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Research Article

FORMULATION, IN-VITRO EVALUATION AND RELEASE KINETIC STUDIES OF CEFIXIME MICROSPHERES

Mallikarjun.V*, Sriharsha.S.N.
*Department of Pharmaceutics, S.R College of Pharmacy, Ananthasagar, Warangal, Andhra Pradesh, India – 506371.
Pacific College of Pharmacy, Pacific University, Udaipur, Rajasthan, India.
Email: mallikarjunvasam@gmail.com

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Abstract

The aim of the study was to formulate and evaluate sustain release microspheres of Cefixime by orifice ionic gelation method. Different formulations were prepared using different ratios of drug and sodium alginate as polymer alone and in combination with co-polymers like carbapol 934P, HPMC K15M using calcium chloride acts as cross linking agent. The particle size, morphology of microspheres were studied using optical, scanning electron microscopy(SEM) and it was shown that microspheres gave particles in the range of 528.87±0.02µm to 828.0±0.08 µm were with more spherical shape, smooth surface and showed good flowing properties. The entrapment efficiency of microspheres was determined and had concluded that as the concentration of polymer increased, the entrapment efficiency was also increased. The entrapment efficiency of all the formulations was found to be in the range of 81.25±0.02% to 89.99±0.03%. The FT-IR studies showed stable character of cefixime in the drug loaded microspheres and revealed the absence of drug-polymer interaction. Data obtained from in-vitro release were fitted to various kinetic models and drug release followed zero order kinetics, the "n" value obtained from Korsmeyer-Peppas model showed that microspheres followed non-fickian drug release mechanism.

Key Words: Cefixime, Sodium alginate, Carbapol 934P, HPMC K15M, Entrapment Efficiency, Ionic gelation method and Microspheres.

Introduction: Microspheres are defined as "Monolithic spheres or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles" (or) can be defined as structure made up of continuous phase of one or

more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level[1]. Microspheres are solid spherical particles ranging in size from 1-1000µm. They are spherical free flowing particles consisting of proteins or synthetic polymers, which are biodegradable in nature.

Microspheres for oral use has been employed to sustain the drug release, and to reduce or eliminate gastrointestinal tract irritation. In addition, mutliparticulate delivery systems spread out more uniformly in the gastrointestinal tract. This results in more reproducible drug absorption and reduces local irritation when compared to single-unit dosage form disintegration of the dose and unwanted intestinal retention of polymeric material, which may occur with matrix tablets on chronic dosing, can also be avoided. Due to its small particles size, are widely distributed throughout the gastrointestinal tract which improves drug absorption and reduces side effects due to localized build-up of irritating drugs against the gastrointestinal mucosa[2].

Cephalosporins exert bactericidal activity by interfering with bacterial cell wall synthesis and inhibiting cross-linking of the peptidoglycan. The peptidoglycan layer is important for cell wall structural integrity. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by transpeptidases known as penicillin binding proteins (PBPs). PBPs bind to the D-Ala-D-Ala at the end of muropeptides (peptidoglycan precursors) to crosslink the peptidoglycan. Beta-lactam antibiotics mimic this site and competitively inhibit PBP cross linking of peptidoglycan. The cephalosporins are also thought to play a role in the activation of bacterial cell autolysins which may contribute to bacterial cell lysis.

Materials & Methods:

Materials: Cefixime was obtained as a gift sample from Dr. Reddy Laboratories Hyderabad, A.P, India. All other excipients are obtained from S.D Fine Chemicals, Mumbai.

Method: Cefixime loaded microspheres formulation was prepared by using Hydroxyl propyl methyl cellulose (HPMC), and Carbapol P934 as copolymer employing Ionic Gelation technique[3]. The interaction between Sodium Alginate and Calcium chloride was used to prepare Calcium alginate microspheres. Cefixime 400 mg was dispersed in the Sodium Alginate 3%, 4% and 5%, the formulations was coded as F1, F2 and F3. In other formulations, Cefixime 400 mg was dispersed in the Sodium Alginate 3%, 4% and 5%, along with (1%) co polymer Carbapol 934P and (1%) co polymer HPMC K15M. These formulations were coded as F4, F5, F6, F7, F8 and F9 with different concentrations of polymer shown in Table 1.

First the polymeric solution was mixed thoroughly with stirrer and then it was sonicated for 15 min to form viscous solution of polymer. To this polymeric solution drug was added and stirred thoroughly with stirrer to form uniform dispersion of drug in the polymeric solution. The resulting dispersion was then added manually drop wise into calcium chloride (5% w/v) solution through a syringe of size no 22G on magnetic stirrer with 100 rpm. The added droplets were retained in calcium chloride solution for 15 minutes to complete curing reaction and to produce rigid microspheres. The microspheres were collected by decantation and then washed thoroughly with distilled water and dried at 45 ºC for 12 hours. The formed microspheres are shown in figure 1.

Table 1: Formulation Table of Cefixime Microspheres

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Cefixime (mg)	400	400	400	400	400	400	400	400	400
Sodium alginate (%)	3	4	5	3	4	5	3	4	5
Carbopol P934 (%)	-	-	-	1	1	1	-	-	-
HPMC K15M (%)	-	-	-	-	-	-	1	1	1
Calcium chloride (%)	5	5	5	5	5	5	5	5	5

Figure 1: Photography of Cefixime loaded microspheres

Evaluation of microspheres:

1. **Production Yield:** The production yield of microspheres of various batches were calculated using the weight of final product after drying with respect to the total weight of the drug and polymer used for preparation of microspheres and % production yields were calculated as per the formula mentioned below[4].

$$\% PY = W_O/W_T \text{ X } 100$$

PY= Production Yield; W_O = practical mass(microspheres); W_T = Theortical mass (polymer+drug)

The production yield of microspheres was shown in Table 3.

2. Drug Entrapment Effiency:

About 100 mg of microspheres was taken and triturated with phosphate buffer pH 6.8 and transferred to 100 ml volumetric flask. It was made up to 100 ml and mixed well. The solution was then kept aside for 12 hrs. It was than sonicated in ultrasonicator and then filtered through membrane filter (0.45µm) and estimated for drug content by measuring the absorbance at 288nm. The drug entrapment efficiency was calculated using the formula[5].

$$\text{Drug Entrapment Efficiency} = \frac{\text{Estimated \% drug content}}{\text{Theoritical \% drug content}} \text{ X } 100$$

The results obtained were given in Table 3.

3. Particle size analysis:

Particle size of different batches of microspheres was determined by optical microscopy. The projected diameter of microspheres from each batch was determined using ocular micrometer and stage micrometer equipped with optical microscope. Analysis was carried out by observing the slide containing microspheres under the microscope. Mean particle size of all formulations were determined. The results were given in the Table 3.

4. Flow property of Microspheres:

The flow properties of prepared microspheres were investigated by measuring the Angle of repose by using fixed funnel method. The value of Angle of repose was calculated by using the formula[6].

$$\text{Angle of repose } (\theta) = \tan^{-1}(h/r)$$

h = cone height, r = radius of circular base formed by the microspheres on the ground. The results were shown in the Table 3.

5. Scanning Electron Microscopy:

Shape and surface morphology of microspheres was studied using Scanning Electron Microscopy(SEM)[6,7,8]. The microspheres were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument. The photographs were taken using a Hitachi-3700N.

The surface morphology of cefixime microspheres were studied by using SEM analysis.

SEM photographs of formulations (F7) were shown in Figure 2, which indicated that the microspheres were discrete, uniform and spherical. The microspheres prepared with HPMC exhibited a smooth textural surface.

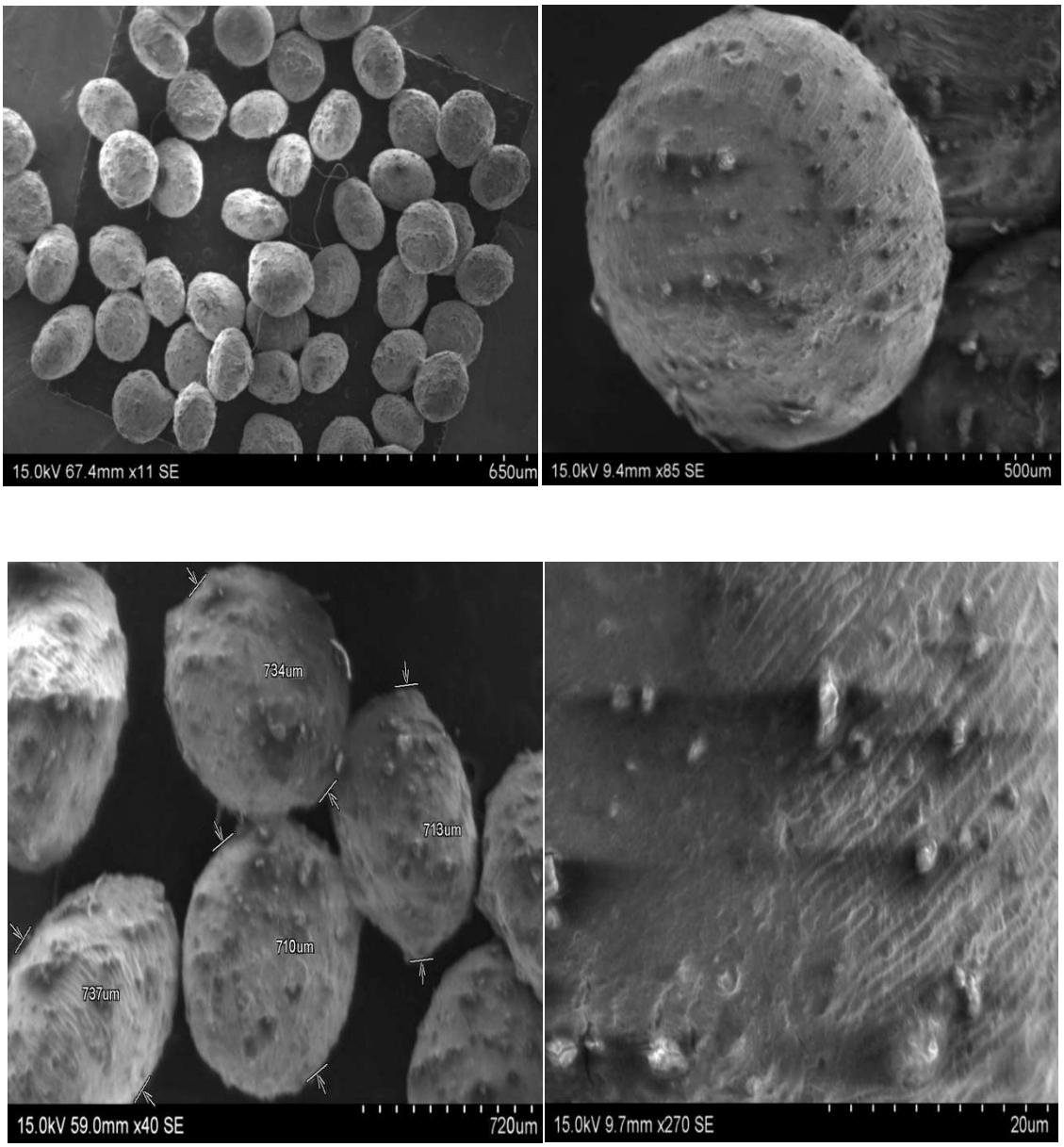

Figure 2: SEM Images of F7 Formulation

6. Degree of Swelling:

The swelling ability of microspheres in physiological media was determined by swelling them in the Phosphate buffer pH 7.2. 100 microspheres were suspended in 5 ml of phosphate buffer pH 7.2, the increase in particle size of microspheres was noted upto 10 hrs and the swelling index was calculated using the formula

$$\alpha = (W_S - W_O) / W_O$$

α is the degree of swelling; Wo is the particle size of microspheres before swelling; Ws is the particle size of microspheres after swelling. The degree of swelling of all the formulations were shown in Table 3.

Results and discussion

Drug Excipient Compatibility Studies:

1. Infrared Spectroscopy Studies (I.R.):

The characteristics peaks for Cefixime were obtained at 1768.75 cm^{-1}, 1667.09 cm^{-1}, 1589.55 cm^{-1}, 1536.88 cm^{-1}, 1381.56 cm^{-1}, 1336.05 cm^{-1}, 1224.08 cm^{-1}, 1188.06 cm^{-1}, 929.23 cm^{-1}, 862.62 cm^{-1}, 799.76 cm^{-1}, 745.88 cm^{-1} and 565.57 cm^{-1} .Similarly all the characteristics peaks are observed in drug-polymers mixture. This revealed that there was no chemical interaction between drug and polymer. All the spectras were shown in the FT-IR graph Figure 3-6.

Table 2: Interpretation data of Cefixime Microspheres

Figure No	**Name of the Compound**	**Functional Group Assigned (wave number in cm^{-1})**				
		C=O str of lactam	C=O str of Amide	C=N str of Oxime	N=O str	$-NH_2$ of carbamate
-	Characteristic peak	1780-1710	1690-1630	1565-1700	1540-1380	1340-1300
11	Drug(Cef-API)	1768.75	1667.09	1589.55	1381.56	1336.06
14	Drug+ Sod alginate+HPMCK15M	1767.68	1658.78	1547.79	1380.82	1337.15

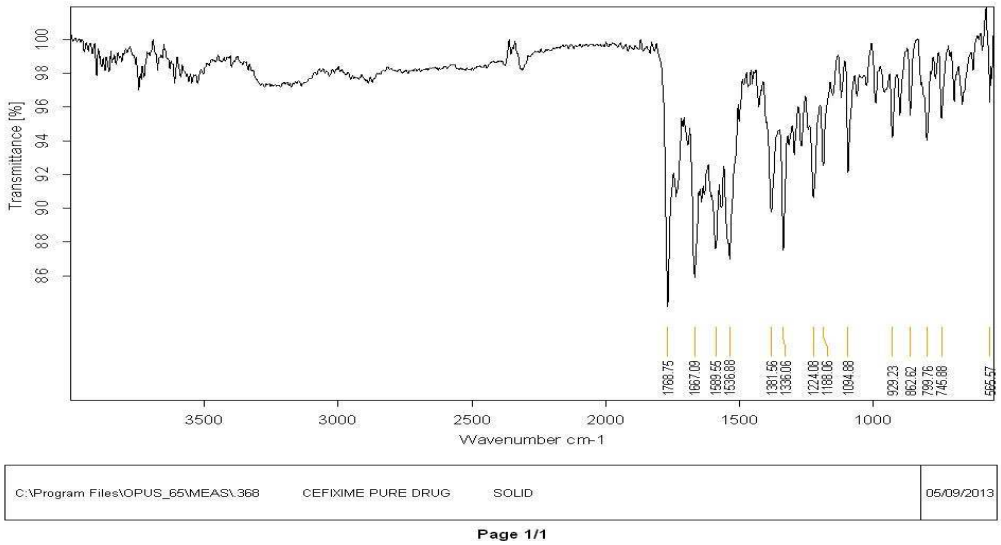

Figure 3: FT-IR Graph of Pure drug Cefixime

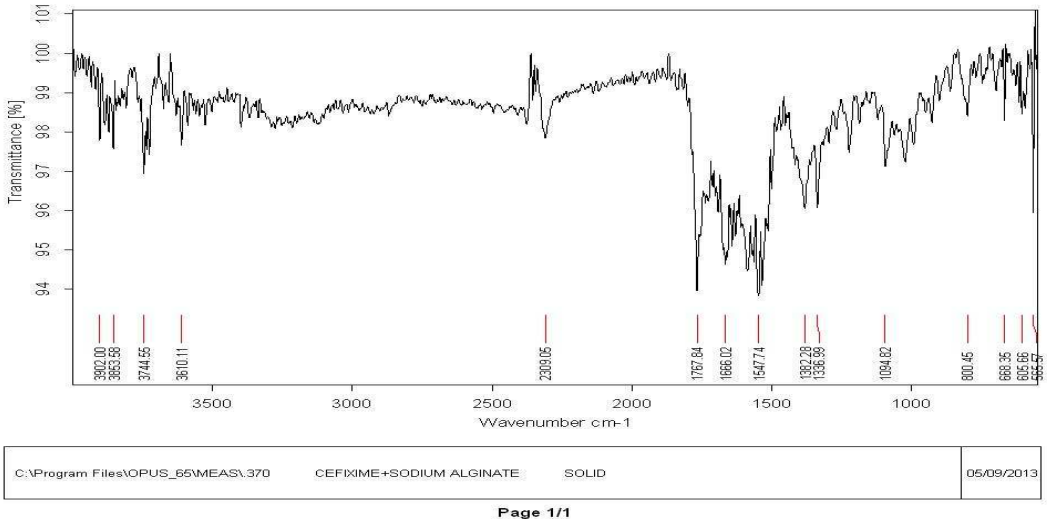

Figure 4: FT-IR Graph of Pure drug Cefixime + Sodium alginate

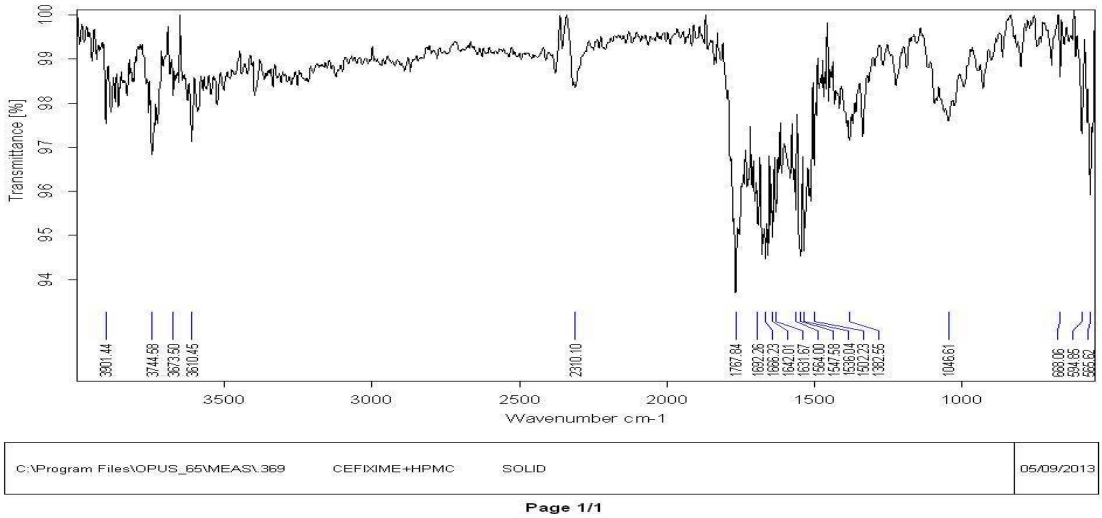

Figure 5: FT-IR Graph of Pure drug Cefixime + HPMC

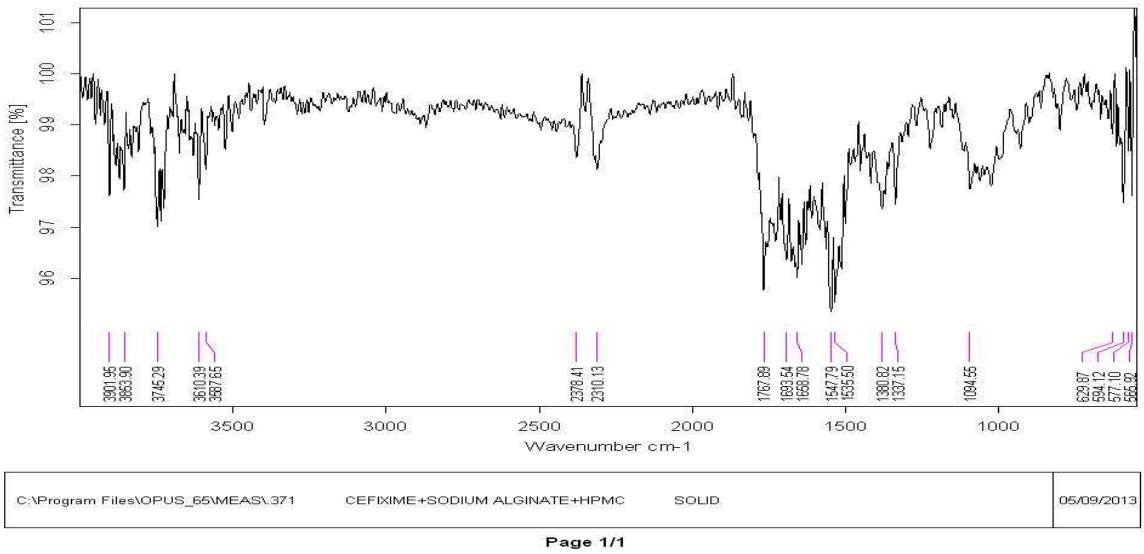

Figure 6: FT-IR Graph of F7 Formulation

Table 3: Physicochemical Characterization of Cefixime Microspheres

Formulation Code	Angle of Repose(θ)	Particle size(µm)	Swelling index	Percentage yield(%)	Entrapment Efficiency
F1	23.1±0.02	528±0.05	0.63±0.01	86.76	81.25±0.02
F2	23.3±0.02	569±0.03	0.65±0.04	86.68	82.17±0.03
F3	23.8±0.03	618±0.02	0.67±0.02	86.67	82.98±0.02
F4	24.2±0.05	690±0.03	0.69±0.03	84.85	83.50±0.03
F5	24.6±0.05	760±0.04	0.70±0.06	84.60	84.78±0.02
F6	24.8±0.04	824±0.08	0.71±0.05	84.65	86.01±0.06
F7	25.1±0.02	642±0.06	0.70±0.05	88.58	88.85±0.04
F8	25.4±0.03	698±0.05	0.73±0.04	88.42	89.60±0.03
F9	25.8±0.03	720±0.03	0.75±0.01	88.31	89.99±0.03

Values are mean ± SD, n=3

7. *In Vitro* Drug Release Studies:

The *In Vitro* dissolution study of Cefixime loaded microspheres was carried out in USP Dissolution Apparatus 1 (Basket type Apparatus). The affect of polymers and its concentration were studied for the release profile of prepared microspheres of Cefixime, the release mainly depended on the polymer concentration and its viscosity. The percentage cumulative drug release (%CDR) was calculated from the amount of drug release and the results are shown in the Table 12 to 20.

Table 4:- *In Vitro* Drug Release Studies of cefixime microspheres(F1-F9).

Time(hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	12.51	9.27	6.5	14.06	11.27	8.47	15.63	11.73	9.62
2	18.79	13.76	11.11	21.20	17.62	14.57	21.79	19.89	17.26
3	28.56	24.25	19.09	28.65	24.92	20.95	29.68	24.35	20.66
4	36.77	31.34	27.17	36.46	31.58	25.95	35.43	30.17	28.18
5	48.61	43.81	39.12	45.32	38.17	32.30	42.60	37.48	33.89

6	57.84	53.90	46.47	50.40	45.35	38.94	50.07	44.15	39.54
7	68.82	63.10	59.07	56.19	51.79	46.89	59.54	50.73	46.98
8	81.33	75.90	71.32	63.79	58.71	53.59	66.99	58.71	52.92
9	92.93	86.21	81.13	69.97	65.44	59.85	72.91	64.36	59.32
10	-	94.62	89.86	77.90	73.22	66.47	79.91	70.18	66.08
11				85.03	81.00	75.75	86.78	78.22	73.09
12				91.24	85.90	81.34	98.79	88.94	82.87

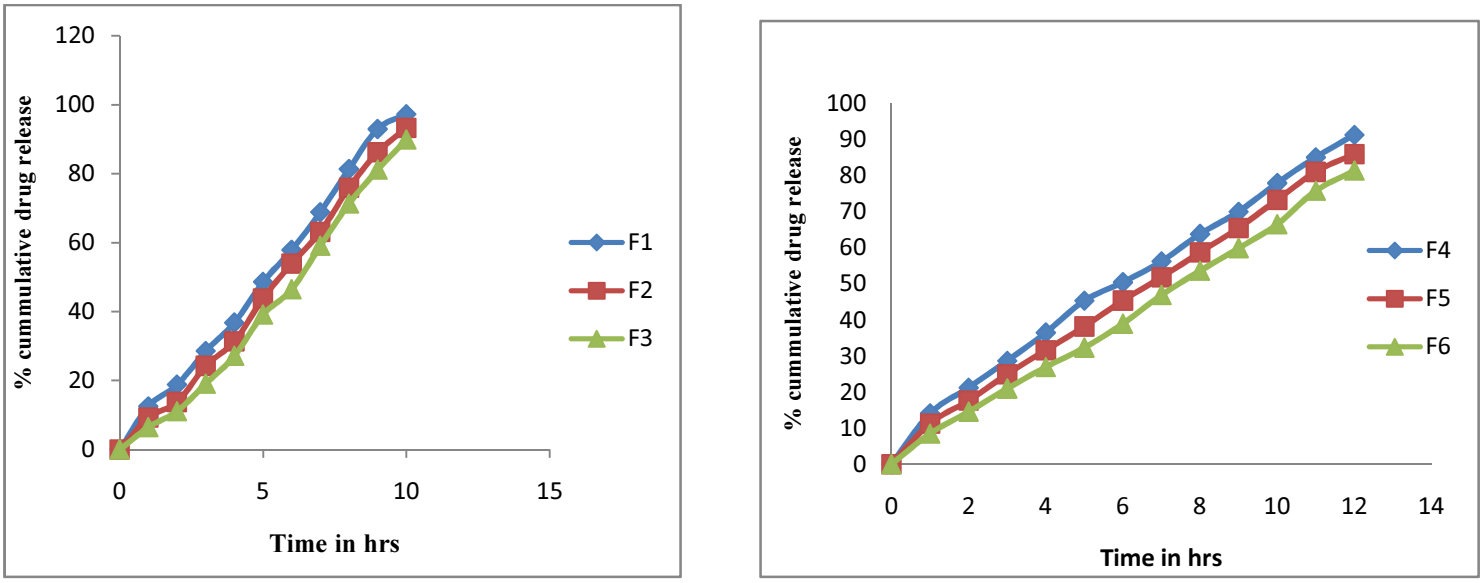

Figure 7: *In-vitro* drug release profile of Formulation F1, F2, F3, F4, F5 and F6

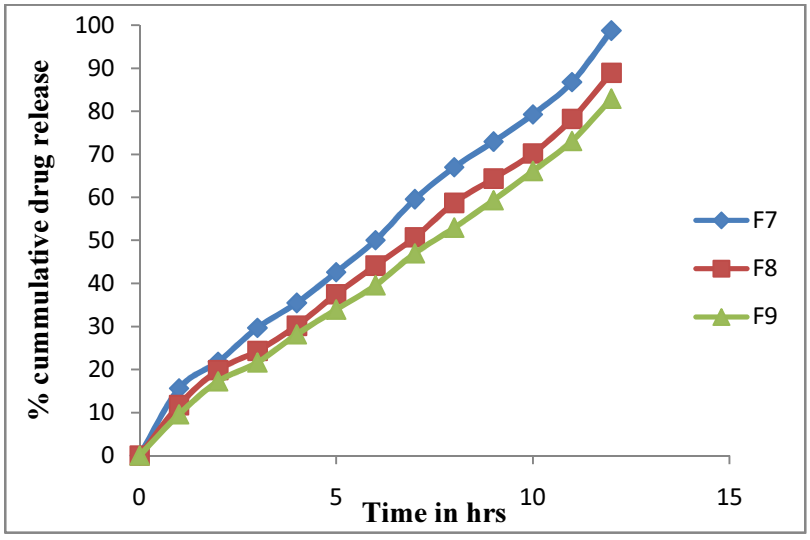

Figure 8: *In-vitro* drug release profile of Formulation F7, F8 and F9

8. Release Kinetics of microspheres:

Table 5:- Drug Release Kinetics data of Formulations F1 to F9

FORMULATION CODE	ZERO ORDER	FIRST ORDER	HIGUCHI	KORSMEYER PEPPAS	
	r^2	r^2	r^2	N	K
F1	0.996	0.837	0.905	0.941	1.035
F2	0.994	0.856	0.885	0.814	0.892
F3	0.988	0.890	0.858	0.912	0.743
F4	0.994	0.917	0.954	0.769	1.112
F5	0.998	0.937	0.937	0.840	1.011
F6	0.994	0.842	0.916	0.924	0.892
F7	0.998	0.922	0.940	0.759	1.131
F8	0.955	0.886	0.930	0.808	1.033
F9	0.996	0.919	0.923	0.854	0.956

9. Stability Studies:

The stability study of the formulation (F7) was performed after 3 months and the effect on various parameters like physical appearance, drug content and in-vitro drug release was studied and results were shown in table 6,7; fig 9

Table 6: Drug content before and after 3months.

FORMULATION CODE	% DRUG CONTENT	
	Initial	After 3 months
F7	88.85	84.93

Table 7: Comparison of in vitro drug release of formulation (F7) initially and after 3 months

Formulation(7)	% cumulative drug release	
Time (hrs)	Initial	After 3 months
1	15.63	16.82
2	21.79	23.75
3	29.68	31.71
4	35.43	37.81
5	42.6	44.25
6	50.07	53.32
7	59.54	61.59
8	66.94	70.02
9	72.91	74.58
10	79.28	80.96
11	86.78	88.65
12	98.71	98.93

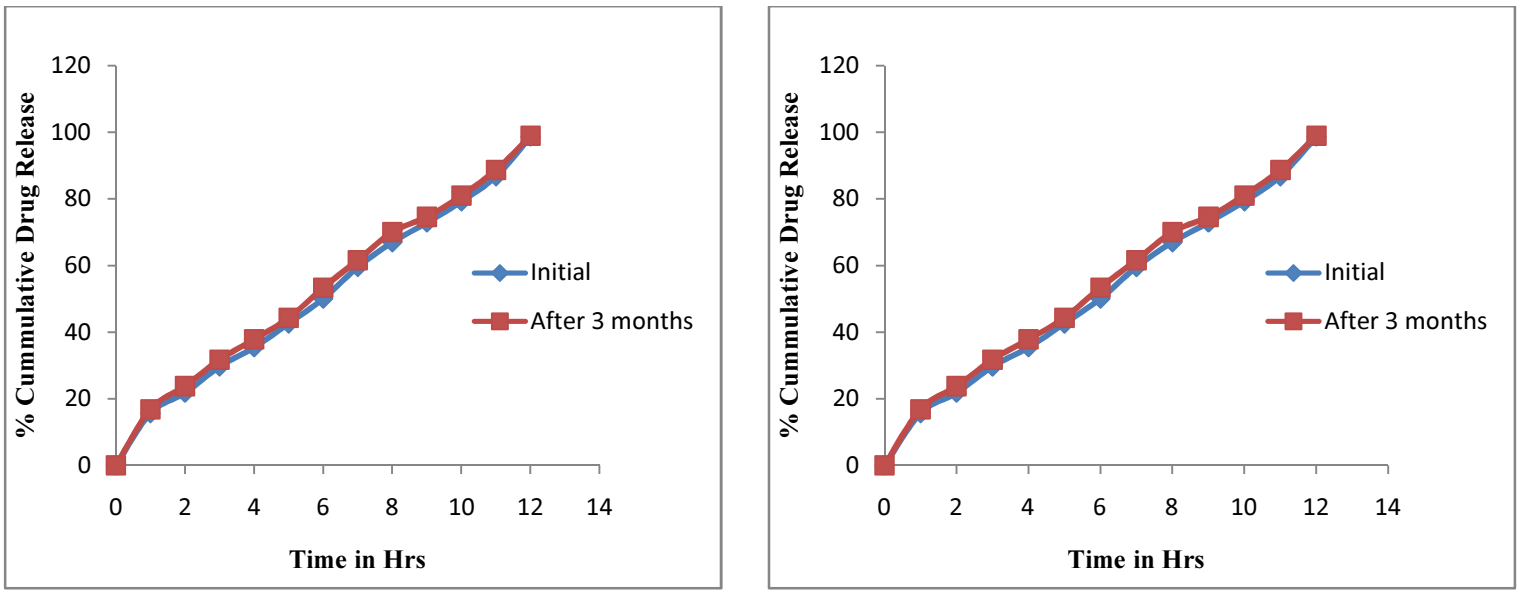

Fig-9: Comparison in-vitro drug release of formulation (F7) initially and after 3 months.

Conclusion

From the experimental results it can be concluded that

- ✓ The percentage entrapment efficiency was higher for F9 formulation i.e. 89.99±0.03%. Practical yield obtained was higher for F7 formulation i.e 88.58%.
- ✓ The particle size analysis revealed that all formulations gave particles in the range of 528.87±0.02 µm to 828.0±0.08 µm and all the formulations showed good flowing properties.
- ✓ SEM analysis of the microspheres revealed that all the formulation was discrete, spherical with ideal surface morphology. More spherical shaped microspheres were formed with copolymer HPMC than other formulation.
- ✓ Increase in the polymer concentration led to increase in the degree of swelling.
- ✓ The data obtained are fitting to various kinetic models indicated that the drug release followed zero order kinetics and F7 showed (r^2) value of 0.998. The n value obtained from Korsmeyer-Peppas model showed that the microspheres followed non-fickian drug release mechanism.
- ✓ The IR spectra revealed that there was no interaction between polymers and drug, hence they are compatible.
- ✓ There was no change in the appearances of the microspheres and no degradation of the drug during storage conditions.
- ✓ From all the parameters studied, it can be concluded that HPMC K15M is the better sustain the drug release than Carbapol P934 for the formulation of sustain release microspheres.

Thus, the formulated microspheres seem to be a potential candidate as sustained drug delivery system for symptomatic treatment therapy of bacterial infection. Therefore, F7 formulation may be used for reducing the dosing frequency there by improving the effectiveness of the drug.

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Correspondence address:
V.Mallikarjun*,
Email: *mallikarjunvasam@gmail.com*