluation, *i.e.,* evaluation is done before filling the powder into the capsules) was subjected to various pre-formulation parameters.

The angle of repose values are found to be in the range of 26.1^o to 31.6^o indicates that the powder blend has good flow properties, *i.e*. powder blend is showing excellent to good flow property. The bulk density of all the formulations were found to be in the range of 0.344 to 0.415 (gm/cm^3). The tapped density of all the formulations were found to be in the range of 0.421 to 0.492 (g/cc). The compressibility Index of all the formulations was found to be ranging between 13.8 to 20.37% which show that the powder has good to fair compressibility. All the formulations have shown

the hausner's ratio ranging between 1.16 to 1.25 indicating the powder has good to fair compressibility; the total evaluation data was tabulated in **Table 3**.

TABLE 3: PRE-FORMULATION PARAMETERS OF BLEND

Formulation Code	Angle of Repose	Bulk Density (g/cc)	Tapped Density (g/cc)	Hausner's Ratio	Compressibility Index (%)
F1	26.4^0	0.387	0.458	1.18	15.5
F2	27.8^0	0.394	0.472	1.19	16.5
F3	26.8^0	0.410	0.480	1.17	14.58
F4	$27.^0$	0.415	0.492	1.18	15.6
F5	28.2^0	0.371	0.450	1.21	17.55
F6	28.3^0	0.375	0.456	1.21	17.7
F7	26.1^0	0.377	0.453	1.20	16.77
F8	26.4^0	0.396	0.463	1.16	14.4
F9	27.5^0	0.356	0.421	1.18	15.4
F10	30.3^0	0.356	0.447	1.25	20.35
F11	31.6^0	0.344	0.432	1.25	20.37
F12	29.3^0	0.372	0.462	1.24	19.48
F13	25.5^0	0.386	0.453	1.17	14.7
F14	27.8^0	0.398	0.462	1.16	13.8
F15	25.7^0	0.375	0.445	1.18	15.7

Fourier Transform Infrared Spectroscopy ((FT-IR) Studies: The IR spectra of diltiazem HCl and optimized formulation, F12 was found to be identical. The characteristic IR absorption peaks of pure diltiazem HCl observed at 2916.58 for -C-H (aliphatic) stretching and for optimized formulation it was found at 2925.95, IR absorption peaks of pure diltiazem HCl observed at 1638.02 for C=O (Amide)stretching and for optimized formulation it was found at 1638.39, IR absorption peaks of pure diltiazem HCl observed at 1283.60 for C-O stretching and for optimized formulation it was found at 1284.17 and IR absorption peaks of pure diltiazem HCl observed at 1475.72 for C=C (aromatic) stretching and for optimized formulation it was found at 1477.70. The FT-IR spectra obtained for pure diltiazem HCl and that of optimized formulation there was no significant shift of peaks this indicated there was no chemical interaction occurred between the drug and the excipients shown in **Table 4** & **Fig. 3A-3B**.

TABLE 4: INTERPRETATION OF FUNCTIONAL GROUP OF OPTIMIZED FORMULA

S. no.	Wave number (cm^{-1}) (FTIR of Diltiazem HCl)	Group	Wave number (cm^{-1}) (FTIR of Optimized formula)
1	2916.58	-C-H(aliphatic) stretching	2925.95
2	1638.02	C=O (Amide)stretching	1638.39
3	1283.60	C-O stretching	1284.17
4	1475.72	C=C (aromatic) stretching	1477.70

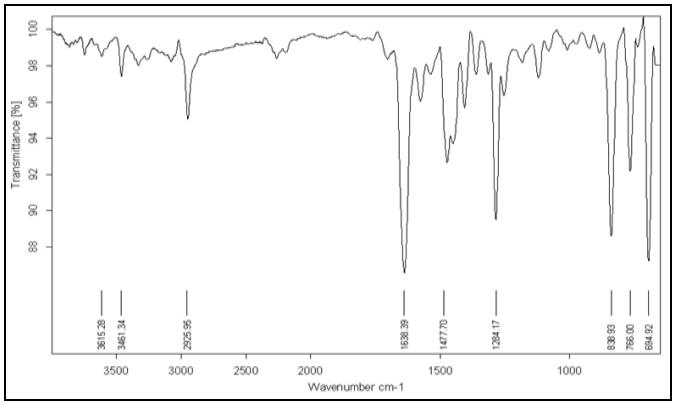


FIG. 3A: FT-IR SPECTRUM OF DILTIAZEM HCl

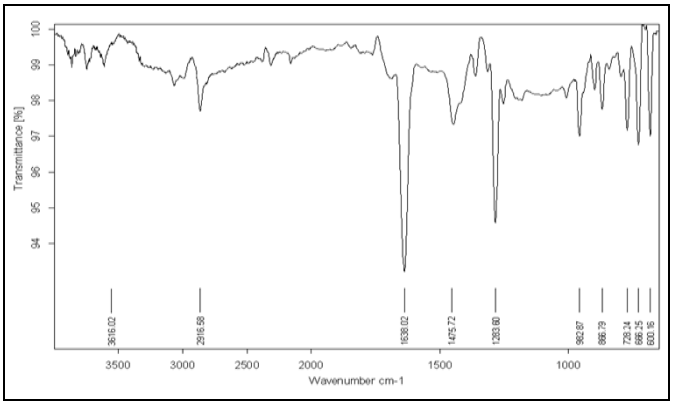


FIG. 3B: FT-IR SPECTRUM OF OPTIMIZED FORMULATION (CARBOPOL)

Differential Scanning Calorimetry (DSC): DSC studies revealed that diltiazem hydrochloride exhibits a sharp endothermic peak at 216.79 ºC corresponding to its melting point which is usually associated with decomposition of the drug. It could also be seen in the optimized formulation at 206 ºC. Also, there was no significant shift of endothermic peak in the optimized formulation to that of the pure drug; this indicates there was no drug-polymer incompatibility was shown in **Fig. 4A** & **4B**.

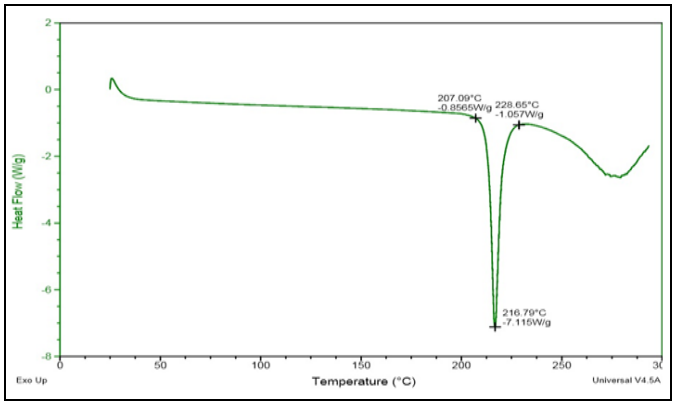


FIG. 4A: DIFFERENTIAL SCANNING CALORIMETRY OF PURE DRUG (DILTIAZEM HYDROCHLORIDE)

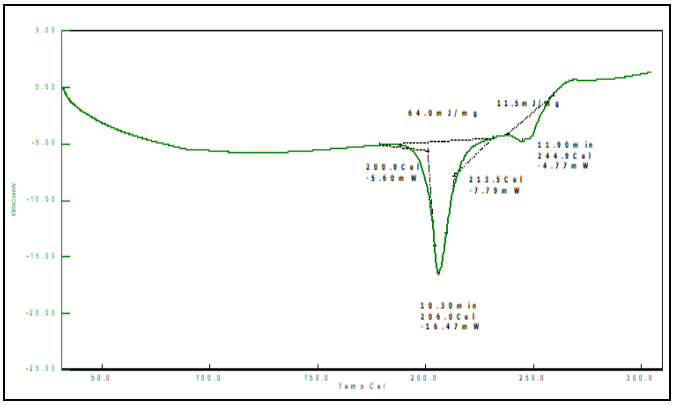


FIG. 4B: DIFFERENTIAL SCANNING CALORIMETRY OF OPTIMIZED FORMULATION

Scanning Electron Microscopy (SEM): The cross-sectional view of the hard-gelatin capsules treated with a crosslinking agent and with and without treating with a cross-linking agent. The difference in the SEM images reveals shown in **Fig. 5A & 5B** that is crosslinking of the amino acids present in the gelatin with cross-linking agent (formaldehyde), thus the cross-linking resulted in the reduction of solubility of gelatin capsules in 0.1NHCl, this leads to the retarding the dumping of the content of gelatin capsules and given enough time for swelling of the polymer-enclosed in the capsule resulted in floating as well as retarding of the release of drug enclosed inside the capsule.

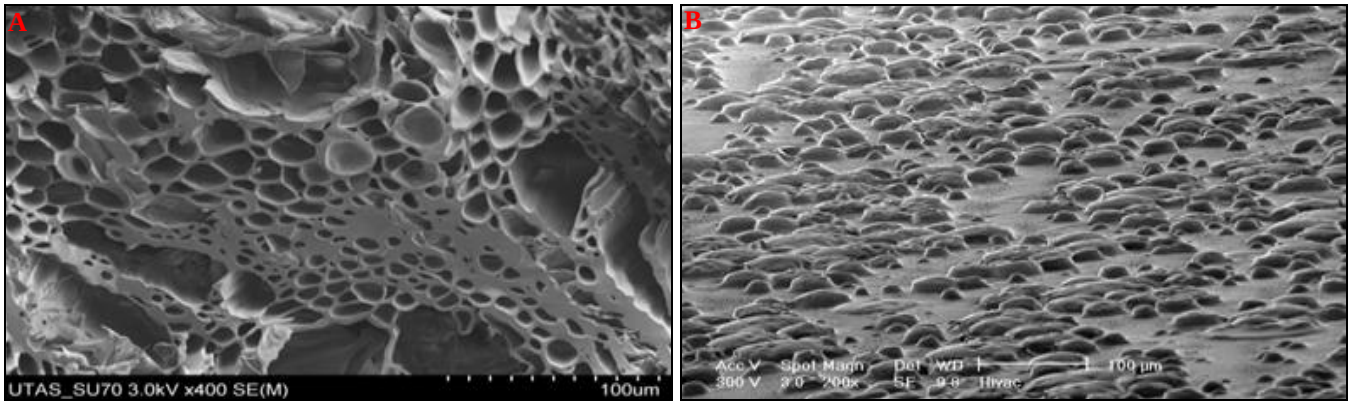
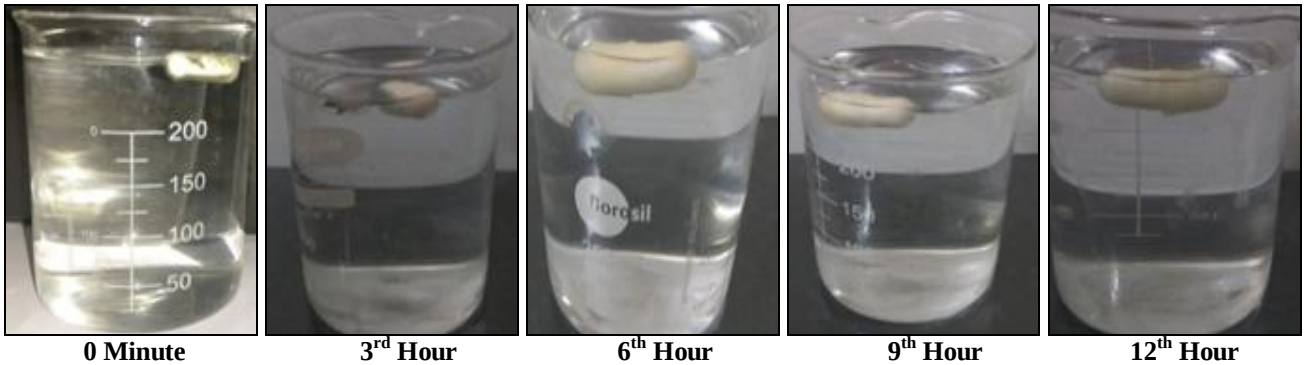


FIG. 5: SCANNING ELECTRON MICROSCOPY. A: NORMAL HARD GELATIN CAPSULE B: CROSS-LINKED HARD GELATIN CAPSULE

Quality Control Parameters for Capsules (Post - filling Evaluation): Quality control tests such as (Post-filling evaluation, *i.e.,* evaluation is done before filling the powder into the capsules) weight variation, water uptake studies, total floating time, assay, percentage of drug release in 0.1N HCl (*In-vitro* drug release) the results were found to be as follows. Weight variation in all the formulations was found to be, 385 ± 1.8 mg to 406 ± 3.5 mg which was in Pharmacopeial limits. The assay was performed, and percent drug content of all the formulations was found to be between 96.9 ± 2.34 and 102.8 ± 2.12% which were within the acceptable limits. All the formulations are showing the total *in-vitro* floating time of greater than 12 hours shown in **Fig. 6**. All the capsules were showing the initial length and diameter were found to be 2.4 cm and 0.7 cm. Moreover, after 12 h of *in-vitro* dissolution, the length and diameter were found to be 3.4 cm, and 1.1 cm values were tabulated in **Table 5**.

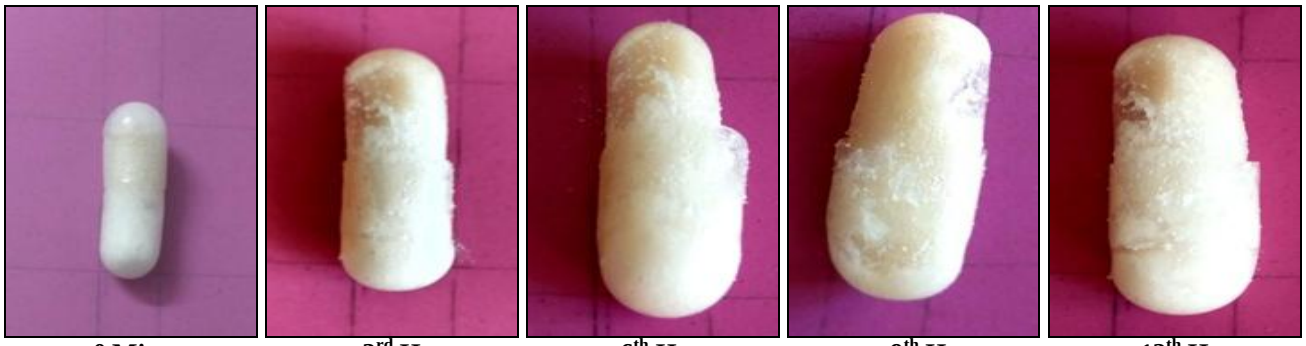


0 Minute **3rd Hour** **6th Hour** **9th Hour** **12th Hour**

FIG. 6: TOTAL FLOATING TIME OF OPTIMIZED FORMULATION F12

TABLE 5: EVALUATION OF FINISHED FORMULATION

Formulation Codes	[a] Weight variation (mg)	[b] Total floating time (h)	Expansion		[c] Drug content (%)
			[b] Initial Length 2.4	[b] Initial Diameter 0.7	
F1	394±1.8	>12	3.5	1.1	97.8±1.98
F2	392±1.8	>12	3.4	1.1	98.5±2.21
F3	402±2.3	>12	3.4	1.1	102.5±1.75
F4	387±1.4	>12	3.4	1.1	97.5±1.54
F5	391±2.2	>12	3.4	1.1	101.7±2.5
F6	385±3.1	>12	3.4	1.1	98.4±01.59
F7	404±5.4	>12	3.4	1.1	99.6±2.15
F8	397±1.9	>12	3.4	1.1	98.7±1.76
F9	389±2.8	>12	3.4	1.1	97.9±2.2
F10	387±2.5	>12	3.5	1.1	99.5±1.58
F11	406±3.5	>12	3.4	1.1	102.8±2.12
F12	393±3.2	>12	3.4	1.1	98.4±1.92
F13	391±2.9	>12	3.4	1.1	96.9±2.34
F14	385±1.8	>12	3.4	1.1	98.2±3.4
F15	394±2.4	>12	3.4	1.1	99.1±2.6



0 Minute **3rd Hour** **6th Hour** **9th Hour** **12th Hour**

FIG. 7: SWELLING OF CAPSULES OF OPTIMIZED FORMULATION-F12

The results of water uptake studies were found to be in the range of 294.1% to 448.4% (Water uptake studies shown in **Fig. 7**, **8.1** & **8.2**).

Water Uptake Studies (Determination of Swelling Index): The capability of the formulation to absorb the water is an important parameter to be considered for the formulation which in turn affects the swelling property of the formulation this plays a crucial role in the rate and kinetics of drug release. The swelling index of all the formulation was represented graphically in **Fig. 7**, **8A** & **8B**.

The weight of the formulation was doubled at the end of 4 h the weight of formulation was increased continuously, and the rate of water uptake was reduced after the 10 h. The percentage of water uptake was enhanced with increasing in the polymer concentration, and it varied with a different polymer. Continuous increase in the water uptake *i.e.* increase in the weight of formulation is due to absorption of water by the polymer in the formulation as wells water absorbed by the crosslinked gelatin capsule.

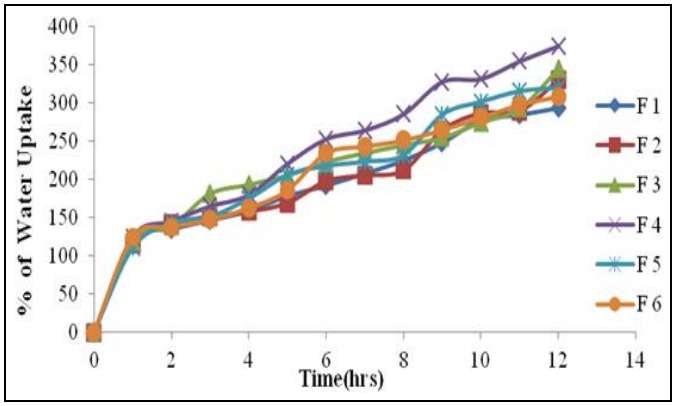


FIG. 8A: GRAPHICAL REPRESENTATION OF WATER UPTAKE STUDIES OF FORMULATIONS F1-F6

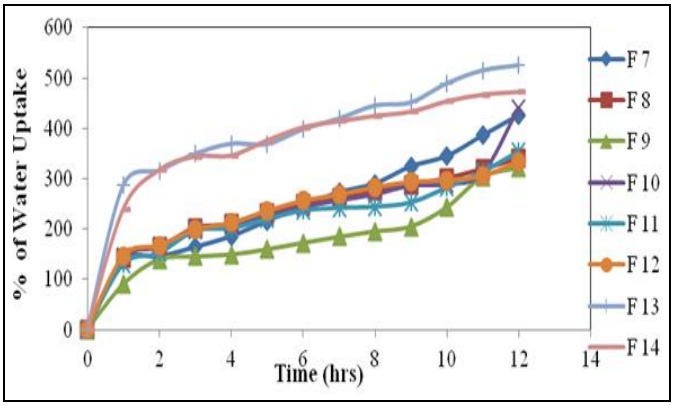


FIG. 8B: GRAPHICAL REPRESENTATION OF WATER UPTAKE STUDIES OF FORMULATIONS F7-F15

***In-vitro* Drug Release of Modified Pulsincap Floating Capsules:** Formulation EF in which the only pure drug was filled into the cross-linked hard gelatin capsule shown the *in-vitro* drug release of 96% within the 4 h, graphically represented in **Fig. 9A** from these studies it was evidenced that cross-linked hard gelatin capsule is not restricting/ hindering the permeation of drug through it (*i.e.*, formaldehyde treated capsule is not a barrier for drug release).

Formulation F1, F2 and F3 containing sodium carboxy methyl cellulose is a polymer of varying concentration (90 mg, 135 mg, 180 mg) have shown the drug release of 102.45 ± 2.63 in 7 h (F1), 99.64 ± 4.61 in 8 h (F2) and 97.94 ± 4.69 in 9 h (F3). Formulation F4, F5 and F6 containing HPMC K_4M as a polymer of varying concentration (180 mg, 135 mg, 90 mg) have shown the drug release of 87.08 ± 3.23 (F4), 92.25 ± 2.56 (F5) and 95.42 ± 3.25 (F6) in 12 h.

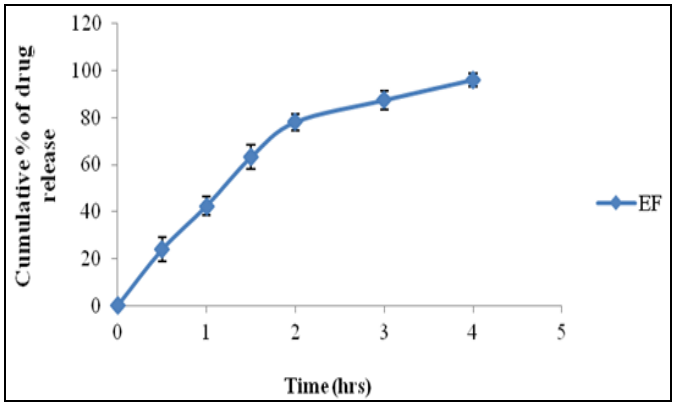


FIG. 9A: GRAPHICAL REPRESENTATION OF *IN-VITRO* DRUG RELEASE FROM THE CROSS-LINKED HARD GELATIN CAPSULE CONTAINING ONLY PURE DRUG

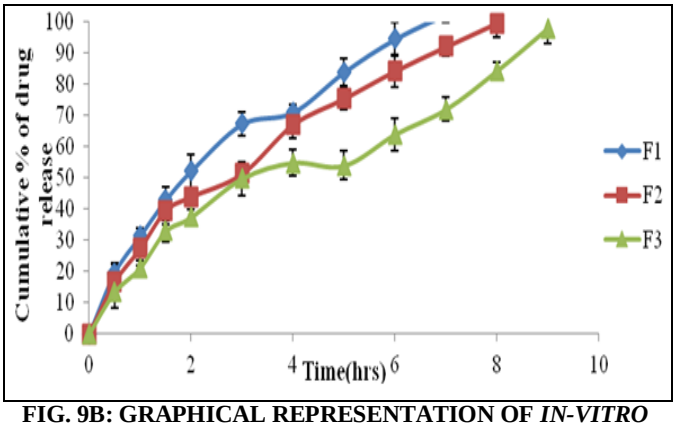


FIG. 9B: GRAPHICAL REPRESENTATION OF *IN-VITRO* DRUG RELEASE FROM FORMULATION CONTAINING DIFFERENT CONCENTRATION OF SODIUM CARBOXY METHYL CELLULOSE

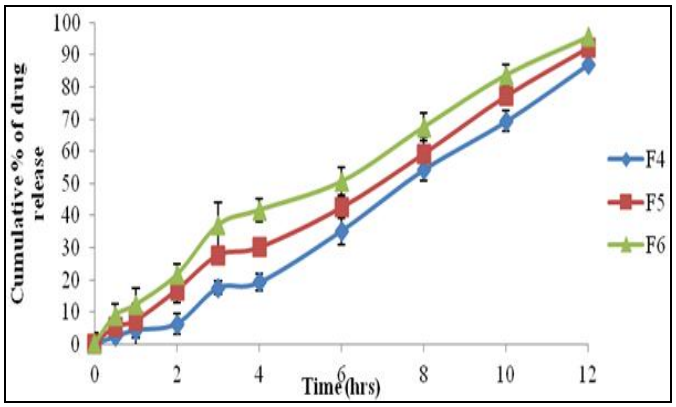


FIG. 9C: GRAPHICAL REPRESENTATION OF *IN-VITRO* DRUG RELEASE FROM FORMULATION CONTAINING DIFFERENT CONCENTRATION OF HPMC K_4M

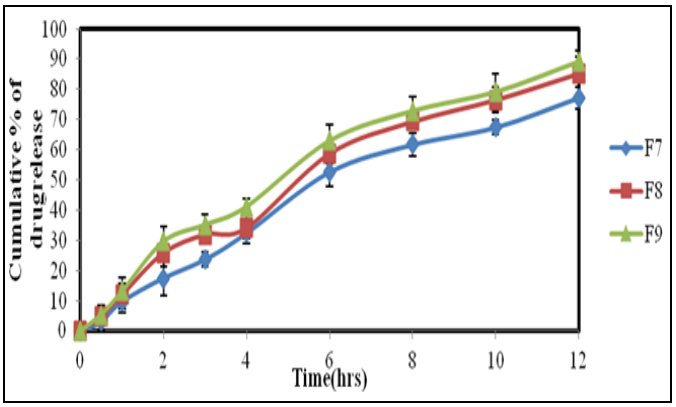


FIG. 9D: GRAPHICAL REPRESENTATION OF *IN-VITRO* DRUG RELEASE FROM FORMULATION CONTAINING DIFFERENT CONCENTRATION OF HPMC $K_{15}M$

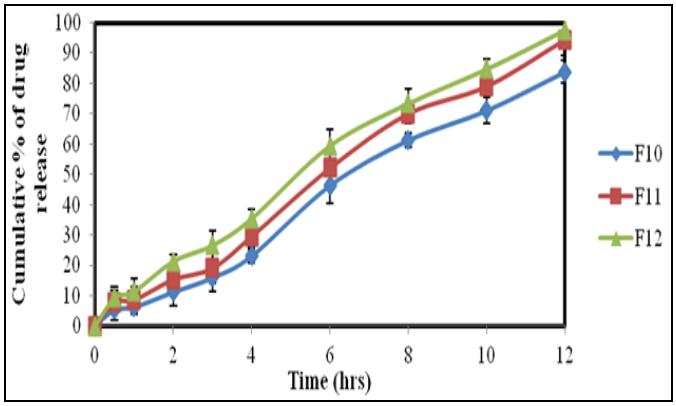


FIG. 9E: GRAPHICAL REPREENTATION OF *IN-VITRO* DRUG RELEASE FROM FORMULATION CONTAINING DIFFERENT CONCENTRATION OF CARBOPOL

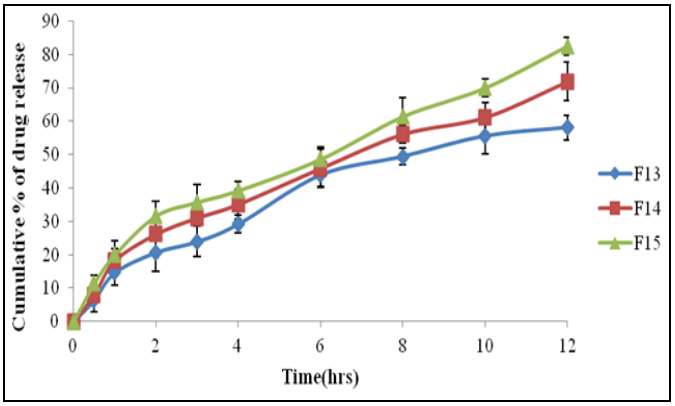


FIG. 9F: GRAPHICAL REPRESENTATION OF *IN-VITRO* DRUG RELEASE FROM FORMULATION CONTAINING DIFFERENT CONCENTRATION OF HPMC $K_{100}M$

Formulation F7, F8 and F9 containing HPMC $K_{15}M$ as a polymer of varying concentration (180 mg, 135 mg, 90 mg) have shown the drug release of 77.23 ± 3.56 (F7), 85.26 ± 5.36 (F8) and 95.42 ± 3.25 (F9) in 12 h. Formulation F10, F11 and F12 containing carbopol as a polymer of varying concentration (120 mg, 90 mg, 60 mg) have shown the drug release of 83.83 ± 3.64 (F10), 91.39 ± 5.34 (F11) and 97.64 ± 3.45 (F12) in 12 h. Formulation F13, F14 and F15 containing HPMC $K_{100}M$ as a polymer of varying concentration (180 mg, 135 mg, 90 mg) have shown the drug release of 58.13 ± 3.65 (F13), 71.89 ± 5.78 (F14) and 82.49 ± 2.64 (F15) in 12 h, **Fig. 9B**, **9C**, **9D**, **9E** & **9F** graphically represents the *in-vitro* drug release from formulation F1-F15.

Order and Mechanism of Drug: The dissolution values obtained from *in-vitro* dissolution studies were subjected to different kinetic models like Zero order, First order, Higuchi and Peppas models. The values obtained are as follow regression coefficient value (R^2) for zero order was found to be 990, R^2 of the first order was found to be 0.8638, so the order of drug release from the optimized formulation follows zero order release as linearity is more for plots of zero order than that of the first order.

R^2 of Higuchi was found to be 0.9446, R^2 of Peppas was found to be 0.9796, *i.e.*, Linearity for the Peppas plots was higher than that of linearity of Higuchi and the value of n, *i.e.*, values of the release exponent 0.7936 this value indicates that mechanism of drug release from the modified pulsincap capsules of diltiazem HCl was non-fickian diffusion, this indicates the drug release from the formulation was governed by polymer rearrangement kinetic data of optimized formulation was shown in **Table 6**.

TABLE 6: KINETICS OF DRUG RELEASE AND MECHANISM OF DRUG RELEASE FROM DOSAGE FORM

Formulation	Zero Order	First Order	Higuchi	Korsmeyer- Peppas		Mechanism of drug release
	R^2 Value	R^2 Value	R^2 value	R^2 Value	n Values	
F12	9904	0.8638	0.9446	0.9796	0.7936	Non-fickian diffusion

***In-vitro* Floating Studies of Capsules with $BaSO_4$:** To make the formulation detectable in the radiographic studies a part of drug/excipients used in the formulation development was replaced by $BaSO_4$ as shown the **Table 2,** as the formulation components were replaced by high-density $BaSO_4$, it is necessary to check for effect of $BaSO_4$ in *in-vitro* total floating time before carrying out the radiographic studies. This study revealed that the percentage of the $BaSO_4$ that was added in the formulation had not altered the total *in-vitro* floating time of capsules, *i.e.*, the formulation with and without $BaSO_4$ have shown the total floating time of 12 h shown in **Table 7**.

TABLE 7: EVALUATION OF CAPSULES WITH $BaSO_4$ FOR *IN-VITRO* TOTAL FLOATING TIME

Parameters	Optimized batch without $BaSO_4$	Optimized batch with $BaSO_4$
In-vitro total floating time	More than 12 h	More than 12 h

***In-vivo* Evaluation (X-Ray Studies):** The gastric residence time of optimized formulation was evaluated by conducting *in-vivo* X-ray studies in healthy human volunteers. From the radiographic images following results were obtained. From the results, it was observed that the mean gastric residence time for the optimized formula of the floating capsule was up to 6 h in the fed state, up to 2 h in fasting state.

The behavior of the floating capsules in the stomach of human volunteers was observed in real time using a radiographic imaging technique (shown in **Fig. 10.1**, **10.2** & **Table 8**). In radiographic images made 60 min after the administration, the tablets were observed in the human stomach. In the next radiograph taken at 2, 4, 6, 8 h (in fed state) & 1, 2, 4 h (Fasting state). The capsules had altered its position and turned around floated on the gastric fluid.

The capsule containing the drug remained in stomach for about 2 h in fasting condition & and remained in the stomach for 6 h in the fed state. In fed condition volunteers were given regular meals and volunteers were asked to take a glass of water (water should not be cold or very warm.) for every half an hour and in fasting. The capsule was administered to volunteers only with a glass of water.

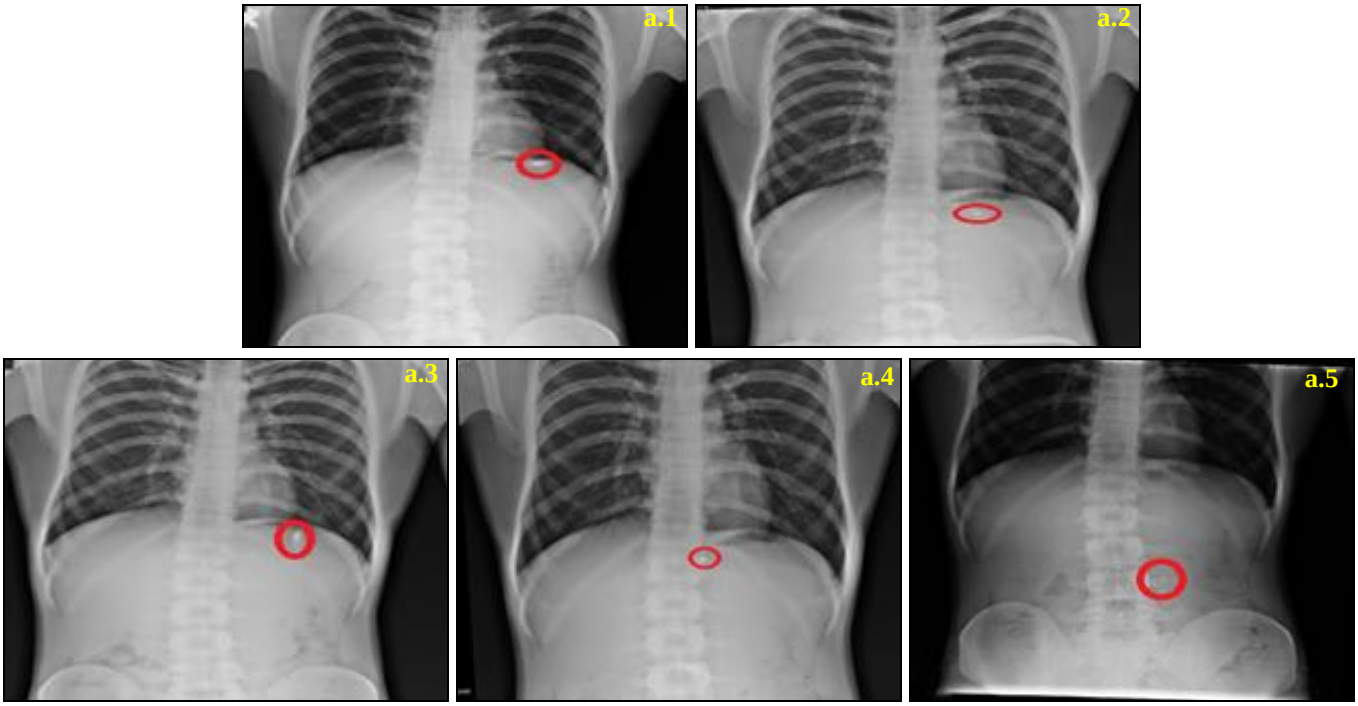


FIG. 10.1: RADIOGRAPHIC IMAGES ARE SHOWING THE PRESENCE OF $BaSO_4$-LOADED FLOATING CAPSULES IN THE GIT AT DIFFERENT PERIODS IN FED CONDITION. THE CAPSULES ALTERED ITS POSITION IN THE STOMACH. Images were taken at: a.1) 1 h, a.2) 2 h, a.3) 4h, a.4) 6h and a.5) 8hr after capsule administration (n=2 subjects).

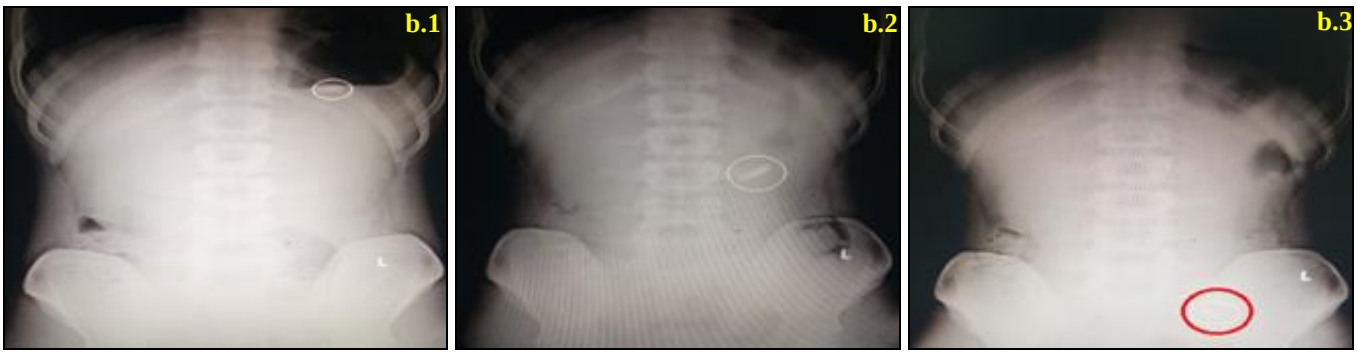


FIG. 10.2: RADIOGRAPHIC IMAGES (1, 2 & 3) SHOWING THE PRESENCE OF A $BaSO_4$-LOADED FLOATING CAPSULES IN THE GIT AND IMAGES (3) SHOWS THE DISPLACEMENT OF CAPSULES FROM STOMACH DUE TO HOUSEKEEPER WAVES IN A FASTING CONDITION. Images were taken after: b.1) 1 h, b.2) 2 h and b.3) 4 h after tablet administration (n=2 subjects).

TABLE 8: RESULTS OF RADIOGRAPHIC STUDIES

Condition	Gastric residence time
Fasting condition	Less than 2 h
Fed state	Up to 6 h

CONCLUSION: Modified pulsincap floating capsules were formulated & evaluated successfully. Results were as follows. The powder blend was subjected to various pre-formulation parameters. The angle of repose values is found to be excellent to good flow property. The compressibility was found good to fair. The weight variation, assay in all the formulations was found was Pharamacoepial limits. All the formulations are showing the total *in-vitro* floating time of greater than 12 h.

Formulation EF in which the only pure drug was filled into the cross-linked hard gelatin capsules shown the *in-vitro* drug release of 96% (Shown in **Fig. 10.1**) within the 4 h (This was done to ensure that drug was diffusing through the cross-linked hard gelatin capsule). Among all the formulation F12 (optimized) containing carbopol have shown the better *in-vitro* drug release, *i.e*. 97.64 ± 3.45 in 12 h, following Zero Order (0.9446) with drug release mechanism of non-fickian diffusion (n=0.7936) and all the evaluation values of powder as well as finished formulation are within limits.

FT-IR studies shown no shifting of major peaks of the pure drug when compared with the formulation (Mixture of drug and excipients) that indicates the absence of incompatibilities between the drug, polymer, and excipients used for the development of formulation. Optimized formulation loaded with $BaSO_4$ capsules behavior in the stomach of human volunteers was observed in real time using a radiographic imaging technique. In radiographic images made 60 min after the administration, the tablets were observed in the human stomach. In the next picture taken at 2, 4, 6, 8 h (in the fed state) & 1, 2, 4 h (Fasting state). The capsules had altered its position and turned around floated on the gastric fluid. The capsule containing the drug remained in stomach for about 2 h in fasting condition & and remained in the stomach for 6 h in the fed state. In fed condition volunteers were given regular meals and volunteers were asked to take a glass of water (water should not be cold or very warm) for every half an hour and in fasting. The capsule was administered to volunteers only with a glass of water.

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CONFLICT OF INTEREST: None declared

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