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EUDRAGIT L100- GELATIN BASED MICROSPHERES FOR SUSTAINED DRUG DELIVERY OF ACECLOFENAC AS ORAL TABLETS- PHYSICOCHEMICAL EVALUATION, KINETICS AND FIT FACTOR

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ABSTRACT

Aim: To prepare Aceclofenac microspheres by solvent evaporation to optimize the release of Aceclofenac for sustained drug delivery. *Method*: The microspheres were prepared by incorporating water phase (gelatin solution) into the organic phase (drug and Eudragit L100 in Dichloromethane: ethanol). The drug-polymer system was subjected to compatibility studies using FTIR and DSC. The microspheres were evaluated for particle size, flow properties, entrapment efficiency and percentage yield. The compressed tablets of microspheres were assessed for physicochemical properties and *in vitro* drug release studies. *Results*: No interaction was observed between the drug and excipients. The physicochemical properties of the tablets were found satisfactory. The *in vitro* dissolution studies signified that as the polymer concentration increased the drug release decreased. *Conclusion*: The

formulation F1-T showed a similar dissolution profile as to that of marketed Aceclofenac sustained release tablet, following zero order kinetics with anomalous drug release mechanism.

Keywords: Calorimetry, FTIR, Kinetics, solvent-evaporation technique, oral drug delivery

INTRODUCTION

Aceclofenac is a non steroidal anti-inflammatory drug (NSAID) which is considered as the first-line therapy in the symptomatic treatment of osteoarthritis and rheumatoid arthritis. The successful treatment of arthritis depends in the maintenance of effective concentration of drug in the body by means of a sustained drug delivery system [1].

Among the different approaches to achieve sustained drug delivery system, microencapsulation appears as the most efficient method in terms of process development and economy. Basically the drug release from these systems is dependent on the amount of polymer concentration, composition and drug particle size. Many sustained delivery systems are formulated to retard the release of drug over a prolonged period of time such that it achieves the Minimum effective concentration (MEC) without causing any undesirable side effect or dose dumping. .

The objective of the present study was to develop an optimized formulation of Aceclofenac microspheres by solvent evaporation technique and study the effect of compressed tablets of microspheres on drug release. The formulations were also evaluated with the marketed product of Aceclofenac sustained release product by means of model independent approach (fit factor) as approved by the FDA [2].

MATERIAL AND METHOD

Material

The following chemicals were used in the preparation of microspheres; Aceclofenac (donated by Highnoon Pharma, Lahore, Pakistan); Eudragit L-100 (Merck, Germany); Eudragit RS-100 (Merck, Germany) ; Gelatin (BDH Chemicals, UK); Dichloromethane (Riedel-deHaen, Germany); Ethanol (Merck, Germany); n-hexane (RDH, Germany); Disodium hydrogen phosphate (Fluka, Germany); Sodium dihydrogen phosphate (Fluka, Germany); Distilled water. All the chemicals used were of analytical grade.

Method

Construction of calibration curve of Aceclofenac

The calibration curve of Aceclofenac was constructed by the stock solution dilution method in phosphate buffer pH 7.4 as reported in literature [3]. Different dilutions

were made from stock solution containing 1, 2, 4, 6 and 8µg/ml of the drug . The samples were taken from each dilution, filtered and analyzed spectrophotometrically (T-80 UV/vis spectrophotometer, PG instrument Ltd.) at wavelength of 275 nm [4].

Preparation of microspheres

For the preparation of organic phase, Eudragit L-100 was dissolved in Dichloromethane and ethanol (3:1) and mixed homogeneously on hot plate magnetic stirrer (Table 1). The water phase was obtained by dissolving gelatin in distilled water and kept in a water bath at 60°C for 40 minutes. Aceclofenac was added in Eudragit L100 solution and dispersion was kept under stirring for 30-35 minutes. Then, the gelatin solution was added drop wise into the organic phase on hot plate magnetic stirrer (800 rpm) to obtain homogenous complex of the two polymers embedding the drug. The dispersion was filtered and obtained microspheres were washed with n-hexane. They were allowed to dry at room temperature for 24 hours and stored in air-tight container till further analysis [5].

Table 1: Formulation of Aceclofenac microsphere containing combination of Eudragit L-100

Formulation Code	EL-100 (gm)	Aceclofenac (gm)	Gelatin (gm)	Dichloromethane (ml)	Ethanol (ml)
F1	1	1	0.5	15.0	5.0
F2	2	1	0.5	30.0	10.0
F3	2.5	1	0.5	37.5	12.5
F4	3	1	0.5	45.0	15.0
F5	3.5	1	0.5	52.5	17.5
F6	4	1	0.5	60.0	20.0

Characterization of microspheres of Aceclofenac

Particle size determination

The microspheres were suspended in liquid paraffin and examined under optical microscope fitted with optical micrometer. Atleast 100 particles were estimated for diameter measurement and average was taken [6].

Percentage yield

Percentage yield was calculated by dividing the weight of obtained microspheres after drying with the total amount of excipients and drug used in the preparation by following equation [7].

Percentage yield = (Weight of microspheres) / (Initial weight of Aceclofenac + Eudragit L100 + Gelatin)*100

Encapsulation efficiency and drug loading

The actual amount of Aceclofenac in microspheres was determined by dispersing accurately weighed amount of microspheres in phosphate buffer pH 7.4 and keeping under magnetic strirring for 24 hours. Sample was withdrawn, filtered and analyzed spectrophotometrically at 275 nm.

Corresponding amount of drug in formulation was estimated by calibration curve (Figure 1). Percentage entrapment efficiency (EE%) was calculated by the following equation [7]:

EE% = (Actual amount of Aceclofenac) / (Theoretical amount of Aceclofenac) *100

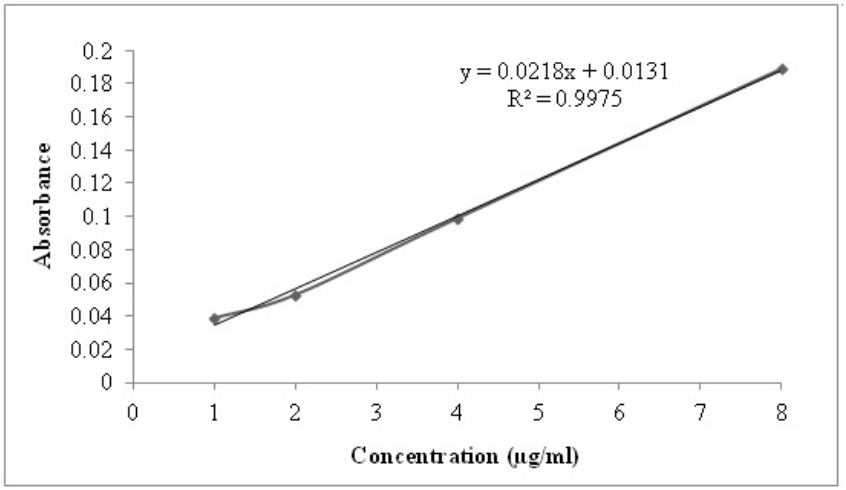

Figure 1: Calibration curve of Aceclofenac in phosphate buffer pH 7.4

FTIR studies

FTIR studies were conducted on pure drug, polymer and drug-polymer matrix mixture. The sample was mixed with potassium bromide and compressed into pellet by applying 1 ton/ unit pressure. Scanning was done in range of 400-4000 cm^{-1} [8].

DSC studies

DSC studies were performed to analyze the thermal behavior of pure substances (Aceclofeanc and Eudragit L100) and drug loaded matrix mixture. A sample of 5 mg was sealed in an aluminum pan and the DSC thermograms were reported at a heating rate of 5– 300^{o}C in atmosphere of nitrogen at flow rate of 50 ml/min [8].

Micromeritic properties

Powder flow properties were evaluated by the Micromeritic tests. Bulk density and tapped density was measured by using 10 ml of graduated cylinder. The preweighed sample was added in the cylinder and volume was noted. The cylinder containing the sample was then tapped 100 times and new volume after tapping was noted. Both densities were calculated by the density formula (mass/ volume).

Carr's index (CI) or compressibility index and Hausner ratio was calculated by [9]:

$$CI\% = (\rho_{tapped} - \rho_{bulk}) / (\rho_{tapped}) * 100$$

$$\text{Hausner Ratio} = (\rho_{tapped} / \rho_{bulk})$$

Compression of tablets

Different batches of tablets were prepared by direct compression of microspheres. Accurately weighed amount of microspheres

(equivalent to 100 mg of drug) were taken and blended with lactose (q.s) with 1% magnesium stearate to make a total tablet weight of 200 mg. The powder was compressed with single punch machine to produce tablets of approximately 9.8 kg/cm^2 hardness. The tablets were evaluated for thickness, diameter and friability [9].

***In vitro* drug release studies from microsphere**

In vitro dissolution studies were using USP basket dissolution apparatus. The tablets were put into the basket containing 900 ml of phosphate buffer pH 7.4, thermostatically controlled at 37±0.1^{o}C with a paddle speed of 50 rpm. After the predetermined time interval, 1ml of sample was withdrawn, diluted, filtered and examined at 275 nm. To maintain the sink conditions dissolution volume was preserved by adding fresh buffer with same amount withdrawn for sampling [10].

The release profiles were fitted according to zero-order kinetics, first order kinetics, Higuchi model and Korsmeyer-Peppas [11] to describe the drug release mechanism.

Fit factor (f_1 and f_2)

The f_1 is also known as the dissimilarity factor. As the value approaches 0 it signifies that the test and the reference profiles are identical and increases proportionally with the dissimilarity between the two dissolution profiles. It is calculated by the following Eq. 9 [12]:

$$f_1 = \left\{\frac{\sum_{t=1}^{n}|R_t - T_t|}{\sum_{t=1}^{n} R_t}\right\} \times 100 \text{.....Eq. 9}$$

The value of factor f_2 lies between 0 and 100. The f_2 is 100 when the test and the reference profiles are identical and as it approaches 0 the dissimilarity increases. The f_2 is also known as the similarity factor and is calculated by Eq. 10 [12]:

$$f_2 = 50\ log\left[\frac{100}{\sqrt{1+\left(\sum_{i=0}^{n}(R_t - T_t)^2\right)/n}}\right] \text{...Eq. 10}$$

RESULT AND DISCUSSION

Particle size

The particle size of microspheres at different drug to polymeric ratio was 91-120±1 µm (Table 2). As the concentration of polymeric material was increased, the mean diameter of the particles also increased. This was attributed to the increase in viscosity of the internal phase which allowed an increase in thickness of the wall of microspheres [13].

Percentage yield and encapsulation efficiency

The Percentage yield of the formulations varied from 61-95% where as the drug entrapment efficiency of the formulations was in the range of 79-91%, indicating high efficiency of drug entrapment by the polymer (Table 2). The EE% increased with the

increase in Eudragit L100 concentration. This may be attributed to the rapid hardening of the droplets following increased Eudragit L100 proportion that results in reduced drug diffusion into the aqueous phase [7].

FTIR study

The FTIR was conducted for the analysis of interaction and compatibility between drug and polymer and for the stability of drug during encapsulation processing (Figure 2). Pure Aceclofenac shows different peaks at 3883.9, 3716.5, 3576.3, 3519.0, 1717.9, 1445.0, 1248.4, 1147.5, 748.2, and 610.6 cm^{-1}. Aceclofeanc have one ester group, one carbonyl group, and secondary amine with characteristic band. The peak at 1717.9 cm^{-1} indicated stretching vibration of C=O carboxylic group attached to methylene group and ether group. The band at 1445.0 cm^{-1} showed the stretching vibration of N-H group. The peaks at 1248.4 cm^{-1} and 748.2 cm^{-1} showed the vibration of C-N group and C-Cl group respectively. The Eudragit L-100 showed different peaks at 3946.0, 3843.1, 3715.8, 3636.4, 3583.0, 1381.1 and 1158.0 cm^{-1}. The IR data of drug-polymer mixture showed that there was no main fluctuation in frequencies of mixture of drug with polymer when compared to pure Aceclofenac and Eudragit L100 [14].

DSC study

DSC results for pure drug, Eudragti L100 and drug-loaded microspheres are presented in Figure 3. The thermogram of Eudragit L100 was characterized by two thermal phases: the first exothermic reaction at about 27°C and the second endothermic centered at about 72°C. The DSC analysis of Aceclofenac showed a characteristic sharp endotherm peak at 151°C. This demonstrated the melting point of Aceclofenac. Similarly the endothermic peaks were observed for formulations mixture at corresponding melting points but the endothermic peak of drug in formulation graphs was shifted slightly towards lower temperature. This may be due to uniform distribution of drug and formation of polymer-drug matrix [8]. The thermal investigation showed good stability and no interaction of drug with polymer was observed.

Micromeritic properties

The formed microspheres of Aceclofenac were white in color. The CI and Hausner ratio (Table 3) showed that powder flow of the formed microspheres was good to fair which indicated that they could be compressed into a tablet with little difficulty [15]. Lactose was added into the microspheres as a bulking agent with 1%

magnesium stearate as a lubricant for tablet formation.

Evaluation of tablets

The tablets were evaluated for their physicochemical properties (Table 4). The tablets of different batches were found to be uniform in diameter (0.368- 0.376 cm) and thickness (0.801- 0.808 cm). The friability of the tablets was below 1% (0.54- 0.72%) and within the official limit. The weight variation of tablets was less than ±7.5% (0.21- 0.34%) which confined to the official limit of USP-NF. Hence, the combination of Eudragit L100, lactose and magnesium stearate can be satisfactorily used together for the preparation of Aceclofenac tablets by direct compression.

***In vitro* dissolution study of Aceclofenac microspheres containing Eudragit L-100**

Aceclofenac release from the microsphere was studied for 12 hours in phosphate buffer pH 7.4. According to the solubility profile indicated in the literature, Aceclofenac is freely soluble in acetone and practically insoluble in water. Aceclofenac shows pH dependant drug solubility i.e. at lower pH (1.2) the solubility is lesser as compared to alkaline pH (6.8 or 7.4) [16]. To further increase the solubility of the drug, various authors have indicated the use of Sodium lauryl sulphate upto 3% w/v [8, 17] *in vitro*.

The formulation F1-T showed the highest drug release at 1:1 (drug: polymer) as shown in Figure 4. As the amount of polymer increased, drug release was decreased (Table 5). Eudragit L100 being a pH-independent and hydrophobic polymer prevented the penetration of dissolution medium into the microspheres as the concentration increased, leading to a slower dissolution and diffusion of the drug molecules from the compacted matrix formulation [18].

The 'model dependant' approach to analyze dissolution profile of formulations indicated that F1-T, F2-T, F4-T followed zero order kinetics i.e. the drug release was independent of the initial concentration [11]. The formulations F3-T, F5-T and F6-T followed Higuchi model (Table 6).

The value of n for F1-T, F2-T, F3-T and F4-T was in range of 0.5-1.0 which showed that the drug release mechanism was non-Fickian (combination of diffusion and erosion). The values of n for formulation F5-T was less than 0.5 which indicated Fickian transport mechanism [19].

The prepared tablets were compared with a marketed product of Aceclofenac (sustained release tablet) prescribed once daily (Figure 5). The f_2 (similarity factor) was 75 for F1-T and F3-T but comparatively F1-T had closer proximity in terms of drug release at t_{12}

(Table 5). This showed the similarity between the dissolution profiles of the two formulations.

Table 2: Particle size, Percentage yield and Entrapment efficiency (EE%) of Aceclofenac microspheres

Formulation	Particle size (µm)	Percentage yield	Theoretical drug loading (%)	Actual drug loading (%)	EE%
F1	**91.93**	**77.14**	**50.0**	**39.90**	**79.80**
F2	**92.31**	**61.3**	**33.3**	**27.30**	**81.98**
F3	**94.86**	**66.2**	**28.6**	**24.74**	**86.50**
F4	**95.23**	**95.5**	**25.0**	**21.78**	**87.14**
F5	**99.16**	**81.6**	**22.2**	**19.91**	**89.68**
F6	**99.73**	**82.1**	**20.0**	**18.20**	**91.00**

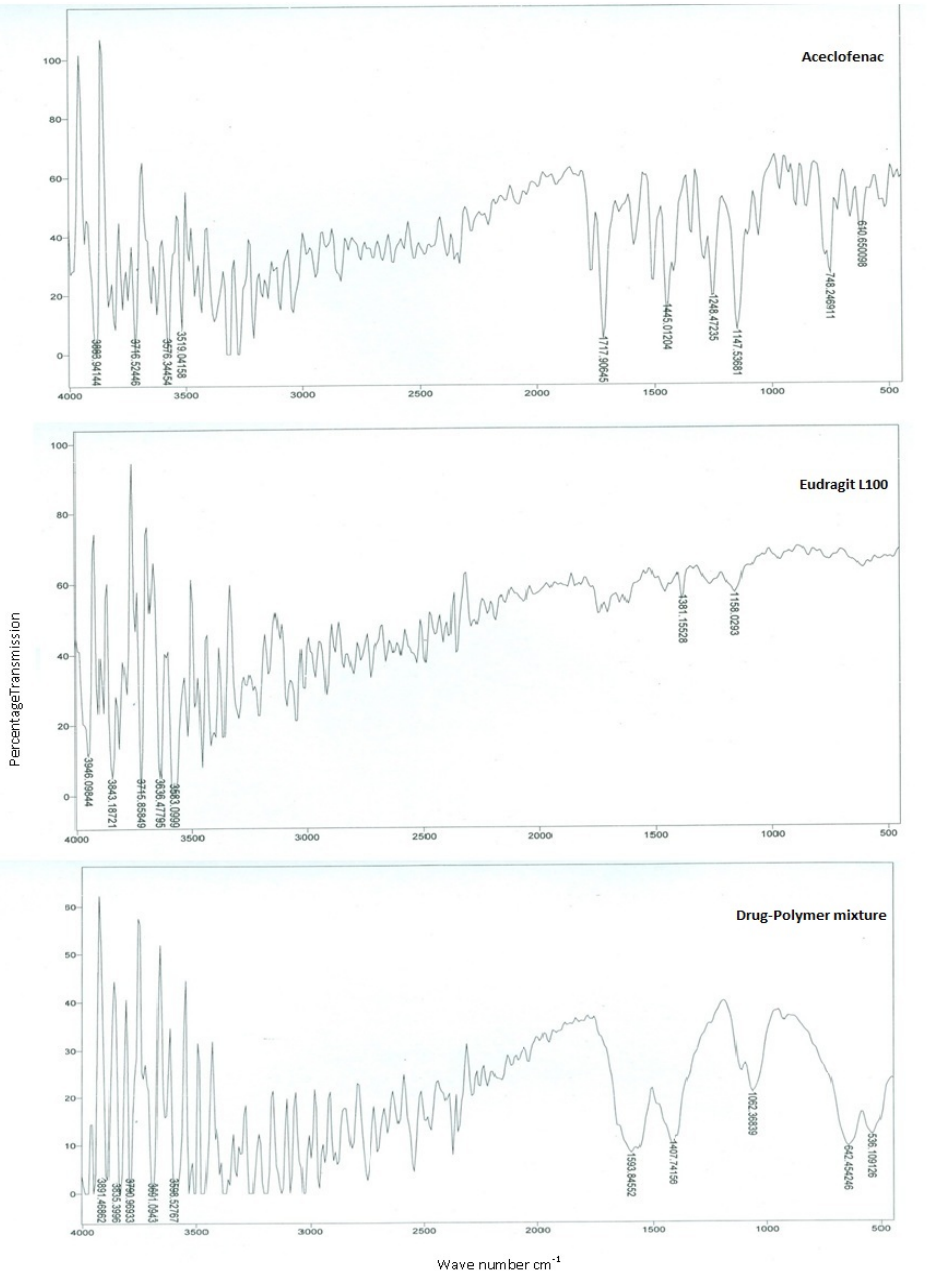

Figure 2: FTIR studies of Aceclofenac, Eudragit L100 and drug-polymer mixture

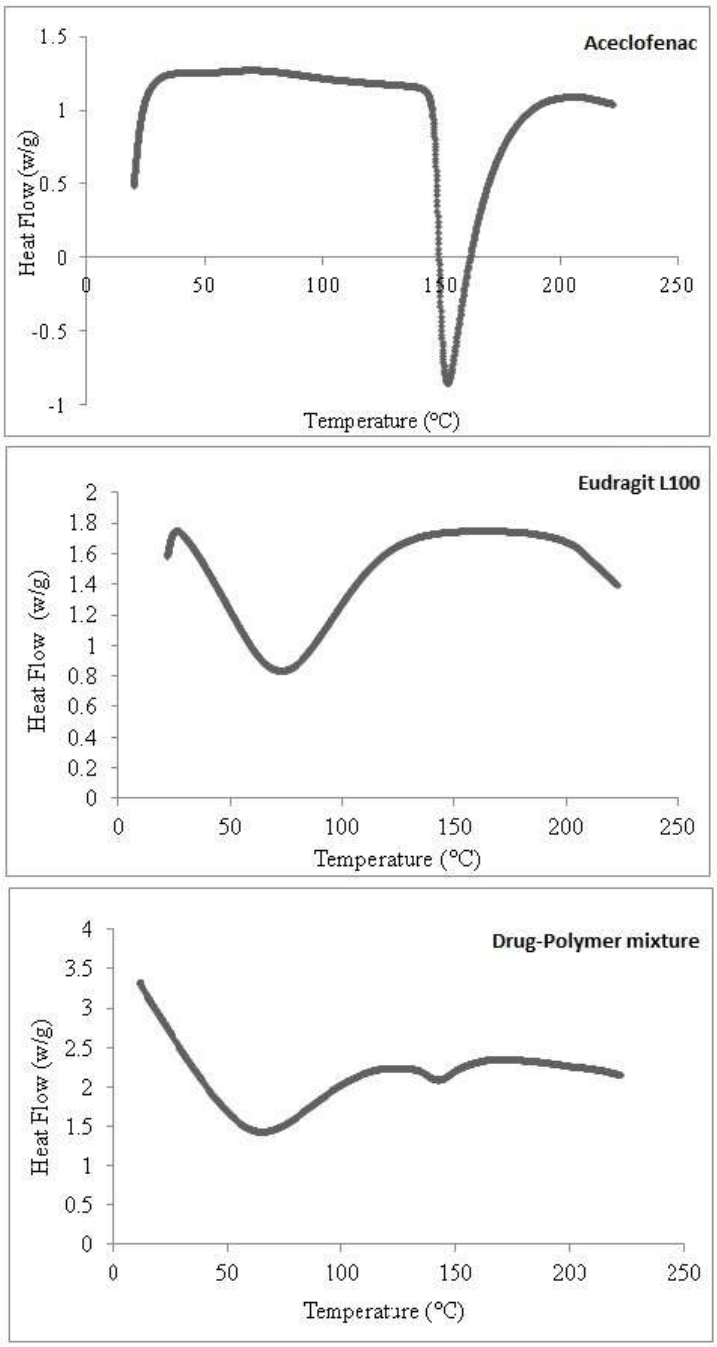

Figure 3: DSC studies of Aceclofenac, Eudragit L100 and drug-polymer mixture

Table 3: Bulk density, tapped density, Carr's index (CI%) and Hausner ratio of Aceclofenac microspheres

Formulation	Bulk density (g/cm^3)	Tapped density (g/cm^3)	CI (%)	Haunser ratio
F1	**0.190**	**0.225**	**15**	**1.18**
F2	**0.120**	**0.149**	**17**	**1.24**
F3	**0.156**	**0.183**	**14**	**1.17**
F4	**0.207**	**0.255**	**19**	**1.23**
F5	**0.196**	**0.224**	**12**	**1.14**
F6	**0.204**	**0.239**	**14**	**1.20**

Table 4: Thickness, diameter, weight, weight variation and friability of Aceclofenac microsphere tablets

Formulation code	Thickness (cm)	Diameter (cm)	Weight variation (mg)	Friability (%)
F1-T	0.801	0.373	0.27	0.72
F2-T	0.804	0.376	0.34	0.60
F3-T	0.805	0.375	0.22	0.63
F4-T	0.805	0.371	0.31	0.71
F5-T	0.808	0.369	0.34	0.54
F6-T	0.803	0.368	0.21	0.68

Table 5: Cumulative percentage drug release and fit factor of Aceclofenac tablets

Time (hr)	F1-T	F2-T	F3-T	F4-T	F5-T	F6-T	Standard
0.15	4.62	4.51	4.34	3.90	4.56	3.52	7.10
0.3	6.10	5.55	6.99	5.88	7.21	9.24	8.58
0.45	7.21	6.05	7.92	7.21	9.08	12.18	9.80
1	9.65	9.88	12.57	8.42	15.57	17.61	11.24
2	13.10	13.46	18.14	12.25	19.26	20.48	14.42
3	17.39	15.96	22.32	16.18	21.47	22.37	18.95
4	21.57	19.02	25.76	18.49	24.32	25.43	22.29
5	24.16	22.67	29.22	21.59	27.62	26.91	27.90
6	28.67	26.36	32.01	25.10	30.70	28.54	33.41
7	33.64	29.52	36.05	27.79	32.97	31.31	39.35
8	37.92	32.09	39.52	31.56	35.64	33.74	42.71
9	41.44	37.37	41.61	34.23	38.84	36.16	45.02
10	44.31	41.24	45.02	38.50	41.61	37.37	46.95
11	49.07	45.50	47.72	42.43	43.48	38.98	50.42
12	54.05	48.99	48.93	47.06	45.74	41.02	53.00
f_1	7	12	8	19	14	21	==
f_2	75	65	75	56	64	55	==

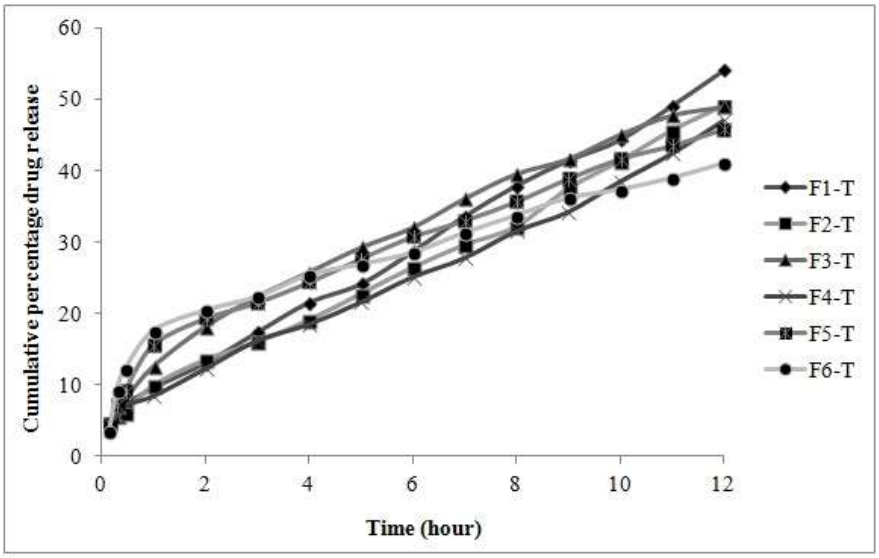

Figure 4: Cumulative percentage drug release of compressed microspheres of Aceclofenac

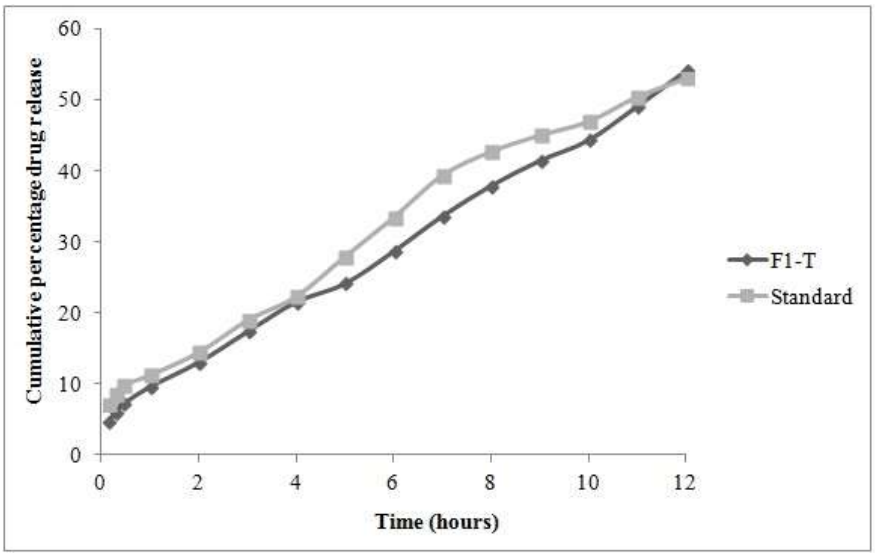

Figure 5: Comparative graph of F1-T and Branded Aceclofenac sustained release tablet

CONCLUSION

The formulation F1-T and F3-T showed a similar drug profile as compared to the Branded sustained release Aceclofenac tablet. Although the EE% of F1-T was lesser than the EE% of F3-T, but percentage yield was greater at 1:1 (drug: polymer) as compared to 1:2.5. The Micromeritic properties and physicochemical properties of compressed tablets of Aceclofenac microspheres at 1:1 was satisfactory and reproducible, making it a better candidate for the development of sustained release drug delivery system for Aceclofenac. The Branded Aceclofenac and F1-T shared zero order drug release kinetics with the anomalous drug release mechanism from matrix system.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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