

FORMULATION DEVELOPMENT AND INVITRO EVALUATION OF EPLERENONE PULSINCAP DRUG DELIVERY SYSTEM

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ABSTRACT

The purpose of the present study was to design and evaluate an Oral, site specific, Pulsatile drug delivery system containing Eplerenone as a model drug, which can be time dependent manner, to modulate the drug level in synchrony. It is used for treat high blood pressure based on chronopharmaceutical considerations. The basic design consists of an insoluble hard gelatin capsule body, filled with powder blend and sealed with a hydrogel plug. The powder blend containing Eplerenone, Crospovidone, Lycoat, MCC and talc was prepared and evaluated for flow properties and FTIR studies. From the obtained results, F6 powder blend formulation was selected for further fabrication of pulsatile capsules. Hydrogel plug was formulated in a combination of hydrophobic polymer like ethyl cellulose with hydrophilic polymers like HPMC K200M in 1:1, 1:2, and 2:1 ratios to maintain a suitable lag period and it was found that the drug release was controlled by the proportion of polymers used. The prepared formulations was evaluated for drug content, weight variation and *Invitro* release studies. FTIR studies confirmed that there was no interaction between drug and polymers and *Invitro* release studies of pulsatile device revealed that increasing ethylcellulose polymer content resulted in delayed release of Eplerenone from the pulsincap after a predetermined lag time of 6hrs. Based on *invitro* studies performed, F9 was found to be optimized formulation.

KEYWORDS: Pulsatile system; time dependent delivery; Eplerenone; Chronopharmaceutics; *Invitro* release studies.

INTRODUCTION

In this century, the pharmaceutical industry is caught between pressure to keep prices down and the increasing cost of successful drug discovery and development. In the form of an NDDS or ChrDDS, an existing drug molecule can “get a new life” thereby increasing its market value and competitiveness and extending patent life.

Among modified- release oral dosage forms, increasing interest has currently turned to systems designed to achieve time specific (delayed, pulsative) and site-specific delivery of drugs. In particular, systems for delayed release are meant to deliver the active principle after a programmed time period following administration. These systems constitute a relatively new class of devices the importance of which is especially connected with the recent advances in chronopharmacology. It is by now well-known that the symptomatology of a large number of pathologies as well as the pharmacokinetics and pharmacodynamics of several drugs follow temporal rhythms, often resulting in circadian variations. Therefore, the possibility of exploiting delayed release to perform.^[1-5]

Chronotherapy is quite appealing for those diseases, the symptoms of which occur mainly at night time or in the early morning, such as bronchial asthma, angina pectoris and rheumatoid arthritis. The delay in the onset of release has so far mainly been achieved through osmotic mechanisms, hydrophilic or hydrophobic layers, coating a drug- loaded core and swellable or erodible plugs sealing a drug containing insoluble capsule body.

Delivery systems with a pulsatile pattern are receiving increasing interest for the development of dosage forms, because conventional systems with a continuous release are not ideal. Most conventional oral controlled release drug delivery systems release the drug with constant or variable release rates. A pulsatile release profile is characterized by a time period of no release (lag time) followed by a rapid and complete release.^[6]

A lot of work is being done to achieve pulsatile release so that the drug release can be delivered according to circadian rhythms of our body. Advancis Pharmaceutical Corp., German town, Maryland, USA has developed once-a-day pulsatile delivery system called Pulsys®,

which enables the delivery of antibiotic amoxicillin in regular concomitant pulses.^[3-7] to get maximum bronchodilating effect in the morning hours. One such example is of a bronchodilator “Uniphyll” (theophylline),^[2] which was developed by Purdue Pharmaceuticals Products L. P., Stamford, USA, and approved by FDA in 1989.

Eplerenone, an aldosterone receptor antagonist similar to spironolactone, has been shown to produce sustained increases in plasma renin and serum aldosterone, consistent with inhibition of the negative regulatory feedback of aldosterone on renin secretion. The resulting increased plasma renin activity and aldosterone circulating levels do not overcome the effects of eplerenone. Eplerenone selectively binds to recombinant human mineralocorticoid receptors relative to its binding to recombinant human glucocorticoid, progesterone and androgen receptors.^[8]

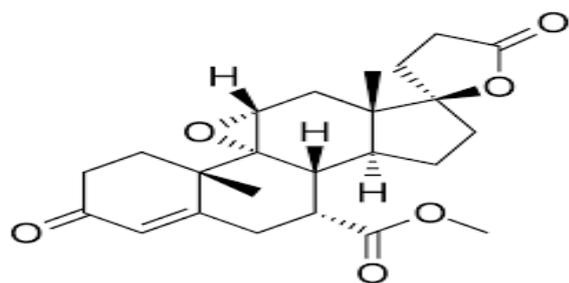


Figure1: chemical structure of Eplerenone

Experimental Work

Eplerenone Pharma Grade from Spectrum Pharma Labs, Hyderabad, Crospovidone, Lycoat, Microcrystalline cellulose, Talc, Ethyl cellulose, HPMC K200M, Magnesium stearate Formaldehyde Potassium permanganate, Potassium dihydrogen Phosphate, Methanol, Sodium hydroxide pellets LR Grade from S d fine chemical Ltd, Mumbai

METHODOLOGY

Preformulation studies^[9-11]

Preformulation testing is the first step in the rationale development of dosage forms of a drug substance. It is one of the important prerequisite in development of any drug delivery system. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. Characterization of the drug is a very important step at the preformulation phase of product development followed by studying the properties of the excipients and their compatibility.

The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be mass-produced.

The following are the various preformulation studies:

Solubility: Solubility is defined as amount of substance

that passes into solution to achieve a saturated solution at constant temperature and pressure. The solvents used are water and methanol. Solubility was determined by adding Eplerenone in small incremental amount to a test tube containing fixed quantity of different solvents. After each addition, the system was vigorously shaken and examined visually for any un dissolved solute particles.

Drug-Excipient compatibility studies

To know the chemical compatibility of the drug spectroscopic technique that is FTIR studies were used. The FTIR spectra were recorded using an IR spectrophotometer (IR-Affinity-1, Shimadzu, Japan). The IR spectra for the samples were obtained by KBr disk method. The samples were prepared by grinding the pure drug, polymer and physical mixture with KBr separately. The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000- 600 cm^{-1} . FTIR study was carried on Eplerenone, physical mixture of Eplerenone and for the best formulation.

UV spectroscopy

The main step in preformulation is to establish a simple analytical method so that all future measurements can be quantitative. Most drugs absorb light in ultraviolet wavelengths (190-400nm), since they are generally aromatic or contain double bonds.

10 mg of Eplerenone was accurately weighed on an electronic balance and dissolved in 2 ml methanol and volume was made upto 10ml with buffer which gives 1000 $\mu\text{g/mL}$ (stock solution I). From the stock solution I, 1 ml is pipetted out then transfer to 10mL volumetric flask and volume was made upto 10mL with buffer which gives 100 $\mu\text{g/mL}$. From 100 $\mu\text{g/mL}$, 1mL was pipetted out and volume was made upto 10ml with buffer to give 10 $\mu\text{g/mL}$ and scanned on a UV scanner between 2000-400nm. The maxima obtained in the graph were considered as λ_{max} for the Eplerenone in respective buffers.

Standard calibration curve for Eplerenone

Eplerenone standard calibration curve was plotted in pH 1.2 buffer. Accurately weighed amount of 10 mg of drug was transferred into a 10 ml volumetric flask and the primary stock solution was prepared by making up volume to 10 ml with pH 1.2 buffer. This gives a solution having concentration of 1000 $\mu\text{g/mL}$ of Eplerenone in stock solution. From this primary stock solution 1 ml was transferred into another 10 ml volumetric flask and made up to 10 ml with pH 1.2, from this secondary stock 0.2, 0.4, 0.6, 0.8, 1, and 1.2ml was taken separately and made up to 10 ml with pH 1.2 buffer, to produce 2, 4, 6, 8, 10 and 12 $\mu\text{g/ml}$ solution respectively. The absorbance was measured at 244 nm using UV spectrophotometer.

Similarly Eplerenone standard graphs were plotted in pH 6.8 phosphate buffer and pH 6.8 phosphate buffer by following the above procedure.

Flow Properties Of Api

Table 1: Standard specifications for comparison of flow properties.

| S. No | Flow property | Angle of repose | Carr's Index | Hausner's ratio |
|-------|----------------|-----------------|--------------|-----------------|
| 1. | Excellent | 25-30 | <10 | 1.00-1.11 |
| 2. | Good | 31-35 | 11-15 | 1.12-1.18 |
| 3. | Fair | 36-40 | 16-20 | 1.19-1.25 |
| 4. | Passable | 41-45 | 21-25 | 1.26-1.36 |
| 5. | Poor | 46-55 | 26-31 | 1.35-1.45 |
| 6. | Very poor | 56-65 | 32-37 | 1.46-1.59 |
| 7. | Very very poor | >66 | >38 | >1.6 |

Pulsincap Desingning^[12-18]

Designing or preparation of pulsincap capsules involves 3 steps:

1. Preparation of cross-linked gelatin capsule.
2. Preparation of powder blends for filling into capsules.
3. Formulation of pulsincap of Eplerenone.

A. Preparation of cross-linked gelatin capsule

Formaldehyde treatment

About 100 hard gelatin capsules size '0' were taken. Their bodies were separated from the caps and placed on a wire mesh. The bodies which were placed on a wire mesh were spread as a single layer. 25 ml of 15% v/v of formaldehyde solution was prepared and placed in a desiccators. To this 5 g of potassium permanganate was added. The wire mesh containing the bodies of the capsules was kept on the top of desiccators' containing formaldehyde liquid at the bottom in equilibrium with its vapor and immediately the desiccators' was tightly closed and sealed. The bodies of capsules were made to react with formaldehyde vapors by exposing them for varying periods of time viz., 2, 4, 6, 8, 10hrs. Then they were removed and kept on a filter paper and dried for 24 hrs to ensure completion of reaction between gelatin and formaldehyde vapors, afterwards the capsules were kept in an open atmosphere, to facilitate removal of residual formaldehyde. These capsule bodies were capped with untreated cap and stored in a polythene bag.

Use of Formaldehyde treatment

The main aim of formaldehyde treatment was to modify the solubility of hard gelatin capsules. Cross-linking of gelatin molecules was achieved by exposing to formalin vapors. Cross-linking involves the reaction of amino groups in gelatin molecular chain with aldehyde groups of formaldehyde by a "Schiff's base condensation" so that the gelatin becomes water insoluble. Formaldehyde reacts with gelatin forming an irreversible complex. The primary amine group present in gelatin reacts with formaldehyde making it irreversibly bound. Potassium permanganate was added to formaldehyde solution so that formalin vapors were produced. When bodies of

hard gelatin capsule were exposed to formaldehyde vapors for different periods of time in a closed dessicator, vapor gets equilibrated with formaldehyde liquid and therefore makes the gelatin water insoluble.

Evaluation of formaldehyde treated capsules

Physical tests

- **Identification attributes:** Suitable size capsules which are lockable were selected. Generally the gelatin capsules when touched with wet hand they become sticky but upon formaldehyde treatment the capsules are observed for the stickiness.
- **Visual defects:** Selected 100 treated capsules and observed for visual defects by physical observation and not more than 15-20 capsules must be distorted.
- **Dimensions:** Variations in the dimensions between the formaldehyde treated and untreated capsules were studied. The length and diameter of the capsules were measured before and after formaldehyde treatment by using Vernier calipers.

Optimization Of Formaldehyde Treated Capsule Bodies Exposed At Various Time Intervals Viz., 2, 4, 6, 8, 10hrs

Formaldehyde treated capsule bodies which were exposed at various time intervals viz., 2, 4, 6, 8, 10hrs were optimized by conducting Disintegration test. The test was performed on both untreated and treated capsules. Formaldehyde treated bodies joined with untreated caps and was tested for disintegration. Disintegration test was carried out by using Hiccon disintegration test apparatus. pH 1.2, pH 6.8, buffers were used as medium and maintained at 37°C throughout the experiment. The time at which the capsules disintegrate are noted.

B. Preparation of eplerenone tablet for filling into capsules

All the ingredients were passed through # 60 mesh sieve separately. The drug & MCC were mixed by adding small portion of each at a time and blending it to get a uniform mixture and kept aside.

Then the other ingredients were mixed in geometrical

order and passed through coarse sieve (#44 mesh) and the tablets were compressed using hydraulic press. Compression force of the machine was adjusted to obtain the hardness in the range of 3-4 kg/cm² for all batches.

The weight of the tablets was kept constant for all formulations F1 to F6 (100 mg).

Table 2: Formulae for preparation of blend for filling of Eplerenone pulsincap.

| Ingredients (mg) | F1 | F2 | F3 | F4 | F5 | F6 |
|------------------|-----|-----|-----|-----|-----|-----|
| Eplerenone | 25 | 25 | 25 | 25 | 25 | 25 |
| Crospovidone | 2 | 4 | 6 | -- | -- | -- |
| Lycoat | -- | -- | -- | 2 | 4 | 6 |
| MCC | 69 | 67 | 65 | 69 | 67 | 65 |
| Mg. stearate | 2 | 2 | 2 | 2 | 2 | 2 |
| Talc | 2 | 2 | 2 | 2 | 2 | 2 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |

C. Formulation of Pulsincap of Eplerenone

The modified release pulsincaps containing 10mg of Eplerenone were prepared by using different excipients and polymers in varying ratios. The formaldehyde treated capsule bodies which were exposed to 6 hrs was optimized and chosen for the pulsincap formulation based on disintegration time. Optimized formulation of Eplerenone tablet was filed into the capsule body. For hydrogel plug formulation, the plug was prepared by using the combination of Ethyl cellulose: HPMC in varying ratios. Initially the total weight of the plug was taken as 200 mg and the ratio of hydrophobic & hydrophilic polymer as 1:1, 1:2, and 2:1.

Method of preparation of Pulsincap dosage form

Preparation of powder blend

Hard gelatin capsules of 'size 0' which were hardened with formaldehyde treatment for 6hrs were chosen for the formulation. The bodies and caps separated manually. Optimized formulation F3 was fitted at the bottom of the capsule body.

Preparation of Hydrogel plug

- Plug was prepared as a compressed tablet and placed at the opening of capsule body. The capsule body was closed by a cap.
- Hydrogel plug was prepared by using different polymers like Ethyl cellulose, HPMC at different concentrations.
- A combination of hydrophobic and hydrophilic polymers were used viz.,
- Ethyl cellulose: HPMC, in different ratios like 1:1, 1:2, and 2:1.
- A tight fit between the plug and impermeable capsule shell is essential to regulate water penetration into the capsule content and the drug release prior to complete erosion of plug material. Ideally plug should erode only from the surface exposed to the release medium.
- Plug ejection can be done by swelling on contact with aqueous fluids (or) pushing out by increased internal pressure (or) erosion (or) by enzyme degradation.

Capsule filling

- Homogeneous mixture of drug and excipients were filled into the 6th hr formaldehyde treated capsule body manually by filling method.
- Then, hydrogel plug in the form of a tablet is placed above the mixture i.e., at the opening of capsule body
- The capsule body was closed by a cap.

Capsule sealing

The joint of the treated capsule body and untreated cap of the capsules was sealed with a small amount of 1% ethyl cellulose ethanolic solution.

Evaluation of tablets^[19-22]

Tablet Dimensions

Thickness and diameter were measured using a calibrated vernier caliper. Three tablets of each formulation were picked randomly and thickness was measured individually.

Hardness

Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. The hardness of the tablets was determined using Monsanto hardness tester. It is expressed in kg/cm². Three tablets were randomly picked and hardness of the tablets was determined.

Friability test

The friability of tablets was determined by using electrolab friabilator. It is expressed in percentage (%). Ten tablets were initially weighed (W_I) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes or run up to 100 revolutions. The tablets were weighed again (W_F). The % friability was then calculated by –

$$\%F = 100 (1 - W_I / W_F)$$
 % Friability of tablets less than 1% was considered acceptable.

Weight Variation Test

Ten tablets were selected randomly from each batch and weighed individually to check for weight variation. A little variation was allowed in the weight of a tablet

according to U.S. Pharmacopoeia. The following percentage deviation in weight variation was allowed.

Table 3: percentage deviation in weight variation.

| Average weight of a tablet | Percentage deviation |
|----------------------------|----------------------|
| 130 mg or less | ±10 |
| >130mg and <324mg | ±7.5 |
| 324 mg or more | ±5 |

In all formulations, the tablet weight is 100 mg, hence 10% maximum difference allowed.

Test for Content Uniformity

Tablet containing 10mg of drug was dissolved in 50ml of 6.8 pH buffer in volumetric flask. The drug was allowed to dissolve in the solvent. The solution was filtered, 2ml of filtrate was taken in 10ml of volumetric flask and diluted up to mark with distilled water and analyzed spectrophotometrically at 244 nm. The concentration of Eplerenone was obtained by using standard calibration curve of the drug. Drug content studies were carried out in triplicate for each formulation batch.

In vitro Disintegration Time

Tablet was added to 900ml of distilled water at 37±0.5°C. Time required for complete dispersion of a tablet was measured.

In vitro Dissolution Study

In vitro dissolution of Eplerenone tablets was studied in USP XXIV dissolution test apparatus. 900ml Phosphate buffer 6.8 (simulated fluid) was used as dissolution medium. The stirrer was adjusted to rotate at 50RPM. The temperature of dissolution medium was maintained at 37±0.5°C throughout the experiment. One tablet was used in each test. Samples of dissolution medium (5ml) were withdrawn by means of syringe fitted with pre-filter at known intervals of time and analyzed for drug release by measuring the absorbance at 244 nm. The volume withdrawn at each time interval was replaced with fresh quantity of dissolution medium. Cumulative percent Eplerenone released was calculated and plotted against time.

Evaluation of Pulsincap Dosage Form:^[23-26]

Invitro release studies

Dissolution study was carried out to measure the release rate of the drug from the pulsincap formulation. Invitro dissolution profile of each formulation was determined by employing USP I apparatus by rotating basket method. In order to stimulate the pH changes along GI tract 2 different dissolution media with pH 1.2, 6.8, 2 buffers were sequentially used, and therefore referred to as "Sequential pH change method". The dissolution media were maintained at a temperature of 37 ± 0.5°C throughout the experiment and the speed of rotation of basket maintained at 50 rpm. 900ml of dissolution medium was used at each time. Eplerenone Pulsincaps was placed in basket in each dissolution

vessel to prevent floating. While performing experiments, stimulated gastric fluid (SGF) pH 1.2 buffer was first used for 2 hrs (since the average gastric emptying time is 2hrs) and then removed and the fresh stimulated intestinal fluid (SIF) pH 6.8 buffer was added and used for remaining hours. 5 ml samples of dissolution fluid were withdrawn at predetermined time intervals with the help of a syringe. The volume withdrawn at each time interval was replaced with 5ml of fresh dissolution medium maintained at same temperature. The filtered samples were suitably diluted whenever necessary and assayed for Eplerenone by measuring absorbance at 244 nm, by UV absorption spectroscopy. %CDR was calculated over the sampling times.

Release Kinetics^[27-28]

Drug release mechanisms and kinetics are the two important characteristics of a drug delivery system in describing drug dissolution profile. Mathematical models are used to evaluate the kinetics and mechanism of drug release from the tablets. The model that best fits the release data is selected based on the correlation coefficient(R) value in various models. The models with high 'R-value is considered as the best fit on the release data.

Various mathematical models are

1. Zero order release model
2. First order release model
3. Higuchi release model
4. Korsmeyer – peppas release model

1. Zero Order Release Equation: The equation for zero order release is

Where,

$$Q_t = Q_o + K_o t$$

Q_o = Initial amount of drug

Q_t = Cumulative amount of drug release at time "t"

K_o = Zero order release constant

T = Time in hours

The zero-order kinetics describes the systems in which the drug release rate is independent of its concentration of the dissolved substance. A graph is plotted between the time taken on x-axis and the cumulative percentage of drug release on y-axis and it gives a straight line.

2. First Order Release Equation: The first order release equation is

$$\text{Log } Q_t = \text{Log } Q_o + K_t / 2.303$$

Where,

Q_o = Initial amount of drug

Q_t = Cumulative amount of drug release at time "t"

K = First order release constant

T = Time in hours

Here, the drug release rate depends on its concentration. The first order kinetics describes the systems in which the drug release rate is concentration dependent. A graph

is plotted between the time taken on x-axis and the log % of drug release on y-axis and it gives a straight line.

3. Higuchi Release Equation: The Higuchi release equation is

$$Q_t = K_H \sqrt{t}$$

Where ,

Q = Cumulative amount of drug release at time “t”

K_H = Higuchi constant

T = Time in hrs

Higuchi described the release of drug from an insoluble matrix as square root of time dependent process. A graph is plotted between the square root of time taken on x-axis and the cumulative percentage of drug release on y-axis and it gives it a straight line.

4. Korsmeyer -Peppas Release Equation: The Korsmeyer –Peppas equation is

$$F = M_t / M = K_m t^n$$

Where,

F = fraction of drug released at time ‘t’

M_t = amount of drug released at time ‘t’

M = total amount of drug in dosage form

K_m = kinetic constant

n = diffusion or release exponent

A graph is plotted between the log time taken on x-axis and the log percentage of drug release on y-axis and it gives a straight line.

Table 4: Diffusion exponents and solute release mechanism.

| Diffusion (n) | Overall solute diffusion mechanism |
|---------------|------------------------------------|
| 0.45 | Fickian diffusion |
| 4 | Anomalous (non fickian) diffusion |
| 0.89 | Case- transport |
| n>0.89 | Super case-II transport |

RESULTS AND DISCUSSION

Preformulation Studies

Solubility: It was determined as per standard procedure.

The results are given in Table 7.1.

Table 5: Solubility studies of Eplerenone in various solvents.

| Solvent | Solubility (mg/mL) |
|--------------|--------------------|
| 0.1 N HCL | 0.925 |
| 6.8pH buffer | 0.986 |
| 7.4pH buffer | 0.862 |
| Ethanol | 3.98 |
| Methanol | 5.46 |

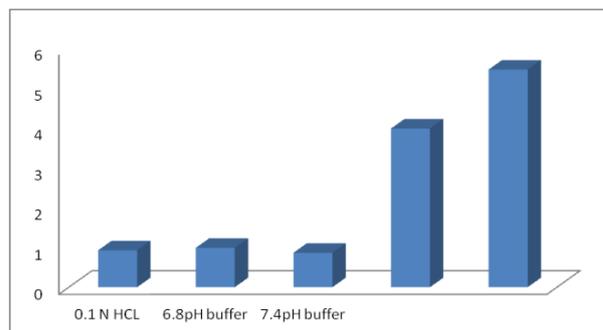


Fig 2: Solubility studies of Eplerenone in various solvents.

❖ **Discussion:** Eplerenone was found to be soluble in 6.8pH buffer and soluble in methanol.

Drug-Excipient compatibility studies: The IR spectrum of pure drug was found to be similar to the standard spectrum of Eplerenone. The spectrum of Eplerenone shows the following functional groups at their frequencies shown in Figure 3.

From the spectra of Eplerenone, combination of Eplerenone with polymers, it was observed that all characteristic peaks of Eplerenone were not altered and present without alteration in the combination spectrum, thus indicating compatibility of the drug and polymers. FTIR spectra of Eplerenone, and Optimized formulation are shown in Figure 3 & 4 respectively.

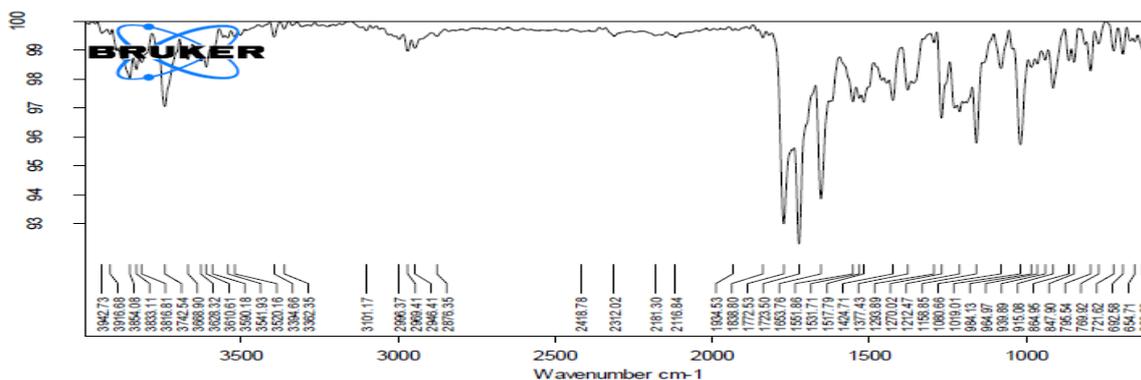


Figure 3: FTIR spectrum of Eplerenone.

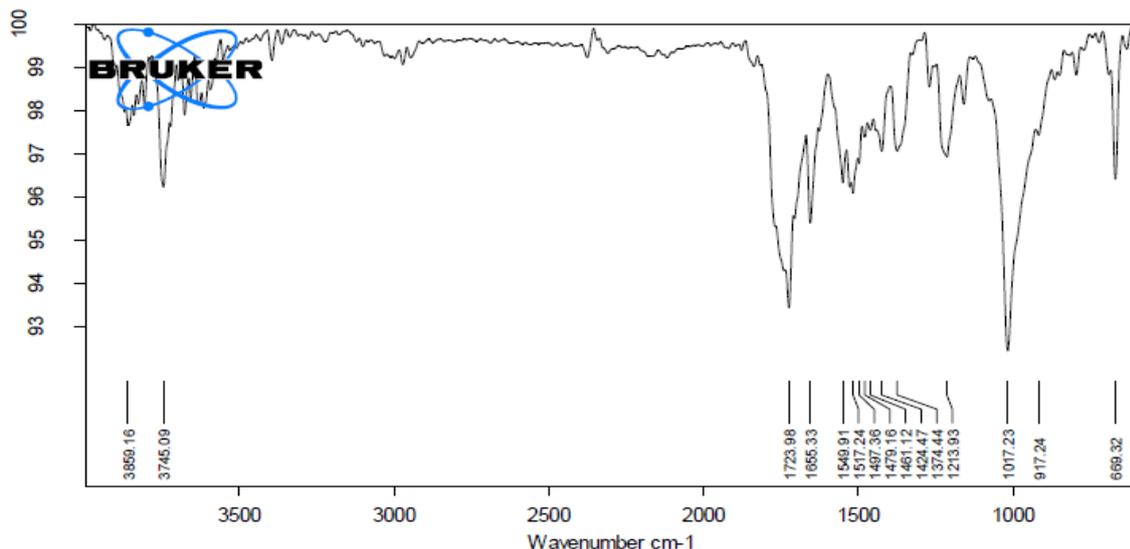


Figure 4: FTIR Spectrum of optimised formulation.

λ_{max} Determination of Eplerenone

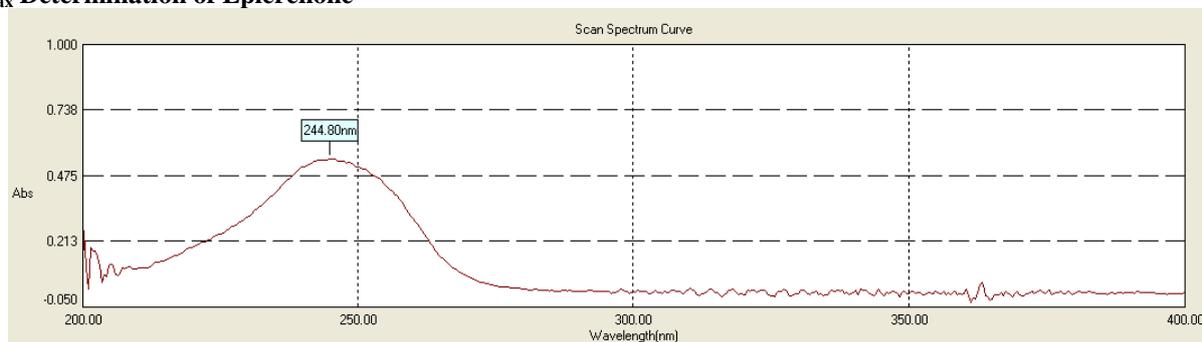


Fig. 5: λ_{max} Determination of Eplerenone.

Standard Calibration Curve

The standard calibration curve of Eplerenone was developed in different pH media such as pH 1.2, and pH 6.8 phosphate buffer. Two buffers were selected in order to mimic the in-vivo conditions of the GIT.

a. Standard Calibration Curve in 1.2 pH

Standard graph of Eplerenone showed linearity at the concentration range of 2-12 μg/mL with correlation coefficient of 0.999. Table 7.2 gives the data of the standard graph and Figure 7.5 shows the standard graph in pH 1.2.

Table 4: Data for calibration curve of Eplerenone in pH 1.2 at 244nm.

| Concentration (μg/mL) | Absorbance |
|-----------------------|------------|
| 0 | 0 |
| 2 | 0.113 |
| 4 | 0.234 |
| 6 | 0.326 |
| 8 | 0.428 |
| 10 | 0.543 |
| 12 | 0.659 |

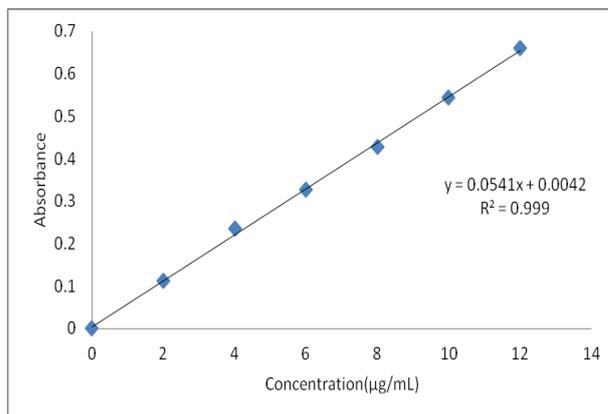


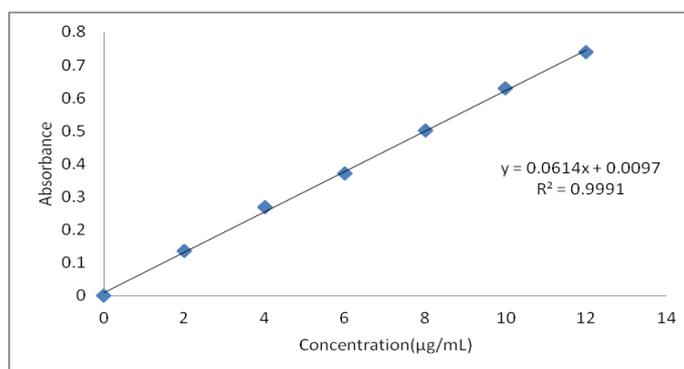
Figure 6: Standard Calibration Curve of Eplerenone in pH 1.2 at 244 nm

b. Standard Calibration Curve in 6.8 pH phosphate buffer

Standard graph of Eplerenone in pH 6.8 phosphate buffer shows linearity in the concentration range of 2-12 μg/mL with correlation coefficient of 0.999. Table 7.3 gives the data of the standard graph and Figure 7.6 shows the standard graph in pH 6.8 phosphate buffer.

Table 5: Data for calibration curve of Eplerenone in pH 6.8 at 244nm.

| Concentration ($\mu\text{g/mL}$) | Absorbance |
|------------------------------------|------------|
| 0 | 0 |
| 2 | 0.135 |
| 4 | 0.269 |
| 6 | 0.372 |
| 8 | 0.502 |
| 10 | 0.628 |
| 12 | 0.739 |

**Figure 7: Standard Calibration Curve of Eplerenone in pH 6.8 at 244 nm.****Flow properties of powder blend****Table 6: Flow properties of powder blend.**

| Formulation Code | Angle of Repose | Bulk Density (g/ml) | Tapped Density (g/ml) | Carr's Index. (%) | Hausner's ratio |
|------------------|-----------------|---------------------|-----------------------|-------------------|-----------------|
| F1 | 29.21 | 0.546 | 0.635 | 14.02 | 1.16 |
| F2 | 26.45 | 0.536 | 0.643 | 16.64 | 1.20 |
| F3 | 27.39 | 0.549 | 0.629 | 12.72 | 1.15 |
| F4 | 30.16 | 0.576 | 0.657 | 12.33 | 1.14 |
| F5 | 29.86 | 0.532 | 0.619 | 14.05 | 1.16 |
| F6 | 28.39 | 0.569 | 0.642 | 11.37 | 1.13 |

Characterization of Tablets**Post Compression parameters**

All the batches of tablet formulations were characterized for official evaluation parameters like Weight variation,

Hardness, Friability, Tablet thickness and drug content and results are shown in the table.

Table 7: Characterization Eplerenone Tablets.

| Formulation code | %Weight variation (mg) | Thickness (mm) | Diameter (mm) | Hardness | Friability (%) | Disintegrating time(sec) | Drug content (%) |
|------------------|------------------------|----------------|---------------|----------|----------------|--------------------------|------------------|
| F1 | 0.25 | 2.01 | 8.01 | 3.26 | 0.15 | 71 | 96.49 |
| F2 | 0.69 | 2.04 | 8.02 | 3.48 | 0.69 | 63 | 97.56 |
| F3 | 0.43 | 2.12 | 8.04 | 3.94 | 0.75 | 54 | 98.13 |
| F4 | 0.97 | 2.01 | 8.06 | 4.05 | 0.85 | 76 | 99.36 |
| F5 | 0.43 | 2.03 | 8.09 | 3.46 | 0.46 | 69 | 95.48 |
| F6 | 0.52 | 2.02 | 8.01 | 3.87 | 0.03 | 47 | 96.43 |

Table 8: % Cumulative drug release of formulations F1-F6.

| Time(min) | F1 | F2 | F3 | F4 | F5 | F6 |
|-----------|-------|-------|-------|-------|-------|-------|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 29.34 | 26.13 | 42.18 | 36.16 | 38.43 | 46.19 |
| 10 | 36.19 | 39.43 | 59.36 | 49.27 | 53.16 | 56.19 |
| 15 | 45.32 | 46.18 | 67.43 | 59.34 | 59.76 | 64.24 |
| 20 | 54.15 | 58.31 | 72.18 | 64.53 | 69.43 | 72.39 |
| 30 | 59.36 | 68.14 | 79.36 | 73.16 | 76.49 | 86.43 |
| 45 | 64.13 | 73.46 | 84.31 | 82.76 | 85.73 | 91.06 |
| 60 | 69.36 | 79.24 | 86.43 | 87.43 | 92.43 | 98.63 |

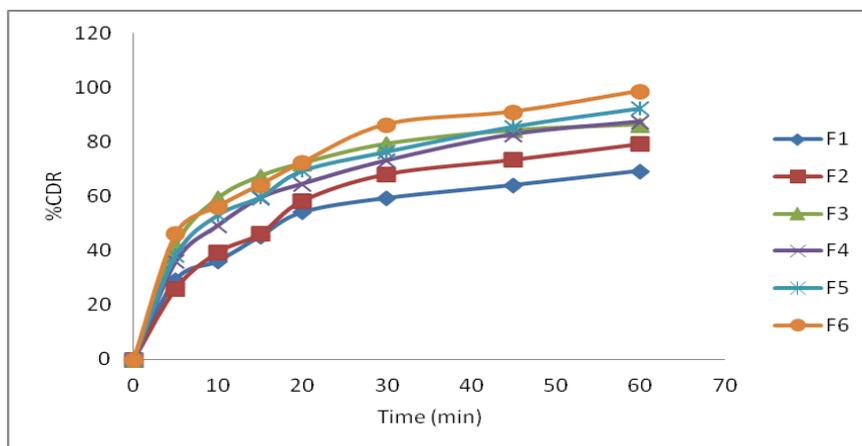


Fig. 8: In vitro drug release of formulations F1-F6.

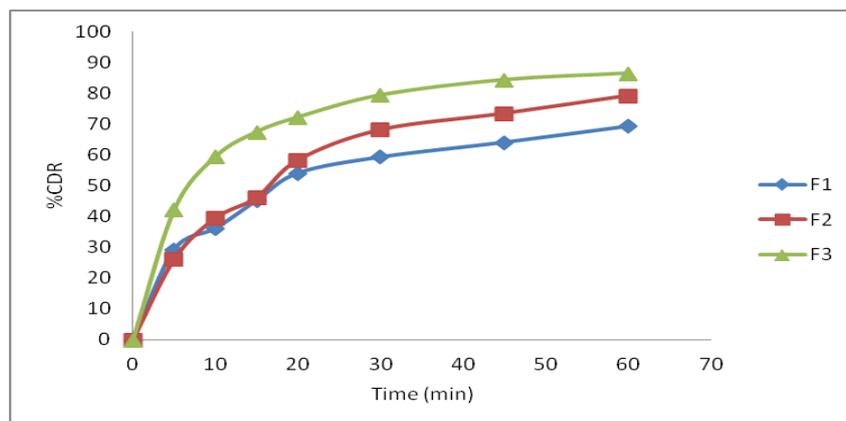


Fig. 9: In vitro drug release of formulations F1-F3.

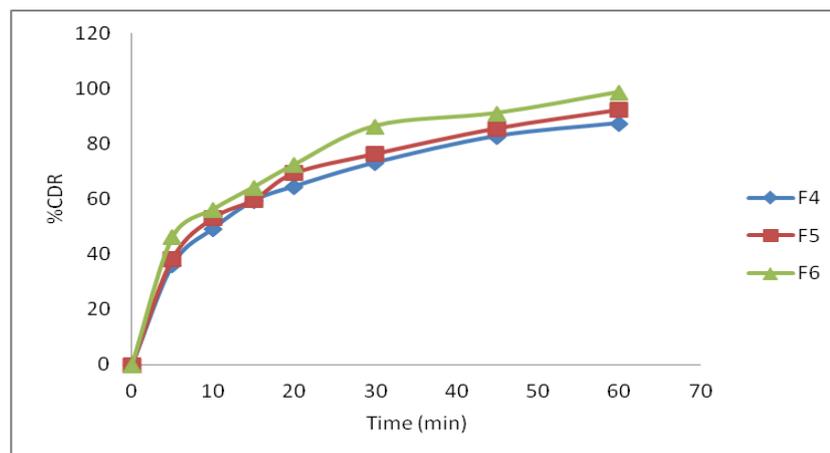


Fig. 10: In vitro drug release of formulations F4-F6.

From the in vitro drug release in studies it was observed that the formulations containing Crospovidone as a super disintegrant in different concentrations like 2,4, and 6%, reveals that the increased in the super disintegrant concentration decreases the drug release time and the F3 formulation containing SSG 6% concentration shows maximum amount of drug release (86.43%) at the end of 60mins.

Whereas formulations containing Lycoat as a super disintegrant in different concentrations like 2,4, and 6%, reveals that the increased in the super disintegrant concentration decreases the drug release time and the F6 formulation containing Lycoat with 6% concentration shows maximum amount of drug release (98.63%) at the end of 60mins.

So, F6 formulation containing 6% concentration of Lycoat shows max. release within 60mins so that it is chosen as optimized formulation.

Evaluation of Formaldehyde Treated Capsules

Physical tests

➤ **Identification attributes:** The size '0' capsules chosen were opaque, with white colored body and red cap. The normal capsule bodies were soft and sticky when touched with wet hand. After treating with formaldehyde, there were no significant changes in the physical appearance of the capsules except for the stickiness. The body of capsule was hard and non-sticking even when touched with wet hand due to treatment with the formaldehyde.

➤ **Visual defects:** Among 100 capsules body which were treated with formaldehyde, about 15 to 20 capsule bodies showed visual defects. They were found to be shrunk and distortion into different shapes due to the complete loss of moisture.

➤ **Dimensions:** Dimensional examination was done by using vernier calipers.

Average capsule length:

- Before formaldehyde treatment (untreated cap and body) : 20.3 mm
- After formaldehyde treatment (treated body and untreated cap) : 19.5 mm

Average diameter of capsule body:

- Before formaldehyde treatment : 7.1 mm
- After formaldehyde treatment : 6.9 mm

Average length of capsule body:

- Before formaldehyde treatment : 17.9 mm
- After formaldehyde treatment : 17.2 mm

❖ **Discussion:** On formaldehyde treatment, the "0" size capsules bodies showed a significant decreases in length and diameter and attained hardness.

Chemical test

➤ **Qualitative test for free formaldehyde:** The formaldehyde treated capsules were tested for the

presence of free formaldehyde by comparing color of sample solution with standard solution. It was found that the sample solution was not more intensity colored than the standard solution inferring that less than 20µg/ml of free formaldehyde was present in 25 capsule bodies.

❖ **Discussion:** Limit test for the presence of residual formaldehyde, indicated that the amount of formaldehyde present in treated capsules was well within limits.

Optimization of formaldehyde treated capsule bodies exposed at various time intervals viz., 2, 4, 6, 8, 10hrs

Table 9: Disintegration test for Treated Capsules.

| Code | Disintegration Time (hrs) | |
|--------------------------|---------------------------|---------------------|
| | 1.2 pH (2hrs) | 6.8 pH (upto 24hrs) |
| C1 (2 rd hr) | 2 | — |
| C2 (4 th hr) | 2 | 1 |
| C3 (6 th hr) | 2 | 5 |
| C4 (8 th hr) | 2 | 7 |
| C5 (10 th hr) | 2 | 12 |

❖ **Discussion:** Basing on the disintegration studies, it was observed that the 6th hr treated capsule (C3) remained intact for 7 hrs so lag time was maintained. C4, C5 remain intact for 9, 12 hrs respectively and therefore they were not selected for the formulation because the required lag time was 6hrs. As the required lag time is 6hrs, C3 (6th hr treated capsule) was selected as optimized time for formaldehyde treatment for further studies.

1. In vitro release studies

Dissolution study was carried out to measure the release rate of drug from prepared pulsincap formulation using USP I dissolution apparatus at 37⁰C using 2 different dissolution media of pH 1.2, pH 6.8 phosphate buffers in order to mimic in vivo GIT conditions. Initially first 2hrs of dissolution was conducted in pH 1.2 buffer, followed by 10hrs of dissolution study in pH 6.8 phosphate buffer.

Table 10: In vitro dissolution data of formulations F7 to F9.

| Time (hrs) | F7 | F8 | F9 |
|------------|-------|-------|-------|
| 0 | 0 | 0 | 0 |
| 1 | 2.36 | 0.36 | 0.16 |
| 2 | 10.41 | 0.96 | 0.64 |
| 3 | 13.49 | 2.58 | 0.97 |
| 4 | 26.48 | 16.43 | 1.23 |
| 5 | 42.16 | 29.54 | 1.54 |
| 6 | 59.63 | 82.41 | 2.68 |
| 7 | 63.18 | 98.36 | 76.35 |
| 8 | 79.42 | | 97.16 |
| 9 | 89.36 | | |
| 10 | 98.46 | | |

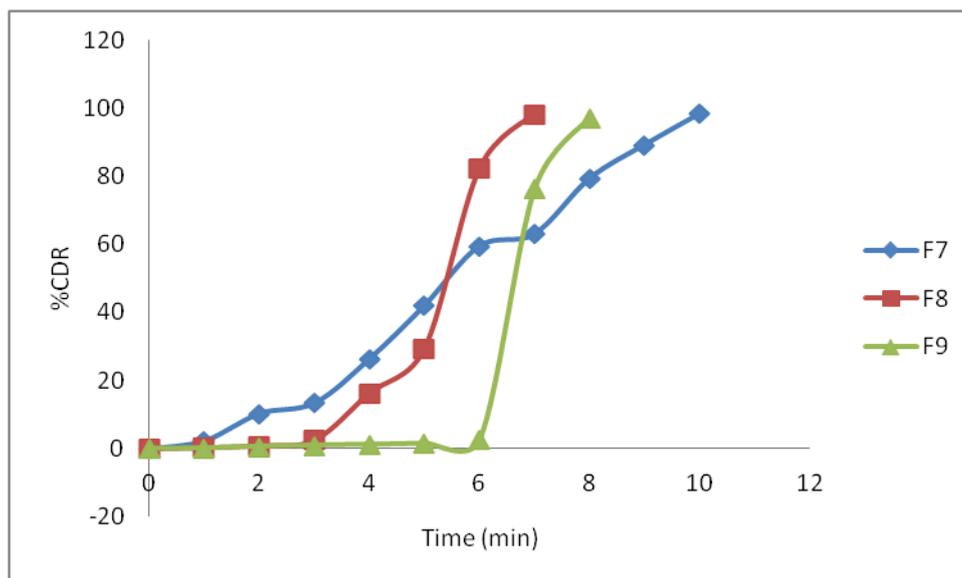


Figure 11 Dissolution plots for formulations F7 to F9

DISCUSSION

All the 3 formulations of Eplerenone pulsincaps were subjected to dissolution studies. Formulations F7, F8, F9 contain the hydrogel plug with combination of hydrophobic polymer and Hydrophilic polymer i.e., Ethyl cellulose: HPMC K200M in the ratio of 1:1, 2:1 & 1:2 of total 200mg weight of the plug.

It was observed that a proper lag time of 6 hours was maintained with minimal upper GIT drug release for the combination of Ethyl cellulose and HPMC K200M hydrogel plug in the 2:1. It was observed that as the concentration of ethylcellulose was increased the release rate of drug was delayed and finally burst release of drug

from the formulation occurred after lag time. So basing on these observations, of all the 3 pulsincap formulations, F9 formulation containing hydrogel plug of ethyl cellulose & HPMC K200M in 2:1 ratio was selected as optimized pulsincap formulation.

Release Kinetics

Dissolution data was fitted in Zero order, First order, Higuchi's and koresmayer peppas equations. The regression coefficient "R" values for zero order, first order, higuchi's and peppas for formulation F9 was found to be 0.553, 0.482, 0.360 and 0.612 respectively.

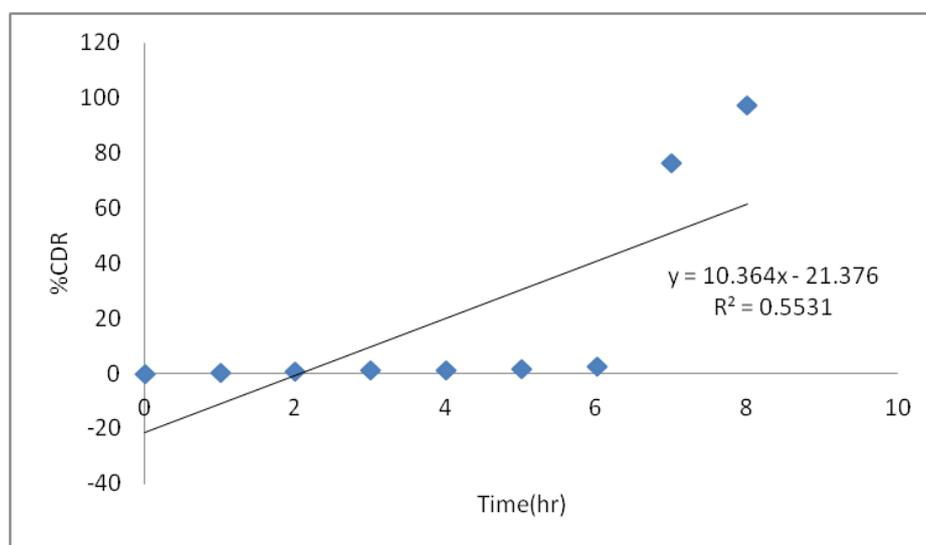


Figure 12: Zero order plot for optimized formulation F9.

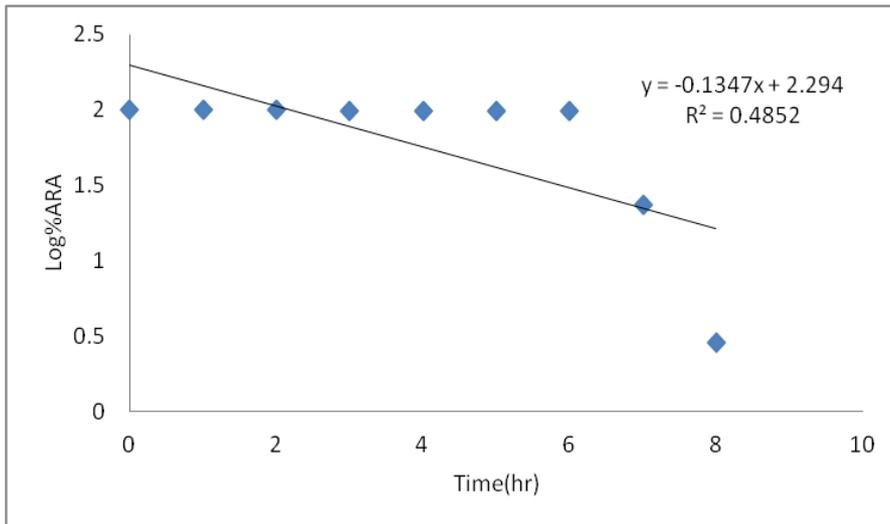


Figure 13: First order plot for optimized formulation F9.

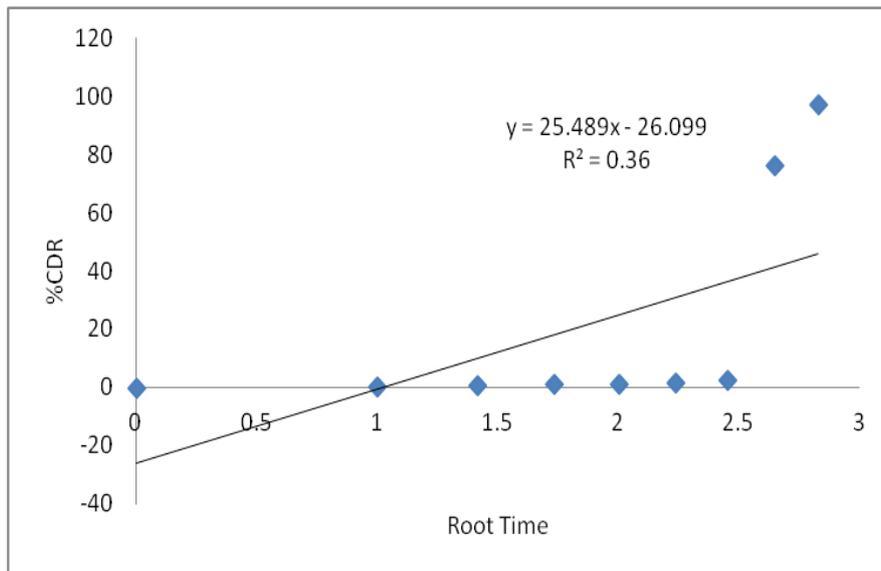


Figure 14: Higuchi's order plot for optimized formulation F9.

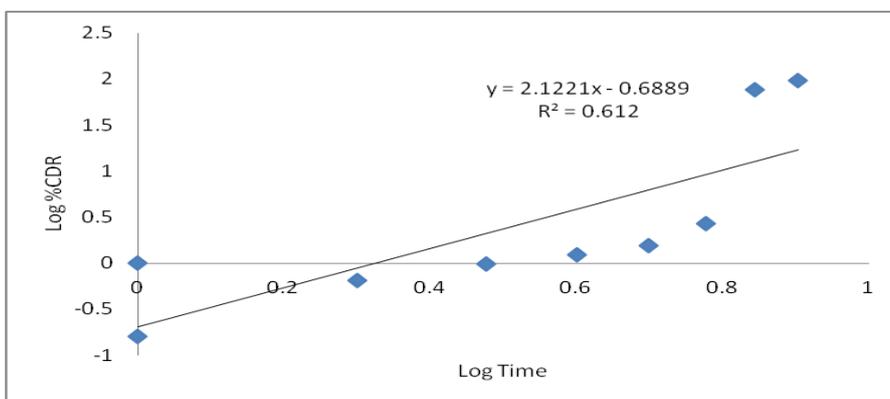


Figure 15: Koresmayer peppas order plot for optimized formulation F9.

DISCUSSION

To analyze the mechanism of drug release from optimized F9 pulsincap formulation, data obtained from the drug release studies was subjected to different kinetic treatments. The correlation coefficient (R) was used as

indicator of the best fitting for each of the models considered. The drug release kinetics for the optimized formulation F9 followed the zero order kinetics and follows super case II transport mechanism.

Summary

In the present study, attempt was made to target the drug to the colon and intentionally delaying the drug absorption from the therapeutic point of view in the treatment of lowering cholesterol.

- Prior to formulation, Preformulation studies were carried out in order to establish compatibility between Eplerenone and excipients by FTIR spectroscopy. The results revealed that the drug and polymers were satisfactorily compatible, without any significant changes in the chemical nature of Eplerenone.
- The capsule bodies were made insoluble by formaldehyde treatment by exposing at various time intervals viz., 2, 4, 6, 8, 10hrs and then optimized by using disintegration studies and finally the optimized treated capsule bodies were then subjected to various physical and chemical tests such as identification attributes, visual defects, dimensional studies and qualitative test for free formaldehyde.
- Total 6 formulation was formulated using super disintegrant in different ratios by direct compression method.
- The formulations were subjected to flow properties and FTIR study. Based on the results obtained F3 containing 6% Crospovidone was considered as the optimum powder blend for fabrication of pulsincap capsule.
- Different concentration of the polymers like HPMC K200M, ethyl cellulose in combination were used for the preparation of hydrogel plug to maintain the suitable lag period and it was found that the drug release was controlled by the proportion of polymers used.
- The powder blend F8 was filled into the 6th hr formaldehyde treated capsule bodies and plugged with hydrogel polymers, 200mg hydrogel plug. The ratios of hydrophobic polymer like ethyl cellulose and HPMC K200M were taken in 1:1, 1:2, and 2:1. Finally after arranging the plug, the joint of the capsule body and cap was sealed with a small amount of 1% w/v ethyl cellulose ethanolic solution. The prepared pulsincaps were evaluated for Invitro studies.
- All the 3 formulations of Eplerenone pulsincaps were subjected to dissolution studies. Formulations F7, F8, F9 contain the hydrogel plug with combination of hydrophobic polymer and Hydrophilic polymer i.e., Ethyl cellulose: HPMC K200M in the ratio of 1:1, 2:1 & 1:2 of total 200mg weight of the plug.

It was observed that a proper lag time of 6 hours was maintained with minimal upper GIT drug release for the combination of Ethyl cellulose and HPMC K200M hydrogel plug in the 1:2. It was observed that as the concentration of Hydrophilic polymer was increased the release rate of drug was delayed and finally burst release of drug from the formulation occurred after lag time. So basing on these observations, of all the 3 pulsincap

formulations, F8 formulation containing hydrogel plug of ethyl cellulose & HPMC K200M in 1:2 ratio was selected as optimized pulsincap formulation

CONCLUSION

From the results obtained from executed experiments it can be concluded that the Preformulation studies like pH, solubility and UV-analysis of Eplerenone were compiling with BP standards. The FTIR Spectra revealed that, there was no interaction between polymer and drug. The solubility studies of empty gelatin capsule bodies, which were cross linked with formaldehyde treatment, revealed that they are intact for 24 hrs, and hence suitable for colon targeting. The polymers like HPMC K200M, and Ethylcellulose can be used as hydrogel plugs to delay the release of Eplerenone. The result of micromeritic properties showed good flow property of the powder blend indicating uniform distribution of drug within the various batches of capsule with negligible loss during the formulation stage. In conclusion, this system can be considered as one of the promising formulation techniques for preparing time specific drug delivery systems and in Chronotherapeutic management of high blood pressure. From the preliminary trials it was concluded that it is possible to formulate the pulsatile drug delivery system by the design of time modified chronopharmaceutical formulation.

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