

# Pulsatile Chronotherapeutic Drug Delivery for Controlling Early Morning Surge in Blood Pressure; Effect of Coating on Eplerenone *In-vitro*, *In-vivo* Release and Urinary Na/K Ratio

Maha Abd El hameed Marzouk<sup>1</sup>, Manal Kamal Darwish<sup>1</sup>,  
Ghada Ehab Yassin<sup>1,2</sup>, Marwa Adel Abd El-Fattah<sup>1</sup>

<sup>1</sup>Department of Pharmaceutics and pharmaceutical technology, Faculty of Pharmacy (girls), Al-Azhar University, Cairo, Egypt, <sup>2</sup>Department of Pharmaceutics, Faculty of Pharmacy, October University for Modern Sciences and Art (MSA), Giza, Egypt

The objective of the present study was to develop time delayed chronotherapeutic formulation of Eplerenone (Ep) to provide rapid drug release after a pre-determined lag time for the treatment of early morning hypertension. Cyclodextrin complexation was used to prepare fast release Ep core tablets. The developed core tablets were then coated with different rate-controlling polymers using compression coating technique. The developed tablets were evaluated for hardness, friability, drug content and swelling index. The *in-vitro* drug release was carried out to study the effect of different coating materials on drug release and lag time. Tablets selected for stability study were those showing lag time of 5–7 hours followed by complete drug release; F2, F3, F7, F8, and F12. The *in-vivo* study was carried out on tablets with the highest  $t_{90}$  as compared to commercial tablets after being administered to healthy human volunteers where plasma Ep and urinary Na/K ratio were determined. Results suggested that this approach was able to provide delayed release Ep formulations that will be useful for patients with morning surge in blood pressure.

**Keywords:** Eplerenone. pH-independent chronotherapeutic delivery system. Early morning hypertension. *In-vivo* study. Urinary Na/K ratio.

## INTRODUCTION

Chronotherapeutic is a treatment method in which *in-vivo* drug availability is timed to match the circadian rhythms of disease to produce maximum health benefit and to minimize adverse effects (Airemwen *et al.*, 2017). Pulsatile drug delivery technologies are most widely used to target the drug as per the circadian behavior of diseases where the drug is to be released rapidly and completely after a lag time (Patil *et al.*, 2018). The role of chronotherapeutic in hypertension management is based on the observation that blood pressure shows circadian mediated variation throughout the day where it tends to

be higher in the early morning hours and lower in the evening hours (Kumar, Suresh, 2018). Pulsatile delivery system can be achieved by coating immediate release core tablets by either film coating or compression coating which dissolves or disintegrates gradually to release the drug after a lag time. Compression coated tablets offer coating technique free of solvents, thus this technique is considered to be safe and inexpensive since it doesn't require special coating equipment and also provides higher stability compared to film coating (Kumar, 2017).

Eplerenone (Ep) is a selective aldosterone receptor antagonist that selectively prevents aldosterone receptor binding which in turn results in the blockage of the renin angiotensin aldosterone system (RAAS), with subsequent inhibition of sodium reabsorption that affects blood pressure and the cardiovascular system (Khamis,

\*Correspondence: M. A. Abd. El-Fattah. Department of Pharmaceutics, Faculty of Pharmacy, Al Azhar university, Nasr City, Cairo, Egypt. E-mail: Marwa\_adel2311@yahoo.com. ORCID: 0000-0002-5275-0810

2019). Ep causes increase in urinary  $\text{Na}^+/\text{K}^+$  ratio and clear evidence suggests the use of urinary  $\text{Na}^+/\text{K}^+$  as a translatable biomarker of mineralocorticoid receptor (MR) antagonism in single-dose studies (Eudy *et al.*, 2011). Ep was designed to enhance selective binding to the MR while minimizing the binding to progesterone and androgen receptors. This epoxy modification of the steroidal backbone reduced progestational and antiandrogenic side effects compared with spironolactone while maintaining aldosterone blocking activity (Kolkhof, Barfacker, 2017).

Improving drug solubility is a major concern of drug formulators, especially for drugs classified as BCS (Biopharmaceutical Classification System) Class II drugs (ex. Ep) where the dissolution rate represents the rate-determining step of the absorption process. Inclusion complexation using  $\beta$ -cyclodextrin ( $\beta$ -CD) was used in this study as a solubility enhancing technique due to the ability of cyclodextrin molecules to include guest molecules inside their cavities forming inclusion complex with enhanced solubility (Khames, 2019).

Several gel-forming polymers were utilized in the coating layer; Polyethylene oxide water-soluble resin (PEO WSR 1105), Hydroxypropyl methylcellulose (HPMC K4M) and Carbopol 71G, the hard gel layer formed by these polymers around the core tablet acts as a barrier that prevents drug release. Avicel and Polyethylene glycol (PEG) were used in varying levels together with the above mentioned gel-forming polymers to control lag time by either acting as pore-forming agent for Avicel or as a channeling agent in case of PEG. Release of drug occurs upon erosion of the matrix in the case of PEO WSR and HPMC or breaking the hydrogel structure formed by Carbopol by the osmotic pressure created when the hydrogel got fully hydrated (Rabadia *et al.*, 2012; Nokhodchi *et al.*, 2012).

The target of the present study was to investigate the effect of different coating polymers on the Eplerenone release from immediate release core tablets with the aim to maintain a lag time of 5 – 7 hours which is desirable for chronotherapeutic application in early morning hypertension to deliver the drug at the time of the hypertension surge.

## MATERIAL AND METHODS

### Materials

Ep was obtained from Hetero Pharma Company (China). Avicel PH 102, HPMC K4M and Polyethylene glycol (PEG 6000) were obtained as gift samples from AUG Pharm for Pharmaceutical Industries (Egypt).  $\beta$ -cyclodextrin ( $\beta$ -CD) was obtained as a gift sample from MUP Pharm for Pharmaceutical Industries (Egypt). Carbopol 71G was obtained as a gift sample from Lubrizol Corporation, Ohio (USA). Polyethylene oxide water-soluble resin (PEO WSR 1105) was obtained from Colorcon Limited, Dartford (UK).

### METHODS

#### Preparation of solid inclusion complex

The inclusion complex was prepared by co-grinding method with  $\beta$ -CD as a complexing agent. Physical mixtures of Ep and  $\beta$ -CD in 1:1, 1:2 and 1:3 molar ratios were prepared by simple trituration of accurately weighed different components using mortar and pestle. All physical mixtures were ground for 30 minutes then sieved through 60 size mesh (Tan *et al.*, 2013).

#### Solubility and phase solubility studies

Ep solubility was assessed by adding an excess amount of Ep to a volumetric flask containing 25 mL pH 6.8 phosphate buffer. The flask was shaken at 37 °C for 24 hours in a shaking water bath (Gallen Kamp, England). The obtained samples were filtered through Whatman filter paper. The filtrate was diluted and analyzed by UV-Vis spectrophotometer (Jenway, model 6405 UV/Vis, England) at 243 nm (Banode *et al.*, 2011) using pH 6.8 phosphate buffer as a blank. Experiment was carried out in triplicate (Zuheir, Samein, Aiash, 2013).

The phase solubility diagram was used to study the effect of cyclodextrin complexation on the drug solubility by measuring stability constants ( $K_c$ ). Phase solubility studies were done according to the Higuchi and Connors method where an excess amount of Ep was

added to 50 mL of aqueous solutions containing various concentrations of  $\beta$ -CD into glass vials. Contents were then shaken for 48 hours at room temperature (Higuchi, Connors, 1965). After equilibrium, samples were withdrawn, filtered, diluted appropriately and analyzed by UV-Vis spectrophotometer at 243 nm (Banode *et al.*, 2011). Measurements were done in triplicate. Kc was calculated from the straight portion of the phase solubility diagram using the following equation:

$$Kc \text{ (in } M^{-1}) = \text{slope} / (\text{Intercept} \times (1 - \text{slope}))$$

Where, M = Molar concentration, Kc = Apparent stability constant (Moghadam *et al.*, 2018).

#### *Characterization of the inclusion complex Differential scanning calorimetry (DSC)*

DSC thermograms of drug,  $\beta$ -CD, physical mixture and the formed inclusion complex were recorded using DSC (Shimadzu, model DSC-50, Japan) to confirm Ep- $\beta$ -CD complex formation (Chen *et al.*, 2018). Also, the DSC thermograms of Ep and its mixture with suggested excipients (1:1 ratio) were recorded to confirm compatibility between Ep and the used excipients by observing changes in the melting point of the drug (Dumpa *et al.*, 2018).

#### *Powder x-ray diffraction (PXRD)*

The X-ray diffraction pattern of the selected inclusion complex prepared by the co-grinding method was compared with that of pure Ep,  $\beta$ -CD and their physical mixture using Diffractometer (X-Ray Generator Philips, P.W. 3710, Netherland). This was performed by measuring  $2\theta$  in the range of  $4^\circ$  to  $50^\circ$ . The structural analysis of the complex and binary systems was performed using an X-ray diffractometer (Inoue *et al.*, 2018).

#### *Formulation of Ep core tablets*

Immediate release core tablets were prepared using the previously developed inclusion complex. An amount equivalent to 25 mg Ep was mixed with

magnesium stearate as a lubricant (1%) and the directly compressible diluent (Avicel pH 102) to 100 mg. The resulting blend was mixed for about 10 minutes and then compressed with a tablet compression machine (single punch tablet press, Shanghai, China) to form a tablet with a diameter of 8 mm and weight of 100 mg each.

#### *Post compression evaluation of core tablets*

The prepared core tablets were subjected to physicochemical evaluation tests. Uniformity of weight was studied where 20 tablets were selected randomly, weighed individually and the average was calculated. Tablet hardness was measured using tablet hardness tester (pharma test, PTB301, Germany) where the maximum force required to break the tablets was measured in kg/cm<sup>2</sup>. Friability was measured using Tablet friability test apparatus (VEEGO, model FT-2D, India) where the % friability was expressed as loss of weight (Swathi *et al.*, 2018).

Drug content was measured by powdering ten tablets and dissolving weight equivalent to 25 mg Ep in pH 6.8 phosphate buffer and measuring the absorbance spectrophotometrically at 243 nm. The test was carried out in triplicate (Swathi *et al.*, 2018).

*In-vitro* disintegration time (DT) was determined using USP disintegration apparatus (VEEGO, Model VTD-3D India) with 900 mL pH 6.8 phosphate buffer at  $37 \pm 0.5^\circ\text{C}$  (Hasan, Khalil, 2017). The test was carried out in triplicate and results expressed as mean  $\pm$  SD.

*In-vitro* dissolution of the prepared core tablet as compared to pure Ep (25 mg) was performed using USP Type II (Paddle type) dissolution apparatus (USP Standard, scientific, DA6D, Bombay-400- 069, India) at a speed of 50 rpm. A volume of 900 mL pH 6.8 phosphate buffer maintained at  $37 \pm 0.5^\circ\text{C}$  was utilized as dissolution medium. Aliquots of 5 mL of dissolution medium were withdrawn at specific time intervals (10, 20, 30, 40, 50, 80, 90 & 100 min) and filtered through Millipore filter paper 0.45 $\mu\text{m}$ . The amount withdrawn was replaced with the same volume of pre-warmed dissolution medium. After filtration, the amount of drug released was determined by measuring the absorbance spectrophotometrically at 243 nm (Kukadiya, Desai,

Swamy S, 2014). The test was carried out in triplicate. Sink condition was adopted throughout the experiment.

#### Formulation of press-coated tablets

Different pH-independent polymers were used as coating layer. Press coating materials (Table I) were weighed mixed for about 10 minutes and used to prepare

press-coated tablets by direct compression where 125 mg of coating material was placed in 10 mm die to make a powder bed. The previously developed core tablet was placed at the center on the polymer bed and the remaining 125 mg of coating material was filled into the die. The contents were then compressed using a tablet compression machine (single punch tablet press, Shanghai, China), to form a tablet with a diameter of 10 mm.

**TABLE I - Suggested composition of the coating layer**

Formula code	PEO WSR 1105 (mg)	Carbopol 71 G (mg)	HPMC K4M (mg)	PEG 6000 (mg)	Avicel pH 102 up to coat (mg)
F1	62.5	-	-	-	250
F2	87.5	-	-	-	250
F3	125	-	-	-	250
F4	187.5	-	-	-	250
F5	250	-	-	-	-
F6	-	25	-	-	250
F7	-	37.5	-	-	250
F8	-	55	-	-	250
F9	-	62.5	-	-	250
F10	-	-	37.5	212.5	-
F11	-	-	62.5	187.5	-
F12	-	-	87.5	162.5	-
F13	-	-	112.5	137.5	-

\*PEO WSR: Polyethylene oxide water soluble resin, HPMC: Hydroxypropyl methyl cellulose, PEG: Poly ethylene glycol.

#### Post compression evaluation of eplerenone press-coated tablets

Press-coated tablets were evaluated for physicochemical tests including uniformity of weight, hardness, friability and drug content as described for core tablets.

The swelling index (SI) was measured where the extent of swelling was measured as % weight gain by the tablet. Preweighed tablet of each formulation was placed in 50 mL of distilled water. At the end of 0.5 h, 1, 2, 3, 4, 5 and

6 hours, the tablet was withdrawn, blotted with filter paper to remove the excess water on the surface and accurately weighed. Swelling index was calculated by the formula:

$$S.I = \{(M_t - M_o) / M_o\} \times 100$$

Where, S.I = swelling index,  $M_t$  = tablet weight at time  $t$  (hour) and  $M_o$  = tablet weight of tablet at time zero (Krishnaveni, Muthukumaran, Krishnamoorthy, 2013). The experiment was carried out in triplicate and the average was calculated.

Dissolution was carried out using USP dissolution apparatus type II using paddle at 50 rpm. A volume of 900 mL of 0.1N HCl (pH 1.2) was used for 2 hours followed by 900 ml pH 6.8 phosphate buffer. The temperature was set at  $37 \pm 0.5$  °C. A volume of 5 mL of the sample was withdrawn at regular time intervals up to 9 hours and replaced with the same volume of pre-warmed fresh dissolution medium. After filtration, the amount of drug released was determined by measuring the absorbance spectrophotometrically at 243 nm (Kukadiya, Desai, Swamy S, 2014). The test was carried out in triplicate. Sink condition was maintained throughout the experiment.

#### *Accelerated stability study*

Stability studies were performed on the selected formulation to assess their stability as per drug content while storing at 30 and 40 °C under 75% RH for 6 months.

Formulations were evaluated for *in-vitro* dissolution at the end of 6 months. The similarity factor ( $f_2$ ) was calculated to compare the dissolution profiles of stored tablets at 30 °C and 40 °C with the fresh tablets according to the following equation:

$$f_2 = 50 * \log \left\{ \left( 1 + \left( \frac{1}{n} \right) \sum_{t=1}^n (R_t - T_t)^2 \right) \right\}$$

Where  $R_t$  and  $T_t$  are the percent of drug dissolved at each time point for fresh and the stored tablets respectively (Dumpa *et al.*, 2018).

#### *In-vivo studies on human volunteers*

##### *Determination of eplerenone in plasma*

##### *Study design*

All procedures followed were carried out following the ethical standards of the responsible committee on human experimentation (the ethical committee, Faculty of Pharmacy, Al-Azhar University) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study. Volunteers were informed about possible risks and adverse effects of taking the drug, Volunteers were

aged 25-33 years, without a history of medical disease and taking no other medication.

The study was designed as an open-label, single-dose study with 4 groups, three volunteers per group (Abdelbary *et al.*, 2014). Human subjects were instructed to fast 12 hours before the study. They remained under controlled dietary and liquid intake until the end of the study. They were observed medically during the study.

Human volunteers were divided into 4 groups:

Group 1: (Control): Subjects did not take any medication (subjected to study before administration of the tablets).

Group 2: Subjects administrated F3 tablets (containing core tablet coated with PEO WSR/ Avicel pH 102 combination) with a sufficient quantity of water.

Group 3: Subjects administrated F8 tablets (containing core tablet coated with Carbopol 71G/ Avicel pH 102 combination) with a sufficient quantity of water.

Group 4: Subjects administrated Eplerenon genericon (commercial tablets) with a sufficient quantity of water.

#### *Collection of blood samples*

Blood samples (5 mL) were collected in heparinized tubes at 0 (predose), 30, 60, 120, 180, 240, 480, 600, 720 and 1440 minutes after dose administration. The plasma was immediately separated by centrifugation at 3,000 rpm for 10 minutes. Labeled plasma samples were then stored at -20 °C until analyzed.

Samples were deproteinated by adding acetonitrile and centrifuged at 3,000 rpm for 10 minutes. The supernatant (10 µL) was then injected to (Agilent LC-MS-MS, USA) using autosampler. Mass spectrometer equipped with an ESI source was set with the drying gas ( $N_2$ ) at a flow rate of 10 L/min, nebulizer pressure of 40 psi, drying gas temperature of 350 °C and capillary voltage of 4,000 V. The eluate was fed to electron sprayer detector operating in the positive ion mode.

#### *Determination of pharmacokinetic parameters*

Pharmacokinetic parameters including  $C_{max}$ ,  $t_{max}$ , area under concentration-time curve (AUC), elimination rate constant (Ke) and elimination half-life ( $t_{1/2}$ ) were

calculated for test and commercial tablets. ANOVA was carried on  $t_{\max}$ ,  $AUC_{0-24}$  and MRT using Tukey-Kramer Multiple Comparison Test.

#### Determination of $Na^+/K^+$ ratio in urine

Urine samples were collected from the same volunteers subjected to the previous study before administration, 4 hours and 8 hours after dosing.

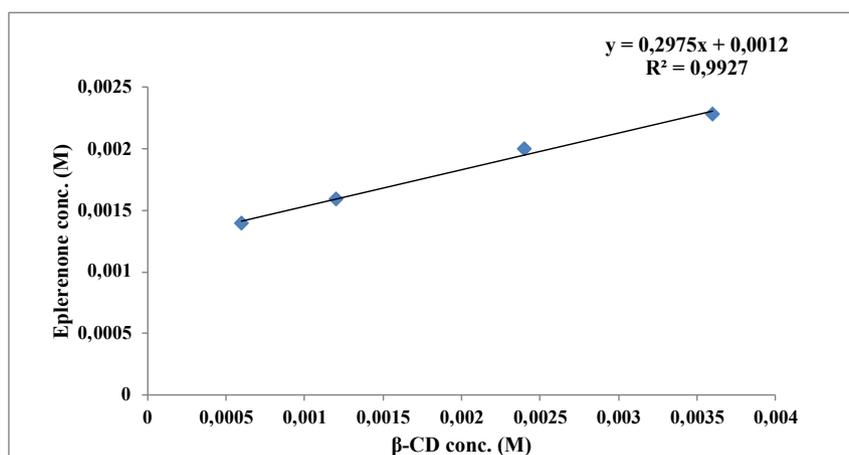
Parameters estimated were urinary  $Na^+/K^+$ , to study time for drug effect on urinary  $Na^+/K^+$  ratio using Flamephotometer (FP 20, Italy) based on atomic emission. Analysis was performed by measuring the emission intensity of solution containing the metal salts and comparing it to standard (Doroodm, FatemehGhasemi, 2017).

## RESULTS AND DISCUSSION

### Solubility and phase solubility studies

The solubility of pure Ep in pH 6.8 phosphate buffer was found to be 0.367 mg/mL which indicated poor Ep solubility according to the USP solubility classification (Al-Shahrani, Ansari, 2018).

Inclusion complexation was done in an attempt to improve Ep solubility (BCS class II) since it is very slightly soluble in water (Khames, 2019). Phase solubility study of Ep (Figure 1) showed that the solubility profile of Ep in distilled water was influenced by  $\beta$ -CD concentration at room temperature. The correlation coefficient squared value ( $r^2$ ) was  $> 0.95$  ( $r^2 = 0.9927$ ) and thus it was regarded as  $A_L$  type (Higuchi, Connors, 1965). Since slope was less than 1, complex formation at 1:1 (Ep to  $\beta$ -CD) molar ratio was suggested (Kfoury *et al.*, 2016).



**FIGURE 1** - Phase solubility diagram of Ep with  $\beta$ -CD.

\* $\beta$ -CD: betacyclodextrin.

### Characterization of the inclusion complex

#### Differential scanning calorimetry (DSC)

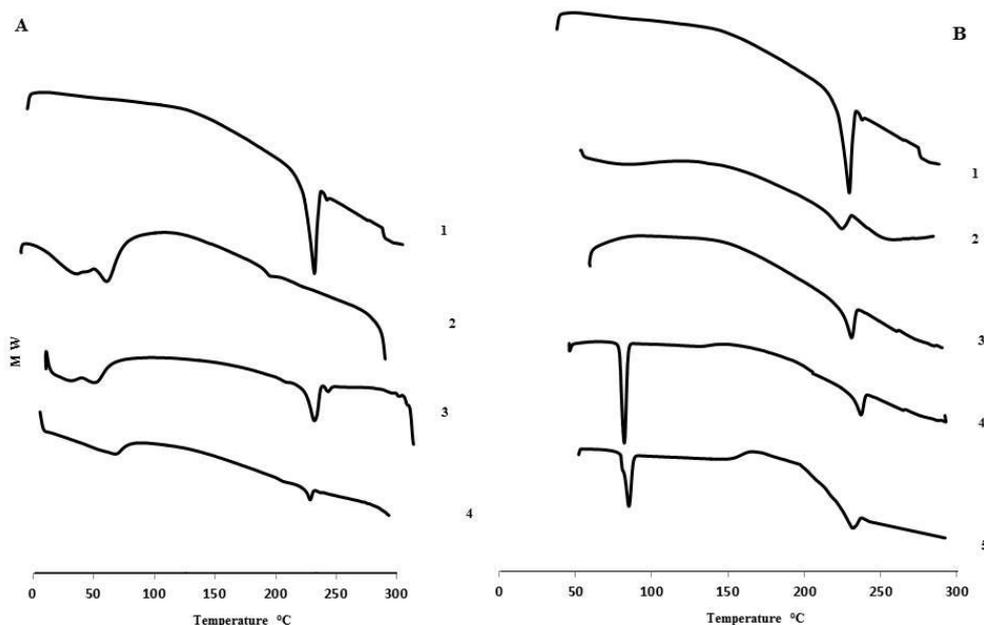
Ep (Figure 2. A. 1) showed an endothermic peak at 236 °C due to its melting while  $\beta$ -CD exhibited (Figure 2. A. 2) a characteristic broad peak of water loss from 60-120 °C (Khames, 2019; Patil *et al.*, 2012). The thermal behavior of the physical mixture (Figure 2. A. 3) was a

combination of the thermal behavior of both. However, the thermal behavior of the inclusion complex (Figure 2. A. 4) appeared significantly different from either pure form or the physical mixture where  $\beta$ -CD showed a broader peak shifted to 82 °C and Ep showed a lower intense peak indicating complex formation and reduction in drug crystallinity (Sherje, Jadhav, 2018).

The melting endotherm of Ep was observed unchanged in the physical mixtures with excipients

(Figure 2. B) suggesting good physical compatibility. Minor changes in the drug endotherm as broadening of the peak or its shifting to higher or lower temperature could

be due to the drug mixing with excipient which lowered the purity of each ingredient in the mixture and indicated no potential incompatibility (Singh, Nath, Mater, 2011).



**FIGURE 2** - (A) DSC thermograms of (1) Eplerenone, (2)  $\beta$ -CD, (3) physical mixture and (4) Eplerenone  $\beta$ -CD complex, (B) DSC Thermograms of (1) Eplerenone, (2) Ep/Carbopol 71 G physical mix., (3) Ep/HPMC K4M physical mix., (4) Ep/PEG 6000 physical mix., (5) Ep/PEO WSR 1105 physical mix.

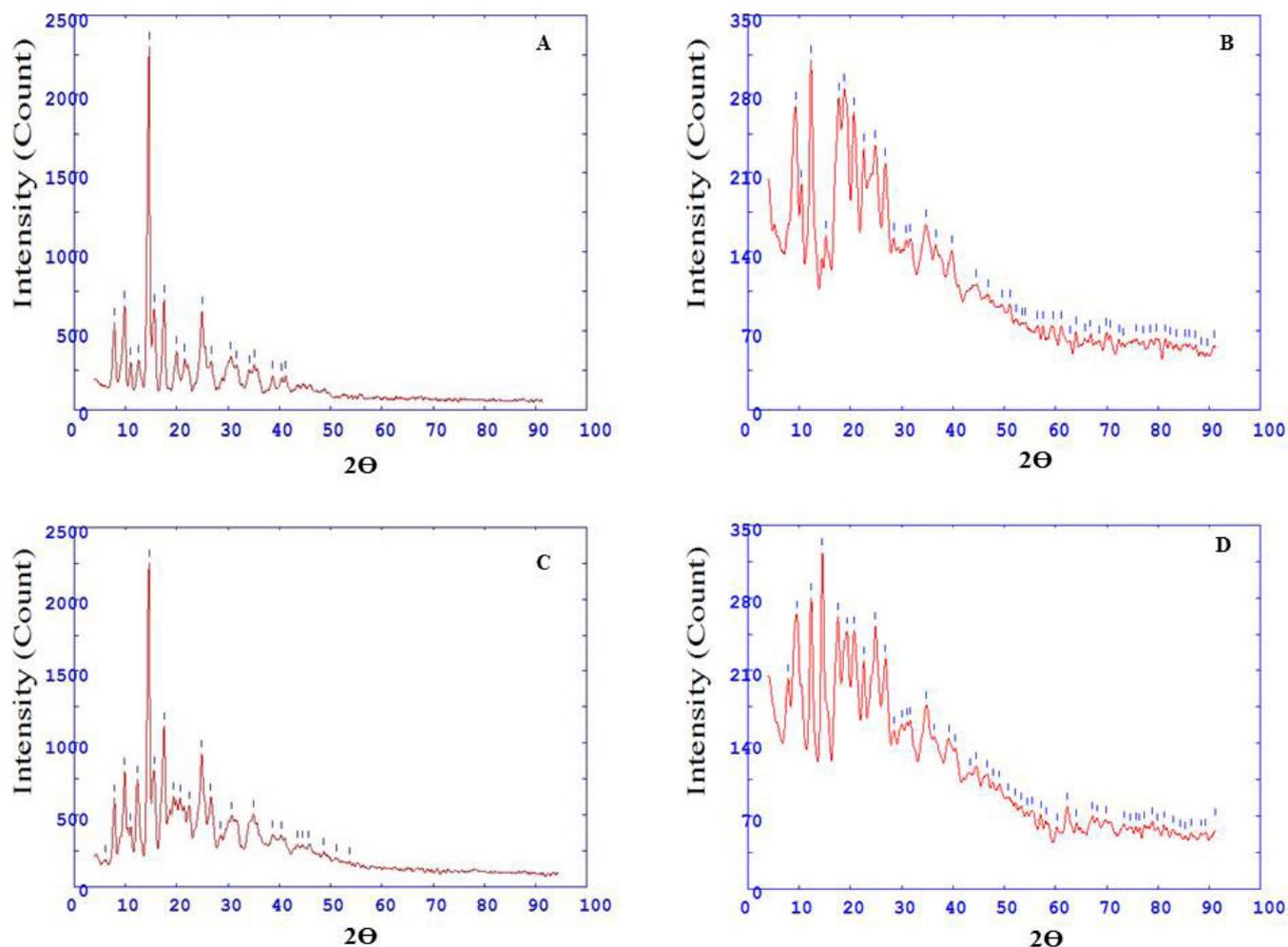
\*PEO WSR: Polyethylene oxide water-soluble resin, HPMC: Hydroxypropyl methylcellulose, PEG: Polyethylene glycol.

#### Powder x-ray diffraction (PXRD)

The diffractogram of Ep (Figure 3. A) showed several sharp peaks at diffraction angles; ( $2\theta$ ) of  $7.813^\circ$ ,  $9.847^\circ$ ,  $11.001^\circ$ ,  $12.583^\circ$ ,  $14.596^\circ$ ,  $15.581^\circ$ ,  $17.543^\circ$ ,  $24.911^\circ$  and  $30.558^\circ$  indicating crystalline form of the drug. Physical mixture diffractogram (Figure 3. C) was simply a superposition of individual components indicating the

presence of Ep in its crystalline form and no formation of new physical form.

The diffractogram of the inclusion complex (Figure 3. D) differed markedly from either pure form or the physical mixture where the intensity of the peaks was markedly decreased which indicated inclusion complex formation and reduction in drug crystallinity (Inoue *et al.*, 2018).



**FIGURE 3** - PXR D patterns of (A) Eplerenone, (B)  $\beta$ -CD, (C) physical mix., (D) inclusion complex.

\*PXR D: powder x-ray diffraction.

### Post compression evaluation of core tablets

All tablets were white in color. Hardness, % friability and thickness were all within acceptable limits as per USP (Table II).

Disintegration time (DT) of the core tablets prepared with Ep-  $\beta$ -CD inclusion complex showed rapid disintegration (Table II) which could be due to high water-soluble components in the formulation ( $\beta$ - CD) (Nagar *et al.*, 2011). For pulsatile release, DT had to be short to obtain the burst effect (Jagdale *et al.*, 2014).

Ep release from core tablets (Figure 4) showed over 80% drug release within the first 10 minutes since complexation with  $\beta$ -CD greatly improved Ep dissolution

as compared to pure Ep (Figure 4) due to increased solubility and rapid wettability of the complexed drug.  $\beta$ -CD exhibits a hydrophilic exterior with a hydrophobic internal cavity where the guest molecule could fit into, thus increasing its affinity toward water and increasing the dissolution (Garrido *et al.*, 2017).

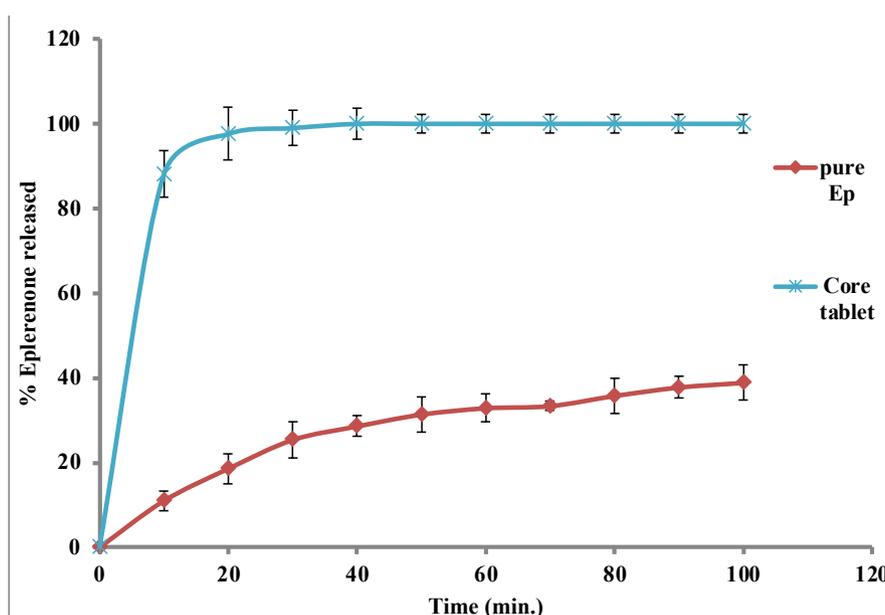
$\beta$ -CD has surfactant-like properties because of its hydrophilic exterior surface which can lower the interfacial tension between poorly soluble drugs and the dissolution medium, leading to enhanced drug dissolution (Jayaprabha, Joy, 2015).

The prepared tablets showed satisfactory hardness, % drug content and fast drug release. So, it was considered suitable to be coated for further studies.

**TABLE II** - Post Compression physical parameters of core tablets

<b>Weight (mg) ± SD</b>	<b>100.05 ± 0.07</b>
<b>Thickness (mm) ± SD</b>	2.27 ± 0.028
<b>Hardness (Kg/cm<sup>2</sup>) ± SD</b>	3.5 ± 0.035
<b>Friability % ± SD</b>	0.55 ± 0.07
<b>DT (sec.) ± SD</b>	48.00 ± 4.24
<b>Drug content (%) ± SD</b>	100.3 ± 0.42

\*DT: Disintegration time.



**FIGURE 4** - *In-vitro* dissolution of pure Eplerenone and Eplerenone from core tablets prepared by the inclusion complex.

### Post compression evaluation of eplerenone press-coated tablets

All tablets were white in color and round. All tablets confirmed the requirement of content uniformity. Hardness, % friability and thickness were all within acceptable limits as per USP (Table III).

In swelling studies (Figures 5 A-C), it was observed that when the tablet comes in contact with water, it undergoes wetting starting from its surface followed by progression into the inner matrix core through microscopic pores (Semalty, Bisht, Semalty A, 2012).

SI was shown to be dependent upon the quantity of the polymer and exposure time of the tablet to the medium; this explains the decreased value of SI in F6

at the end of the 2<sup>nd</sup> hour due to decreased Carbopol 71 G level which led to faster tablet erosion (Harikrishnan, Madhusudhan, Santhiagu, 2016).

A direct correlation between swelling and lag time was observed and thus formulations with maximum swelling indices had longer lag time (Domala, Eedara, Dhurke, 2014).

During dissolution, it was found that the polymers used act by gel formation. PEO WSR 1105 and HPMC K4M containing tablets (Figure 6 A, C) got hydrated upon contact with dissolution media and formed a gel layer around the core. The rate of drug diffusion of the gel layer and tablet erosion control the drug release rate (Nokhodchi *et al.*, 2012; Shah, Chaudhary, Mehta, 2014). The hydrogel formed by Carbopol 71G (Figure 6 B) when

got fully hydrated; the osmotic pressure built within broke up the structure by sloughing off discrete pieces of the hydrogel (Rabadia *et al.*, 2012).

Increasing level of PEO WSR 1105 and HPMC K4M (F4, F5, and F10) results in higher lag time due to increased rigidity of the gel layer which preserved the inner core. Increasing Carbopol level (F9) also increased lag time since the gel layer formed became stronger, with fewer regions of low microviscosity in swollen tablets (fewer interstitial spaces between the microgels) (FCR Tablets- Lubrizol Pharmaceutical Bulletin 31, 2011).

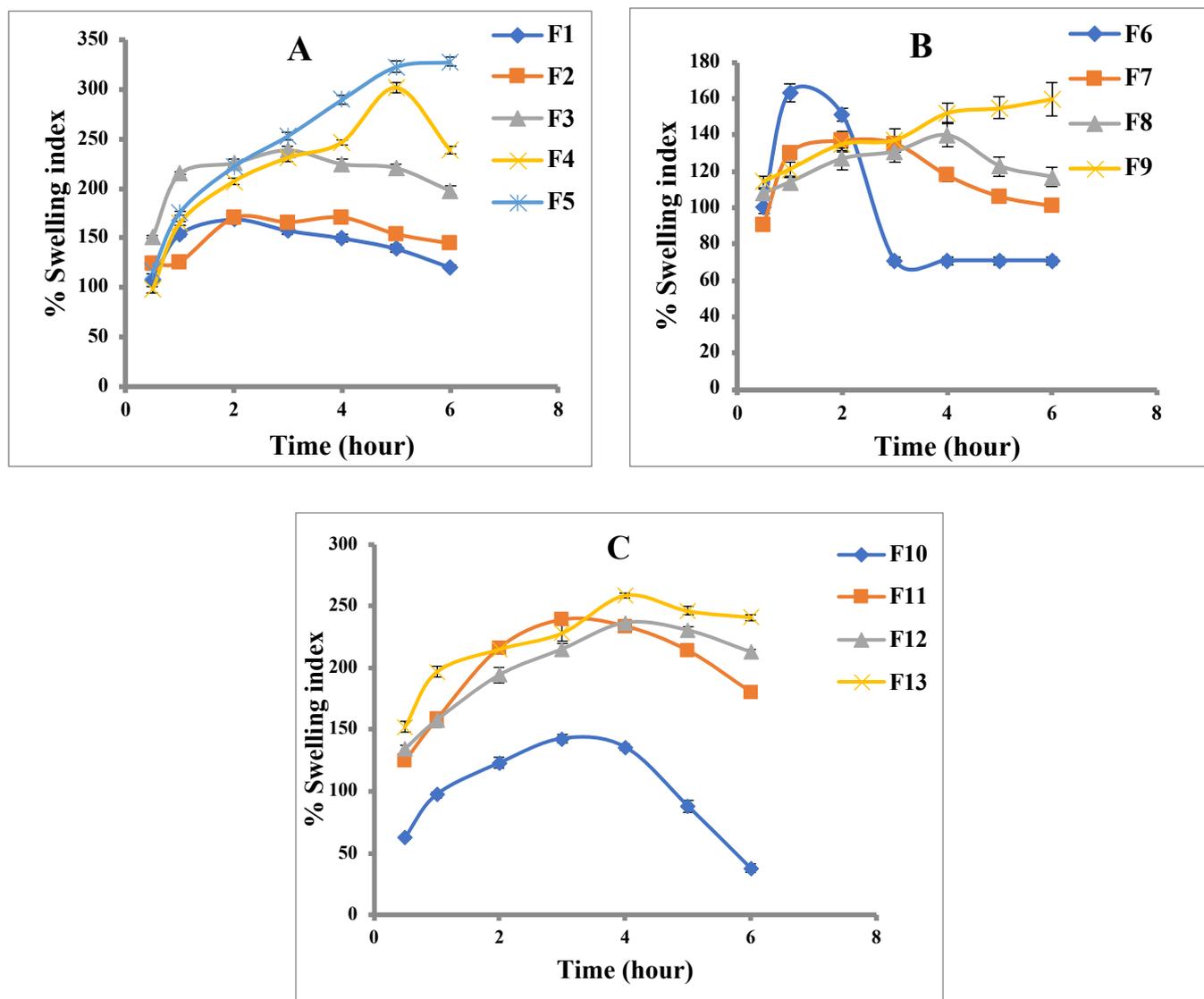
On the other hand, decreased Carbopol level as in F6 (10 % Carbopol) led to disappearance of lag time since its low concentrations hinder crosslinks formation thus forming less viscous gel (FCR Tablets- Lubrizol Pharmaceutical Bulletin 31, 2011).

Avicel 102 enhances water diffusion into the gel by wicking mechanism; it imparts its disintegrating action through porosity and capillary action. Avicel particles themselves act as pore-forming agents providing pathways for liquids to the tablets (Latha *et al.*, 2011). PEG 6000 was used as a channeling agent since it is soluble and tends to leach from formulation leaving tortuous capillaries in tablets (Balaji, Umashankar, Kavitha, 2014).

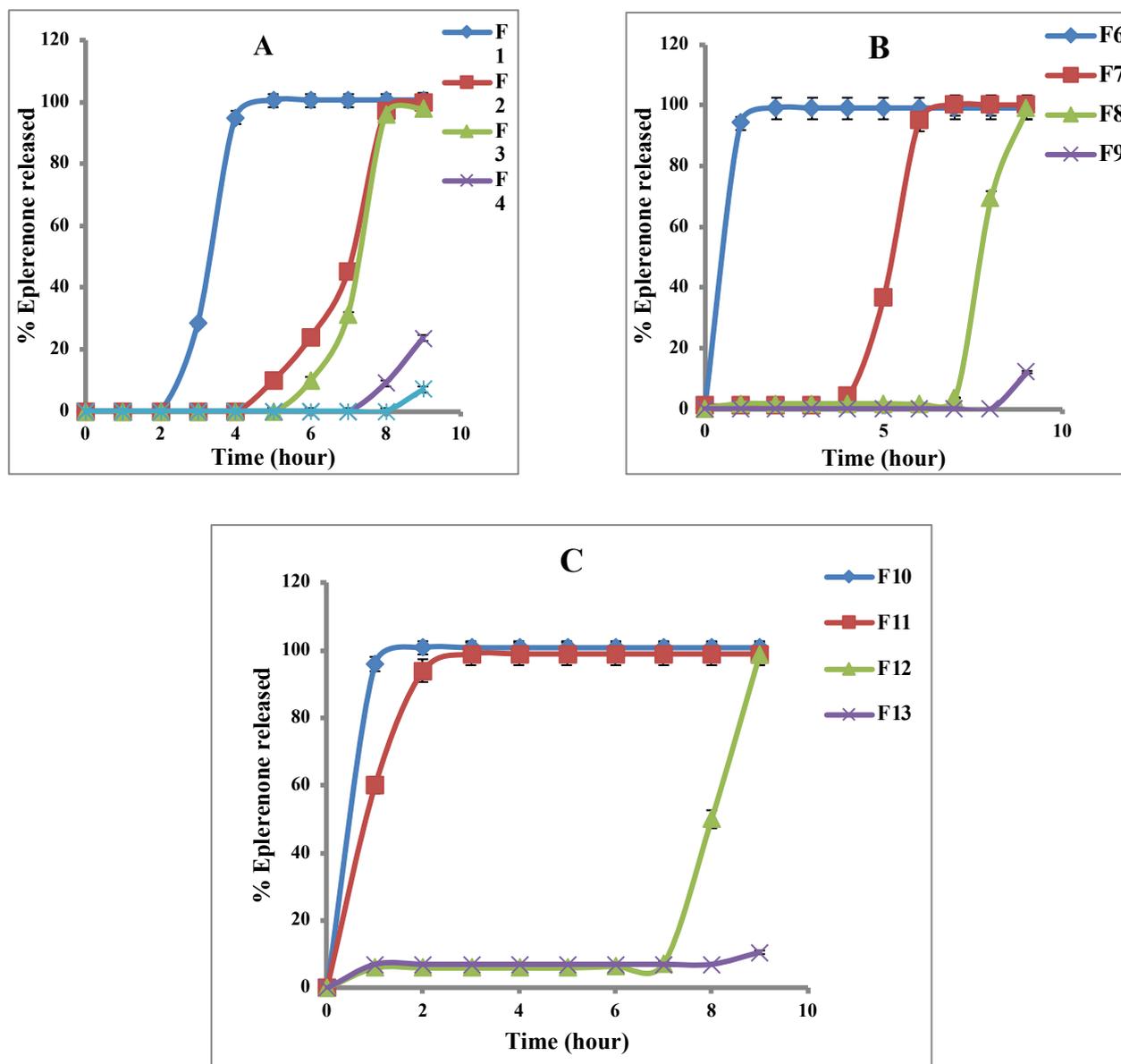
With adjusting the level of the controlling release polymer with either Avicel or PEG 6000 level, lag time of 5, 6, 5, 7 and 7 hours followed by complete drug release was obtained with F2, F3 (35 and 50 % PEO respectively), F7, F8 (15 and 22 % Carbopol respectively), and F 12 (35 % HPMC and 65 % PEG) making them suitable for chronotherapeutic application (Pagar, Vavia, 2012).

**TABLE III** - Physical parameters of Eplerenone press-coated tablets

Formulae	Weight (mg) ± SD	Thickness (mm) ± SD	Hardness (Kg/cm <sup>2</sup> ) ± SD	Friability (%) ± SD	Drug content (%) ± SD
F1	345.40±0.57	4.45±0.010	6.01±0.90	0.140±0.040	101.4±1.04
F2	348.30±2.80	4.50±0.028	7.06±0.40	0.058±0.032	100.9±0.14
F3	348.70±1.40	4.52±0.070	7.56±0.36	0.029±0.065	100.7±0.14
F4	349.60±0.10	4.53±0.051	8.13±0.06	0.010±0.028	99.89±0.12
F5	349.96±0.06	4.65±0.023	9.19±0.17	0.020±0.110	100.4±0.60
F6	350.53±0.08	4.47±0.026	7.38±0.58	0.200±0.005	99.60±1.20
F7	351.20±1.70	4.46±0.050	7.87±1.00	0.105±0.001	101.0±0.65
F8	350.56±1.20	4.49±0.058	8.04±0.47	0.028±0.025	100.5±1.50
F9	351.30±1.50	4.41±0.011	8.60±0.31	0.045±0.050	99.6±1.13
F10	350.70±1.40	4.61±0.010	4.32±0.55	0.257±0.005	100.8±0.70
F11	346.30±1.15	4.54±0.011	4.83±0.80	0.287±0.010	99.30±0.46
F12	349.97±1.27	4.60±0.005	5.60±0.45	0.257±0.052	100.2±0.28
F13	349.70±1.00	4.58±0.015	5.66±0.20	0.245±0.049	100.0±0.75



**FIGURE 5** - (A-C) Swelling behavior of press-coated tablets containing PEO WSR 110, Carbopol 71G and HPMC K4M respectively.



**FIGURE 6** - (A-C) *In-vitro* release of Eplerenone from tablets coated with PEO WSR 1105 (F1- F5), Carbopol71G (F6- F9) and HPMC K 4M (F10- F13) respectively.

### Accelerated stability study

Formulations subjected to stability testing (based on suitable lag time and drug release) (F2, F3, F7, F8, and F12) showed drug content within the pharmacopeial requirements with higher  $t_{90}$  for F3 and F8 (884.4 and 752.07 days respectively) compared to other formulations and thus chosen for further investigation.

Similarity factors were found to be between 60- 88 for tablets stored at 30 °C and between 65- 88 for tablets stored at 40 °C which indicated similarity between stored

and fresh tablets, regarding dissolution profiles since values were greater than 50 (Dumpa *et al.*, 2018).

### *In-vivo* studies on human volunteers

#### *Determination of eplerenone in plasma*

Ep plasma drug concentration is presented in Figure 7. Pharmacokinetic results are depicted in Table IV. Significant difference was observed for  $t_{max}$  (time to maximum plasma concentration; peak time)

(9.33 and 10 vs 4 hours) and MRT (mean residence time) (10.06 and 10.45 vs 5.55 hours) between tested tablets (F3 and F8) compared to commercial ones according to the ANOVA results (Table V) indicating

presence of lag time and delayed-release behavior for the investigated formulations. The bioavailability of the test formulations was significantly higher compared to reference ones.

**TABLE IV** - Mean pharmacokinetic parameters after oral administration of commercial and tested Eplerenone tablets

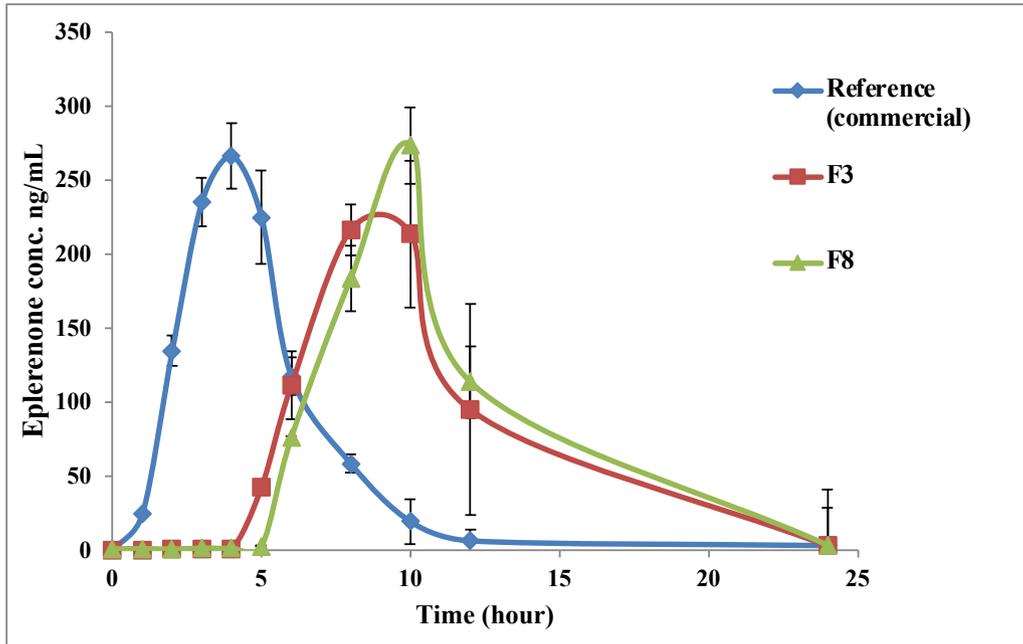
Parameter	Formulations± SD		
	Commercial	F3	F8
$t_{max}$ (hr)	4± 0.00	9.33300± 1.151	10
$C_{max}$ (ng/mL)	268.013± 19.996	258.077± 14.902	273.700± 23.985
Ke (hr <sup>-1</sup> )	0.19300± 0.015	0.21700± 0.032	0.21000± 0.000
$t_{1/2}$ (hr)	3.39000± 0.385	3.22700± 0.529	3.34300± 0.046
AUC <sub>0-inf</sub> (ng.hr/mL)	1360.387± 60.343	1783.2197± 169.006	1868.357± 134.611
AUC <sub>0-24</sub> (ng.hr/mL)	1343.98± 61.929	1761.847± 183.003	1852.303± 131.796
AUC <sub>24-inf</sub> (ng.hr/mL)	16.4040± 1.661	21.3737± 1.5007	16.0530± 1.941
AUMC <sub>0-24</sub> (ng.hr <sup>2</sup> /mL)	7170.317± 588.640	17549.067± 2801.14	19160.677± 1265.135
AUMC <sub>24-inf</sub> (ng.hr <sup>2</sup> /mL)	393.720± 39.937	512.900± 161.912	385.287± 50.582
AUMC <sub>0-inf</sub> (ng.hr <sup>2</sup> /mL)	7564.033± 551.063	18062.023± 3111.646	19545.963± 1295.126
MRT (hr)	5.55700± 0.156	10.0600± 0.612	10.4530± 0.251
Vd (L)	44.923± 14.007	11.2870± 2.568	11.5100± 1.360
TCR (mL/min)	142.917± 27.390	40.0670± 5.823	39.8330± 5.314
$C_{max}/AUC_{0-24}$ (hr <sup>-1</sup> )	0.1997	0.14670	0.14830
Relative bioavailability (%)		131.1	137.4

\*F3: contains 50 % PEO WSR in the coating layer, F8: contains 22 % Carbopol 71 G in the coating layer.

**TABLE V** - The statistical analysis (ANOVA) with respect to  $t_{max}$ , AUC<sub>0-24</sub> and MRT of Eplerenone using Tukey-Kramer multiple comparison test

Groups	$t_{max}$	AUC <sub>0-24</sub>	MRT
Reference vs F3	***	*	***
Reference vs F8	***	**	***
F3 vs F8	NS	NS	NS

\*\*\*: extremely significant, \*\*: very significant, \*: significant, ns: non-significant. P< 0.001

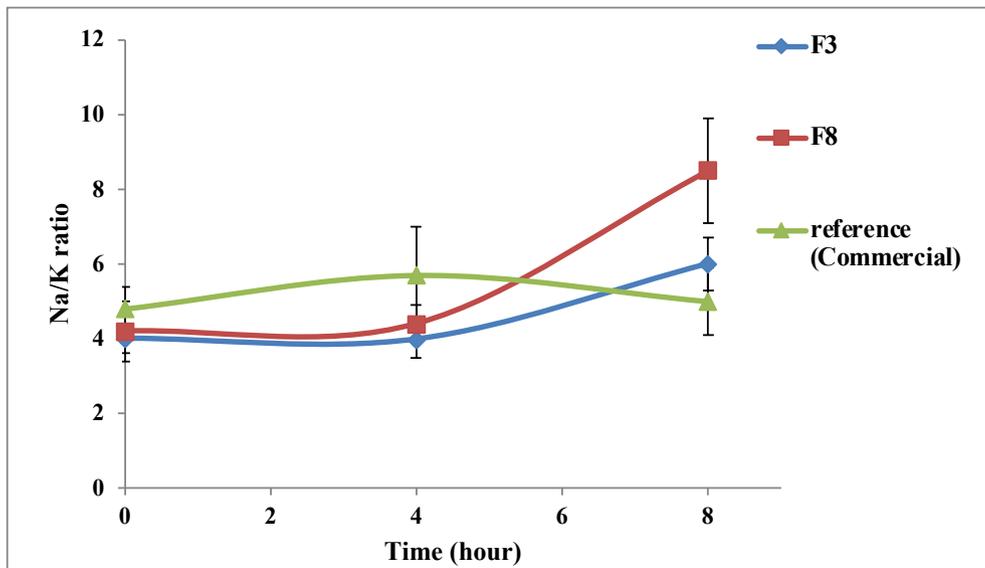


**FIGURE 7** - Mean Eplerenone plasma concentration (ng/mL)/ time curve  $\pm$  SD in subjects receiving commercial and tested tablets. \*F3: contains 50 % PEO WSR in the coating layer, F8: contains 22 % Carbopol 71 G in the coating layer.

*Na<sup>+</sup>/K<sup>+</sup> ratio in urine*

Ep is a selective aldosterone receptor antagonist that blocks mineralocorticosteroid receptor causing increase in urinary Na<sup>+</sup>/K<sup>+</sup> in a dose-dependent manner (Eudy *et al.*, 2011).

Na<sup>+</sup>/K<sup>+</sup> level was increased 4 hours after an oral dose of the commercial tablet and 8 hours after an oral dose of either F3 or F8 tablets (Figure 8) indicating the absence of Ep action 4 hours after oral dose of tested formulations and hence delayed drug release.



**FIGURE 8** - Na<sup>+</sup>/K<sup>+</sup> ratios after oral administration of commercial and tested Eplerenone tablets. \*F3: contains 50 % PEO WSR in the coating layer, F8: contains 22 % Carbopol 71 G in the coating layer.

## CONCLUSIONS

In the present study, press-coated pulsatile chronotherapeutic delivery systems were formulated to produce drug release after a pre-determined lag time. Promising lag time and release behavior suitable for morning hypertension were obtained in several formulations of which F3 and F8 (containing 50% PEO WSR 1105 and 22% Carbopol 71G respectively) were considered most stable. The Ep plasma level confirmed delayed release pattern for the tested formulations as compared to the commercial ones. Also, the  $\text{Na}^+/\text{K}^+$  ratio showed a delayed rise in tested tablets compared to commercial ones which indicate the effectiveness of the chronotherapeutic approach in the treatment of early morning hypertension. So, this study provides useful means for obtaining drug release timed as per chronotherapeutic objectives.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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