Archives of Pharmacy Practice

Research Article

ISSN 2045-080X Vol.3 , Issue 3, 2012

Effect of polymers on *in-vitro* performance of Eplerenone sustained release matrix tablets

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Citation: Reddy Sunil, Vinay Katakam, Jagan Mohan Somagoni, Pavan Panakanti, Sathish Dharani, Madhusudan Rao Yamsani. **Effect of polymers on *in-vitro* performance of Eplerenone sustained release matrix tablets** Archives of Pharmacy Practice. 2012; 3(3) pp 223-228.

Abstarct

Objectives: The intention of the present study was to design and assess oral sustained drug delivery systems for Eplerenone, using Cellulose and natural polymers as release modifiers in the form of matrix tablets.

Material and methods: Matrix tablets containing cellulose polymers like HPMC K4M, HPMC K15M, NaCMC and natural polymers like Guar Gum, Xanthan Gum, and Karaya Gum were prepared by wet granulation technique using PVP K60 as a tablet binder.

Results: The optimized formulation (F1) contains 1: 0.70 ratio (D: HPMC K4M) and (F4) contains 1:1 ratio (D: Guar gum) respectively. The *in-vitro* release kinetic studies of prepared matrix tablets with both the polymers were studied. The kinetic treatment illustrate that the optimized formulation (F1 and F4) followed zero order kinetics with release exponent (n) 0.87. Drug content in the tablets and amount of drug released were estimated by reported HPLC method. The FT-IR and DSC studies did not show any interaction of drug with the excipients used in the formulation.

Conclusion: The results clearly indicated that Eplerenone could be successfully prepared using an appropriate ratio of cellulose polymers like HPMC K4M, and natural gums like Guar gum in the form of matrix tablets

Key words

Eplerenone, Hydroxypropyl methylcellulose (HPMC), Sodium carboxy methyl cellulose, Sustained release matrix tablets, Guar gum.

Manuscript History

Article Received on: 3rd March, 2012
Revised on: 15th May, 2012
Approved for Publication: 19th June 2012

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Introduction

Present research work was undertaken to fabricate low cost sustained release matrix tablets of Eplerenone. There were no reports on sustained release formulations of eplerenone therefore, an attempt was made to formulate eplerenone in the form of a sustained release dosage form and study the influence of hydrophilic matrix forming agents on the *in vitro* release profiles from developed controlled/modified release matrix tablets. Eplerenone (EP) is methyl hydrogen 9, 11-epoxy-17- hydroxy-3-oxopregn-4-ene-7, 21-dicarboxylate, γ-lactone and a highly selective aldosterone blocker [1]. Eplerenone is used for treatment of hypertension and heart failure. Eplerenone is a steroid nucleus- based anti mineral corticoid that is chemically and enzymatically inter convertible to an open lactone ring form. In basic medium, Eplerenone is hydrolyzed to the open lactone ring form [2]. Eplerenone is a suitable candidate for Sustained - release (SR) administration as a result of its dosage regimen (twice or thrice a day) [3]. Unavailability in the market as SR dosage form, good absorption in the entire GI tract, relatively short plasma half-life of approximately 4 hrs [4-5]. Necessitate the use of Eplerenone as sustained release formulation to improve patient compliance.

Matrix system is the release system, which prolongs and controls the release of drug that is dissolved or dispersed in polymer matrices [6]. Hydrophilic polymers such as (HPMC, NaCMC etc.) are widely used in the formulation of modified-release oral dosage forms. This popularity can be attributed to the polymers for the reason that they are non-toxic nature, available with different chemical substitution, having good compressibility and different hydration rates with a choice of viscosity grades. Three aspects of HPMC govern its performance in an extended-release matrix system. First and prominent feature is rapid formation of a viscous gel layer upon hydration. Subsequently viscosity of the rate controlling polymer used and the third aspect is integrity of Matrix during dissolution testing was maintained. Once the gel layer is formed, viscosity of the gel layer regulates the overall rate of drug release [7].

Natural gums are polysaccharides consisting of multiple sugar units linked together to create large

molecules. Upon hydrolysis they yield simple sugar units such as arabinose, galactose, glucose, mannose, xylose or uronic acids, etc. The polysaccharide gums represent one of the most abundant industrial raw materials and have been the subject of intensive research due to their sustainability, biodegradability and biosafety [8].

Natural gums like Guar gum, Karaya gum and xanthan gum gained researchers interest in tablet formulation because of their swelling capability, simplicity and cheap production process. Guar gum is a natural macromolecular galactomannan. The potential of guar gum as an inexpensive and flexible carrier for oral extended release drug delivery has been highlighted [9].

Xanthan gum is a high molecular weight extracellular polysaccharide produced by fermentation process from microorganisms (Xanthomana campestris). The viscosity of the xanthan gum solution is nearly independent of pH and temperature. Xanthan gum is biodegradable and biocompatible and forms gel in water. These properties have led to an increase in use of xanthan gum for fabrication of modified release dosage form [10].

Gum Karaya is a vegetable gum produced as an exudate by trees of the genus Sterculia. Chemically, gum karaya is an acid polysaccharide composed of the sugars galactose, rhamnose and galacturonic acid [8].

Materials And Methods

Chemicals and reagents

Eplerenone was generous gift from AET, (Hyderabad, India). NaCMC, Hydroxypropyl Methyl Cellulose (HPMC K4M, K15M) were obtained from ISP, (Hyderabad, India). Guar gum, Xanthan gum and Karaya gum were obtained from Dabur, (Delhi, India). All other chemicals used were of analytical grade.

Preparation of Eplerenone sustained release matrix tablets

Eplerenone sustained release matrix tablets were prepared by wet granulation technique with varying ratios of hydrophilic polymers (HPMC K4M, HPMC K15M and NaCMC) and natural gums (Guar gum, Xanthan gum and Karaya gum). In this process accurately weighed quantities of drug filler blend was mixed with varying concentrations polymer in a mortar and mixed slightly with pestle. The obtained drug-polymer mixture was sifted through sieve # 40 to get uniform particle size and collected in a polythene bag and further mixed for about 3 minutes to ensure a homogenous blend. This was granulated with water containing 0.6 % of PVP K60 and dried the granules between 50^0C to 60^0C in tray drier for 15-20 min. After drying, obtained granules were passed through # 25 mesh. Accurately weighed Aerosil and Magnesium stearate were sifted through sieve #60 and mixed uniformly with blend, and lubricated for 5 minutes in a polythene bag. Final lubricated blend was compressed into tablets with 6±2 kpa of crushing strength using 8 mm concave punches and corresponding dies on 16 station rotary compression machine (Riddhi, Ahmedabad, India). The total weight of the tablet was 230mg. The qualitative and quantitative compositions of tablets were shown in Table 1.

Table 1: Composition Eplerenone Sustained Release Tablet Formulations

Ingredients (mg)/ Formulation	F1	F2	F3	F4	F5	F6
Eplerenone	70	70	70	70	70	70
HPMC K4M	50	---	---	---	---	---
HPMC K15M	---	50	----	---	---	---
NaCMC	---	---	50	---	---	---
Guar Gum	---	---	---	70	---	---
Karaya Gum	---	---	---	---	70	---
Xanthan Gum	---	---	---	---	---	70
MCC PH 102	98.7	98.7	98.7	78.7	78.7	78.7
PVP K 60	1.3	1.3	1.3	1.3	1.3	1.3
Aerosil	05	05	05	05	05	05
Mg stearate	05	05	05	05	05	05
Total tablet weight (mg)	**230**	**230**	**230**	**230**	**230**	**230**

Characterization of tablets

Compressed tablets were characterized for weight variation and uniformity in thickness using analytical balance (DENVER APX 60, Denver Instrument GmbH, Germany), and digital micrometer (Mitutoyo, Japan), respectively. Crushing strength was measured with Pfizer hardness tester, friability with Roche type friabilator. The drug content in each formulation was determined by triturating the tablets. 10 tablets were taken into a clean, dry mortar and crushed the tablets to fine powder. Accurately Weighed powder equivalent to 70mg of Eplerenone (230 mg) was taken into a 200ml clean, dry volumetric flask, to that 70ml of mobile phase was added and sonicated for 15 minutes, and made up to volume with mobile phase and filtered through 0.45 microns syringe filter, this solution 5 ml was diluted with mobile phase and analyzed by using HPLC at 240 nm.

FTIR study

Pure drug and physical mixture (F1 & F4) were subjected to FTIR studies. Physical mixture was prepared by simple blending. The IR spectra for the test samples were obtained using potassium bromide disk method using an FTIR spectrometer (PERKIN ELMER FT-I Insf. USA).

DSC Study

Pure drug and physical mixture (F1&F4) were studied for Differential scanning calorimetry (DSC) using DSC 822e/TGA SDTA 851e (SWITZERLAND) instrument equipped with a thermal data system.

Calculation of the loading and sustained dose [11].

The total dose of Eplerenone for biphasic delivery was calculated with available pharmacokinetic data [12]. As per the zero order release principle, the rate of delivery must be independent of the amount of drug remaining in the dosage form and constant over time. The release from the dosage form should follow zero-order kinetics, as

shown by the equation

K^0_r = Rate in= Rate out = k_e×Cd×Vd. Eq. (1)

Where K^0_r is the zero-order rate constant for drug release (amount per time), k_e is the first order rate constant of overall drug elimination (per hour), Cd is the desired drug level in the body (amount per volume), and Vd is the volume in which the drug is distributed. The elimination half-life of Eplerenone is 4 h (k_e= 0.693/4=0.173h^{-1})

The loading dose is required to give initial rapid burst of dose so as to attain therapeutic range immediately after dosing

Loading dose (DL) = Css avg×Vd / *F* = (0.2495×66.5) / 0.69 = 24mg Eq. (2)

Where DL = Loading Dose, Css avg = Average Steady State Concentration (0.2495 mg/ml), Vd = Apparent Volume of Distribution (66.5 l), F = Fraction of Dose Absorbed (0.69).

The maintenance dose was (D_M) = K^0_r× H,

Eq. (3)

H = Total desired time for sustained action in hours,

Drug availability rate (Rate of drug input) K^0_r= K_e× D_L

Eq. (4)

K_e is the overall elimination rate constant (per hour), K^0_r= 0.1732 ×24 = 4.158mg / h. Thus K^0_r (Rate of drug input) is 4.158 mg / h, which should also have been equal to the elimination constant so as to maintain the steady state condition. For a system in which the maintenance dose releases drug by a zero order process for a specified time, the total dose is as follows:

Total dose = D_T = D_L*+D_M,

Eq. (5)

D_L*is corrected loading dose

D_M = K^0_r×H= 4.158 ×12 = 49.89 mg

If the maintenance dose begins the release of the drug at the time of dosing, it will add to that which is provided by the initial dose, thus increasing the initial drug level. In this case, a correction factor is needed (K^0_r× T_P) to account for the added drug from the maintenance dose. This correction factor is the amount of drug provided during the period from t=0 to the time of the peak drug level, T_P. If D_L is 24mg, K^0_r is 4.158 mg/h, and T_P is 1.5h, then the corrected loading dose (D_L*) = D_L- (K^0_r×T_P) = 4.158 ×1.5= 17.763 Thus, the total dose required (D_T) =D_L*+D_M= 17.763 +49.89 = 67.653 mg

The total dose of Eplerenone was rounded off to 70 mg (17.763 mg loading dose and 49.89 mg maintenance dose).

Drug release kinetics

The description of dissolution profiles has been attempted using different release models. The data were evaluated according to the following equations.

Zero order: $M_t = M_o + K_o t$ Eq. (6)

First order: $\ln M_t = \ln M_o + K_1 t$ Eq. (7)

Higuchi model: $M_t = K_H \sqrt{t}$ Eq. (8)

Korsmeyer –Peppas model: $M_t/M_o = K_k t^n$ Eq. (9)

Where M_t is the amount of drug dissolved in time t, M_o the initial amount of drug, $K_1$is the first order release constant, K_0 the zero order release constant, K_H the Higuchi rate constant, K_k the release constant and *n* is the diffusional release exponent indicative of the operating release mechanism. The correlation coefficient r was used as an indicator of the best fitting, for each of the models considered table 2

Table 2: Correlation coefficient (R^2), release exponent (n) values for different kinetic models

Formulation Code	Zero order R^2	First Order R^2	Higuchi R^2	Peppas (n)
F1	0.99	0.82	0.99	0.87
F2	0.88	0.91	0.91	0.81
F3	0.89	0.93	0.97	0.72
F4	0.99	0.86	0.99	0.86
F5	0.91	0.80	0.95	0.78
F6	0.87	0.92	0.85	0.70

Stability studies of sustained release matrix tablets

The stability study was carried out for optimized formulations F1 and F4. The tablets were placed in amber colored bottle and were stored for six months at a temperature of 40 ^{0}C ± 2^{0}C and RH 75% ± 5%. The tablets were evaluated for any change in physical appearance and percent cumulative drug release after one, three and six months. The obtained results were compared with the stability data for zero time and room temperature (25^{0}C ± 2^{0}C) and relative humidity (60% ± 5%RH) as per ICH guidelines [13].

Swelling studies

The Eplerenone sustained release matrix tablets were dipped in pH 6.8 phosphate buffer at room temperature. The swollen weight of the tablets was determined at predefined time intervals. The wet weight of the swollen tablets was determined by blotting them with filter paper to remove moisture adhering to the surface, immediately followed by weighing on an electronic balance [14].

The swelling index was calculated by the following equation

Swelling Index = $(W_t - W_0)/W_0$

Eq. 10

Where W_0 is the initial weight of tablet, and W_t is the weight of tablet at time t.

***In-Vitro* Dissolution**

The Eplerenone release from different formulations was determined using a USP XXIV basket apparatus Type 1. The dissolution medium was 900 ml buffer (pH 1.2 buffer for 2 hours and 6.8 phosphate buffer for 10 hrs) at 37± 0.5 °C; stirrer speed 50 rpm. The formulations prepared were subjected to dissolution tests for 12 h. Sample (5 ml) was withdrawn at predetermined time intervals, filtered through filter paper (0.45 µ) and replaced by an equal volume of dissolution medium. Drug content in the dissolution sample was determined by HPLC. The results were expressed as mean ±S.D (n=6).

HPLC Analysis

The quantitative determination of Eplerenone was performed by HPLC, which has gradient elution capability, a UV spectrophotometer detector and auto sampler was used (Waters Allience 2695 separations module, Waters 2487 dual λ absorbance detector of equivalent). A Stainless steel column (inertsil ODS 3 V 250mm, 4.6mm, 5µ ID) 250mm long, 4.6mm internal diameter filled with octadecylsilance chemically bonded with silica gel particles of 5 µm diameter. Quantitation was performed according to the earlier reported method with a slight modification [3]. Mobile phase consisted of mixed buffer of pH 3.2 (potassium dihydrogen orthophosphate) and organic mixture (Acetonitrile and Methanol in the ratio of 90:10.) (V/V) in the ratio of 50:50 and filtered through 0.45 µ and degassed. The filtered mobile phase was pumped at a flow rate of 1 ml/min. 10 µl of sample was injected into the column and the retention time of Eplerenone was found to be 10 min. The elute was detected by HPLC at 240 nm. (***Fig.1. Typical Chromatogram***)

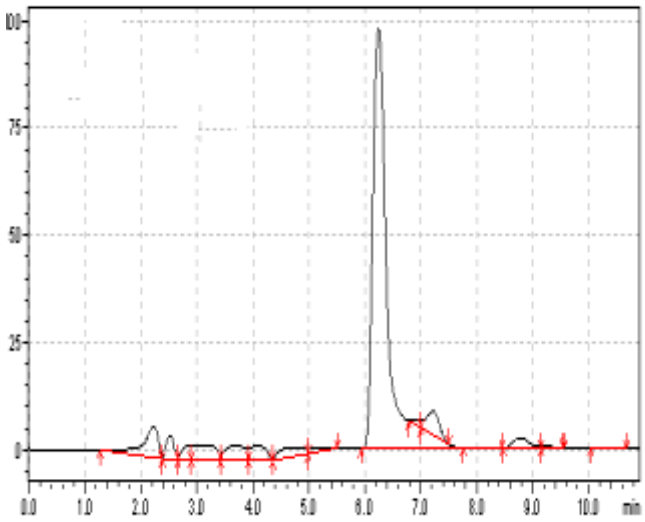


Fig.1. Typical Chromatogram obtained from Eplerenone tablet solution

Results

Physical properties of the compressed granules matrix system

The sustained release matrix tablets were prepared by granulation using PVP K60 as a binding agent using Methocel (K4M, K15M and NaCMC), guar gum, xanthan gum, gum Karaya, microcrystalline cellulose, aerosil and magnesium stearate as glidant and lubricant, respectively. The results of the physical characteristics of sustained release matrix tablets are shown in Table 3

The weight and thickness of the all the tablets were uniform as it was evidenced from RSD values, which were less than 3 indicating uniformity of weight and thickness. Hardness of tablets was found to be between 5.49 to 6.32 kg/cm^2 indicating sufficient crushing strength. The friability was below 1% for all formulations, indicating good mechanical resistance of the tablet. The drug content varied between 69.59 to 70.50 mg in all tablets with low standard deviation indicating content uniformity of the prepared batches.

Table 3: Physical Characteristics of Sustained Release Matrix Tablets

Formulation Code	Hardness* (kg/cm^2)	Thickness** (mm)	Average Weight # (mg)	Friability¥ (%)	Drug content® (%)
F1	6.17±0.45	3.61±0.07	230.37±1.89	0.38±0.06	100.67±0.25
F2	6.75±0.21	3.83±0.06	231.09±1.12	0.40±0.08	99.41±0.34
F3	6.06±0.69	3.46±0.04	230.65±1.20	0.67±0.07	101.28±0.21
F4	5.69±0.85	3.71±0.05	230.05±1.51	0.54±0.05	100.43±0.12
F5	5.09±0.47	3.93±0.08	230.27±1.89	0.57±0.06	100.60±0.28
F6	4.89±0.31	3.66±0.06	231.29±1.22	0.87±0.02	101.71±0.34

* All values are expressed as mean ± SE, n=10
** All values are expressed as mean ± SE, n=10
# All values are expressed as mean ± SE, n=20
¥ All values are expressed as mean ± SE, n=12
® All values are expressed as mean ± SE, n=6

Swelling Index

Tablet composed of polymeric matrices build a gel layer around the tablet core when they come in contact with water. This gel layer governs the drug release. Kinetics of swelling is important because the gel barrier is formed with water penetration. Swelling is also a vital factor to ensure sustained release matrix tablets. Swelling index studies were carried out for the optimized formulations containing HPMC K4M (F1) and Guar gum (F4) to understand the influence of swelling on drug release. The swelling index values for HPMC K4M formulations were found to be 75 % where as more than 85 % swelling was observed for formulation containing Guar gum in 6.5 hrs. Swelling index was improved, with increasing time whereas weight gain by tablet was proportional to rate of hydration up to 6.5 hrs, afterwards it reduces gradually due to disappearance of outermost gelled layer of tablet into dissolution medium. This is represented graphically in figure 2.

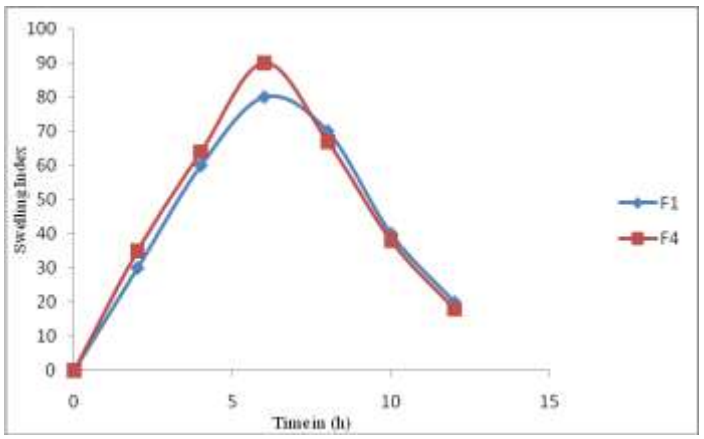


Fig. 2. Swelling index of formulation F1& F4

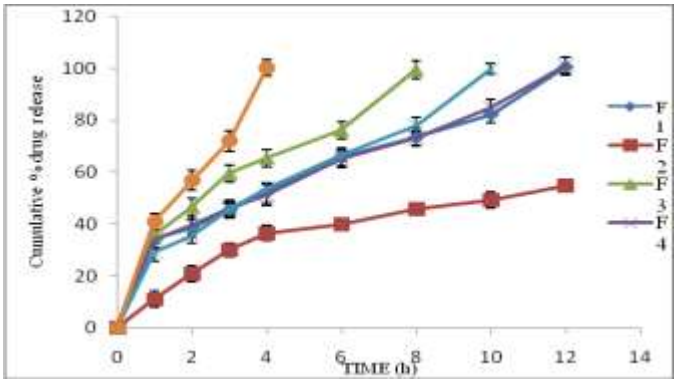
In view of the fact that swelling was more in natural gums, it offers further fluid penetration in to the gel matrix, and thickness of the gelled layer increases, which permit water soluble drugs to diffuse out through the gel barrier, as a result of which the drug release cannot be extended for a longer period of time. All the tablets of different formulations were subjected to various evaluation tests such as thickness, hardness, weight variation and drug content in prepared tablets. The thickness of all the prepared tablets with cellulose polymers as well as natural gums ranged from 3.46±0.07 mm to 3.93±0.08 mm. The average percentage deviation of 20 tablets of each formula was less than ±5%. Drug content was found to be uniform among different batches of the tablets and ranged from 99.41±0.34 % to 101.71±0.34 %. The Hardness of tablets formulated with cellulose polymers like HPMC K4M, and Na CMC ranged from 6.06 ± 0.69 to 6.75 ± 0.21 kg/cm^2. Whereas hardness of the tablets formulated with the natural gums like guar gum, Karaya gum and xanthan ranged from 4.89 ± 0.31 to 5.69 ± 0.85 kg/cm^2 respectively. The observed low hardness values of formulations containing natural gums are as a result of the poor flow properties and insufficient compressibility nature of natural gums [15]. A hardness of 6.17 ± 0.45 kg/cm^2 and 5.69 ± 0.85 was recorded for the optimized formulations (F1&F4) containing cellulose polymer and natural gum, HPMC K4M, Guar gum in the ratio 1:0.70 and 1:1, with excellent flow properties and superior compressibility.

The hardness is not an absolute indicator of strength. An additional measure of a tablet's strength is friability. The accepted limit is below 1% friability of the optimized formulations (F1&F4) containing cellulose polymer and natural gum (HPMC K4M and Guar gum) have shown good compressibility of polymer blend with retarding characteristics. These parameters provide justification and suitability of cellulose polymers (HPMC K4M) and natural gums (Guar gum) as sustained release matrix forming components.

The results of dissolution studies of formulations F2, F3 composed of cellulose polymers alone , HPMC K15M(1:0.71) and Na CMC (1:0.71) respectively (drug-to-polymer ratio) showed 10.99% and 35.77% drug release at the end of 1 hr. Whereas formulations F5 and F6 containing Karaya gum and Xanthan gum alone (1:1), (1:1), respectively (drug-to-polymer ratio) showed 29%, 40%, drug release at the end of 1 hr. Formulation F1 contains HPMC K4M (1:0.71) (drug-to-polymer ratio) showed 34%, 52%, 73% and 100% drug release and formulation F4 contains Guar gum (1:1) (drug-to-polymer ratio) showed 33.87%, 51%, 73% and 101% drug release at the end of 1,4,8 and 12th h respectively according to theoretical release profile. (Shown in Fig. 3).

The n values of peppas were in the range of 0.70 to 0.87 indicating that the drug release was anomalous diffusion. In general drug release from compact matrices follows a Fickian mechanism, but sometimes because of the presence of other additives, a mixture of diffusion (case – I) and chain relaxation (case – II) is seen. However, when drug diffusion does not follow a Fickian mechanism, it is too similar to a zero order continuous and homogenous release kinetic.

Fig.3. Drug release profiles of Eplerenone SR matrix tablets. (mean ± SD, n = 6).



FTIR studies

To study any interaction between drug and polymers used in the development of sustained matrix tablets IR spectroscopy was carried out for the formulations F1and F4. The IR spectra of the (F1 and F4) formulations showed the same absorption bands as the physical mixtures and pure drug indicating absence of interaction between Eplerenone and methocel and gums (Fig. 4). It presumably suggests that the drug molecule is present in an unchanged state in the sustained release matrix tablet.

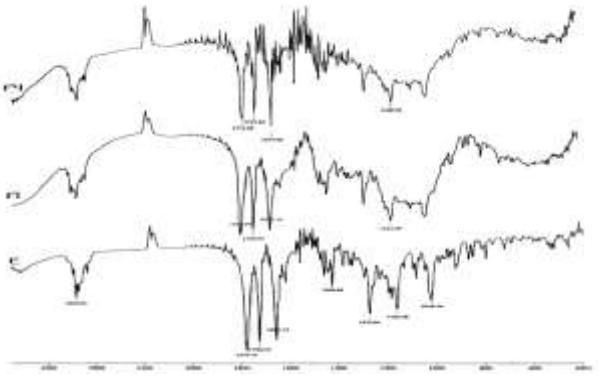
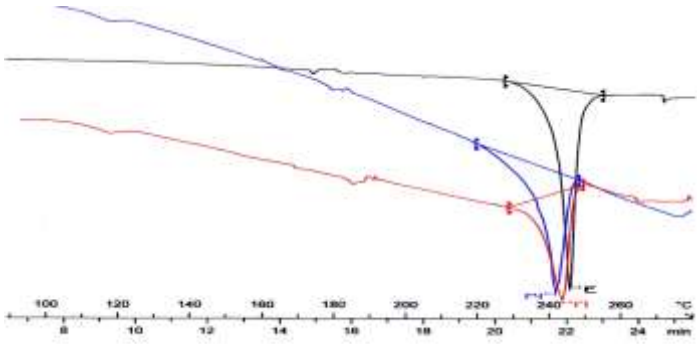


Fig.4. FT-IR Spectra of pure drug, physical mixtures of cellulose and natural gums

Differential scanning colorimetry

The DSC thermogram of the pure drug, physical mixture of drug and polymer which was kept at 40ºC and 75%RH revealed that an endothermic peak of melting of drug appears at about 245.07ºC indicated that there was no incompatibility between drug and excipients (Fig 5).

Fig 5. DSC thermogram of (E) pure drug (F1) physical mixture and (F4) physical mixture



Conclusion

The formulated tablets showed acceptable weight variation, hardness, drug content uniformity with sustained release matrix characteristics. The *in-vitro* release profiles of optimized formulation F1 and F4 showed desired sustained release characteristic and the drug release was sustained for 12 hrs. Indicating cellulose polymers and natural gums like guar gum are prospective excipients for the formulations of aqueous soluble drugs like Eplerenone for oral sustained release matrix tablets.

Acknowledgement

The authors would like to thank AET Laboratories (Hyderabad, India) for providing the gift sample of Eplerenone, and one of the authors (Reddy Sunil) is thankful to the University Grants Commission, New Delhi (RGNFS) India for providing Senior Research Fellowship.

References

1. Mahon, M.C. Recent studies with eplerenone, a novel selective aldosterone receptor antagonist. *Curr. Opin. Pharmaco, 2001;* 1: 190–196.
2. Rane, V.P. Patil, K.R. Sangshetti, J.N. stability-indicating rp-hplc method for analysis of eplerenone in the bulk drug and in a pharmaceutical dosage form. *Acta. Chromatogr, 2009;* 4: 619–629.
3. InspraTM: eplerenone tablets. Amended proposed US PI September 27, 2002 [product monograph]. Peapack (NJ): *Pharmacia Corporation*; 2002.
4. Martin, J., Krum, H. Eplerenone (GD Searle & C0). *Curr. Opin. Invest. Drugs*, 2001; 2: 21- 524.
5. William B White. Assessment of the novel selective aldosterone blocker eplerenone using ambulatory and clinical blood pressure in patients with systemic hypertension**.** *American Journal of Cardiology***,** 2003;1:38-42.
6. Brode, G.L, Goddard, E.D, Harris, W.C, Salansky, G.A. Cosmetic and Pharmaceutical Applications of Polymers. New York: *Plenum Press*, 1991; 117.
7. Chiu, C.W, Henley, M.J., Zallie, J.P. Bulking agents and processes for preparing them from food gums, *United States Patent* No. 6, 229,924.2001.
8. Vikas, R, Parshuram, R, Ashok, K., Tiwarya, R.S. Modified gums: Approaches and applications in drug delivery. *Carbohydrate Polymers*, 2011;83:1031–1047.
9. Tsuchiya, H.M., Hellman, N.N., Koepsell, H.J., Corman, J,et. Factors affecting molecular weight of enzymatically synthesized dextran. *Jour. Amer. Chem. Soc*, 1955;77:2412-2419.
10. Kim, C., Yoo, B. Rheological properties of rice starch - xanthan gum mixtures *J. Food. Eng,2006;*75: 120–128.
11. Meka, L, Thadisetty, A, Vobalaboina, V., Yamsani, M.R. Design and evaluaiton of a novel matrix type multiple units as biphasic gastro retentive drug delivery systmes*. AAPS Pharm.Sci.Tech, 2008;*9: 1253-1261.
12. Chyung S.C, Berry L.M. Pharmacokinetics and metabolism of Eplerenone after oral administration to humans. *Drug. Metab. Dispos*, 2003; 31:1448-1455.
13. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Stability testing of New Drug Substances and Products. QIA (R2), August, 2003.
14. Chowdary, K.P, Monhapatra P. (2006). Evaluation of olibanum and its resin as rate controlling matrix for controlled release of diclofenac, *Indian.J. Pharm*, 2006; 8: 497- 500.
15. Food and Drug Administration. *FDA Guidance for industry.* Washington, DC:U.S. Department of Health and Human Services,1997;8:32