IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
November 2019 Vol.:16, Issue:4

Formulation and *In Vitro* Evaluation of Ciprofloxacin Hydrochloride Mucoadhesive Microspheres

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

SM Shahidulla[1], Saniya Fatima*[1]

Deccan School of Pharmacy, Dar-Us-Salam, Aghapura, Hyderabad, Telangana- 500001.

Submission: 21 October 2019
Accepted: 27 October 2019
Published: 30 November 2019

Keywords: Mucoadhesive microspheres, Ciprofloxacin HCl, Hydroxy Propyl Methyl Cellulose K100, Carbopol 934P, Ethylcellulose, solvent evaporation method

ABSTRACT

The aim of the present work is to study the prepared Ciprofloxacin HCL in mucoadhesive pharmaceutical form by two type of formulations methodology *i.e.* with solvent evaporation method and ionic gelation method. The main objective of the research work is to study the drug and polymer interaction. The formulations were characterized for their physicochemical parameters like swelling ratio, water uptake, gel fraction, percentage yield, drug content, SEM analysis, drug entrapment efficiency and size analysis. FTIR studies reveal the drug excipients compatibility. *In-vitro* drug release studies revealed that the high % drug release for SF6 and IF6 was 99.12% up to 10 hrs. Entrapment efficiency, particle size for SF6 was found to be 96±1%, 1.93±0.09, respectively. Swelling ratio and water uptake by SF6 formulation were 9.6±0.05 and found to be good swelling ratio, high water absorbing ability and high % adherence. Results from stability studies indicate that the formulated mucoadhesive microspheres are stable for a period of 3 months under two different conditions at 25±2°C / 65±5%RH and 40±2°C / 75±5%RH. Among all formulations, SF6 showed controlled drug release up to 14 hrs and considered as ideal formulation.

INTRODUCTION

Microspheres are defined as homogeneous, monolithic particles in the size range of about 1µm-1000µm and are widely used as drug carriers for controlled release. These systems have significant importance in biomedical applications. Microspheres can be produced for protection of core material, reduction of gastric irritation decrease in volatility, conversion of liquid to pseudo-solid, cell microencapsulation and for designing pulsatile drug delivery systems. Administration of the drug in the form of microspheres usually improves the treatment by providing the localization of the active substances at the site of action and by prolonging release of drugs.[1]

✓ Mucoadhesive microspheres include microparticles and microcapsules of 1 to 1000 µm in diameter consisting either entirely of mucoadhesive polymer or having an outer coating with adhesive property.

✓ Incorporating mucoadhesiveness to microspheres leads to efficient absorption and enhanced bioavailability of drug.[2]

Mucoadhesion is a promising approach in the design of the drug delivery systems to prolong the residence time of the dosage form the site of application or absorption and to facilitate intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of drugs. Several studies reported mucoadhesive drug delivery systems in the form of tablet, films, patches and gels for oral, buccal, nasal, ocular, and topical routes.[3]

Ciprofloxacin hydrochloride is a broad-spectrum antimicrobial carboxy fluoroquinolone. It is used for the treatment of urinary tract contaminations, intense uncomplicated cystitis, lower respiratory tract contaminations, intense sinusitis, skin and skin structure diseases, bone and joint contaminations, typhoid fever (enteric fever), uncomplicated cervical and urethral gonorrhea, and inhalational bacillus anthracis (post-exposure). It is soluble in water and belongs to class III (High solubility, low permeability) with a bioavailability approximately70%, protein binding 20 to 40%. It undergoes hepatic metabolism including CYP1A2. Hence the objective of the present work was to formulate the mucoadhesive microsphere of Ciprofloxacin hydrochloride by solvent evaporation method to improve residence of dosage form in GIT, reduced dosing frequency and enhance bioavailability.[4-8]

MATERIALS AND METHODS

Ciprofloxacin hydrochloride was obtained from Drugs India, HYD, Ethylcellulose, Tween 80 from SD fine chemicals, Mumbai, Carbopol 934 P, Liquid paraffin from Qualigens fine chemicals, Mumbai, Hydroxy Propyl Methyl Cellulose - K100 from Ontop pharmaceuticals, Bangalore.

Drug polymer compatibility studies:

1. Fourier Transform Infrared Spectroscopy (FTIR):

Ten milligrams of drug alone, mixture of drug and polymer were weighed and mixed properly with KBr uniformly. A small quantity of the powder was compressed into a thin semitransparent pellet by applying pressure. The IR-spectrum of the pellet from 450-4500cm^{-1}.[9]

2. Differential Scanning Calorimetric (DSC):

Differential scanning calorimeter (DSC) was performed using Perkin Elmer instruments, (Perkin Elmer DSC-7, Norway, USA) to study the thermal behaviour of Ciprofloxacin hydrochloride and mixture of drug and polymers. (Table No.7, Figure No. 9 and 10)[10]

Preparation of Ciprofloxacin hydrochloride by solvent evaporation technique:

Accurately weighed quantity of Ciprofloxacin was dissolved in distilled water. (Table No. 1) This was poured into solution containing different volume of HPMC K100, Carbopol 934P, ethylcellulose which was maintained at room temperature and subsequently stirred at 1000 rpm on magnetic stirrer for two hours to remove the volatile solvent. To this polymeric solution, ethanol was added. The resultant dispersion was extruded through a syringe (Needle no. 18) into 250 ml of liquid paraffin containing 1% w/v tween 80 as emulsifying agent. The stirring was continued until ethanol was evaporated in about 2 to 3 h. After the complete evaporation of solvent, the microspheres were hardened as discrete particles. The microspheres were separated by filtration using Whatman filter paper and washed three times with distilled water, dried at 45^{o}C and stored in a desiccators. Then the obtained microspheres were collected and performed further studies.[10]

Table No. 1: Compositions of Ciprofloxacin hydrochloride mucoadhesive microspheres

Formulation Code	Drug (mg)	Polymers				
		EC (mg)	Carbopol 934P (mg)	HPMK 100 (mg)	Liquid paraffin (ml)	Tween 80 (1%)
SF0	500	500	-	-	250	2.5ml
SF1	500	500	-	250 (1:05)	250	2.5ml
SF2	500	500	-	500 (1:1)	250	2.5ml
SF3	500	500	-	750 (1:1.5)	250	2.5ml
SF4	500	500	250 (1:05)	-	250	2.5ml
SF5	500	500	500 (1:1)	-	250	2.5ml
SF6	500	500	750 (1:1.5)	-	250	2.5ml

Physicochemical evaluation:

1. Swelling ratio

Swelling of mucoadhesive microspheres was carried out in triplicate by gravimetric method. (Table No. 2, figure no.1) Known weight of mucoadhesive microspheres were taken and immersed in pH 7.4 phosphate buffer solution at 37ºC. The difference in weight has given the amount of pH 7.4 phosphate buffer solution uptake by mucoadhesive microspheres after definite time intervals (60 min).[10]

$$\text{Swelling ratio} = W_t - W_o / W_o$$

Where, W_t = weight of mucoadhesive microspheres at time.

W_o = initial weight of mucoadhesive microspheres.

2. Water uptake

Known weight of mucoadhesive microspheres for were taken and immersed in excess of distilled water at 37ºc.(Table no. 3, figure no. 2) The difference in weight has given the amount of water uptake by mucoadhesive microspheres for definite period of time.[11]

Water uptake= W_S/W_D

Where, W_S = weight of swollen mucoadhesive microspheres.

W_D = weight of dried mucoadhesive microspheres.

3. Gel fraction:

To extract the insoluble parts of mucoadhesive microspheres (*i.e.*, the gelled part), the prepared mucoadhesive microspheres were soaked in water for 48 h. Then they were taken out and washed with hot water to remove soluble part, dried and weighed. Gel fraction was determined from equation given below.[11] (Table no.4 and Figure no: 3 and 4)

Gel fraction=$W_e/ W_o \times 100$

Where, w_o = weight of dried mucoadhesive microspheres after crosslinking.

w_e= weight of Sample after extraction of soluble parts

4. Size analysis by optical microscopy

After drying at 37ºC for 48 hours, the mean diameter of the dried beads was measured by using an optical micrometer fitted with a calibrated eyepiece.[12]

5. Surface morphological studies by SEM analysis

The surface morphological studies and shape of the dried mucoadhesive microspheres were examined by using scanning electron microscopy. (Figure no 5)[13]

6. Percentage yield

Percentage yield was calculated using equation given below:

%yield = Practical yield / Theoretical yield × 100[13]

7. Drug content

100 mg equivalent weight of Ciprofloxacin HCl mucoadhesive microspheres were triturated using mortar and pestle. Then the triturated mucoadhesive microspheres were placed in volumetric flask and the volume was made upto 100 ml with pH 7.4 phosphate buffer

solution and kept aside for 24h. The absorbance were measured at 275 nm spectrophotometrically. The drug content was calculated by using the formula.[14]

Theoretical drug content = (Weight of drug / total weight of microspheres) × 100

Practical drug content = Concentration × dilution factor × Conversion factor

8. Drug entrapment efficiency

The 100 mg equivalent mucoadhesive microspheres were soaked and digested in 100 ml of pH 7.4 phosphate buffer solution for 24 h and then the solution was filtered. The absorbance was analysed spectrophotometrically at 275 nm after a necessary dilutions.[15]

Drug Entrapment efficiency = (Actual drug content /Theoretical drug content) ×100

9. *In vitro* drug release studies:

In each formulation100 mg Ciprofloxacin HCl equivalent mucoadhesive microspheres were taken and immersed in 900 ml of pH 1.2 HCl buffer for first two hours. Then acidic buffer is replaced with fresh buffer of 900 ml of pH 7.4 phosphate buffer which mimic stomach and intestinal pH conditions.

The aliquots of 5 ml were withdrawn at one hour time interval and replaced with equal volume of dissolution medium in order to maintain the sink condition throughout the study. Then the samples were filtered and analyzed spectrophotometrically at 275 nm after a suitable dilution. The cumulative amount of drug release was calculated. (Table no.5 Figure no: 6)[16]

10. *In-vitro* mucoadhesion test:

In-vitro mucoadhesion test was performed by using *in vitro* Wash- Off test. 100 particles of mucoadhesive microspheres were counted and placed on strip of goat intestine which was adhered to glass slide by using cyanoacrylate glue. Then this slide consisting of particles was placed in disintegration apparatus for eight hours of time. After eight hours number of particles being adhered was counted and % of Adherence was calculated by using the formula. (Table no.6 Figure no 7 and 8)[17]

Na = (N/No) X 100

Where,

Na = is the adhesion number

No = is the total number of applied particles

N = number of particles attached to the substrate.

11. Stability studies:

Stability studies were performed at a temperature of 30±2^{0}C/65±5%RH &40±2^{0}C/75±5%RH, over a period of three months (90 days) on the mucoadhesive microspheres of ciprofloxacin hydrochloride (Table no.8). [18]

RESULTS AND DISCUSSION

Swelling studies was done in triplicate using phosphate buffer. The swelling ratio of optimised formulation SF6 was found to be 9.6±0.05 in 360 mins.

Table No. 2: Swelling ratio data for Solvent Evaporation formulations

Formulation Code	**Time in mins**					
Formulation	**60**	**120**	**180**	**240**	**300**	**360**
SF0	1.2±0.05	2.6± 0.1	4.5±0.1	5.4± 0.15	7.2± 0.05	7.6± 0.05
SF1	2.2± 0.05	3.6 ±0.05	4.4±0.05	6.4± 0.05	7.3± 0.05	8.2 ±0.05
SF2	2.7± 0.11	4.3± 0.05	6.2± 0.11	7.5± 0.1	10.7± 0.05	10.7± 0.05
SF3	0.5± 0.15	2.6± 0.15	3.6± 0.05	4.3± 0.05	6.6± 0.05	6.6± 0.05
SF4	1.3± 0.1	3.4± 0.15	4.7± 0.05	5.4± 0.05	7.3± 0.11	7.3± 0.11
SF5	1.7 ±0.1	4.6± 0.1	6.5± 0.15	7.5± 0.05	9.6± 0.05	9.6± 0.05
SF6	1.0±0.1	2.6± 0.1	5.3±0.11	9.4±0.05	9.6 ±0.05	9.6±0.05

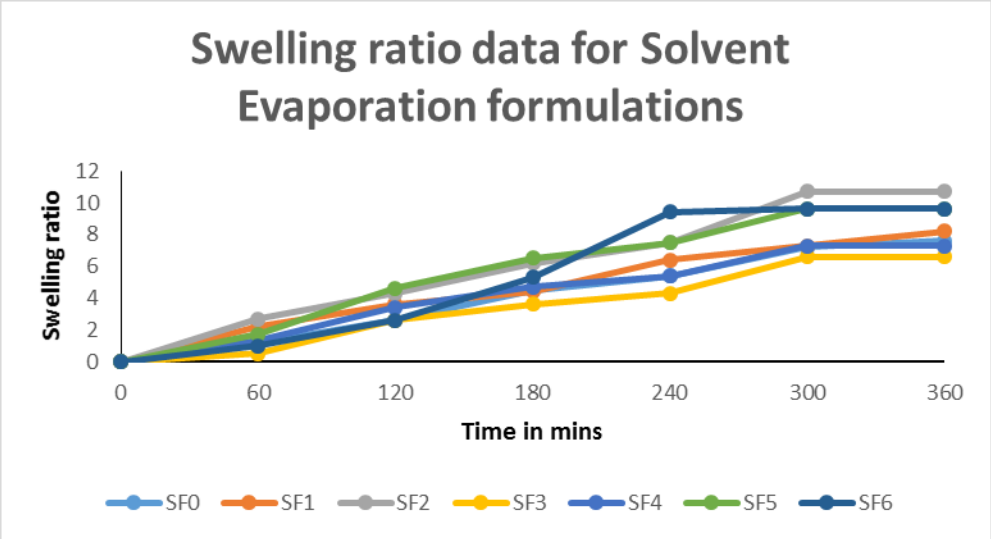

Figure No. 1: Swelling ratio for solvent evaporation

The ability of mucoadhesive microspheres to absorb water and swell without losing its structure is done by water uptake studies. The water uptake of SF6 was found to be11.6± 0.05 in 360 mins.The observed results were shown in the ***table 3.***

Table No. 3: Water uptake data for Solvent Evaporation formulations

Formulation code	Time in mins					
	60	**120**	**180**	**240**	**300**	**360**
SF0	2.4±0.05	3.5± 0.1	5.3± 0.1	6.2± 0.05	8.5± 0.05	8.6± 0.05
SF1	3.2±0.05	4.2±0.05	5.3± 0.05	7.4± 0.05	8.5± 0.05	9.2± 0.05
SF2	3.5±0.11	5.2± 0.05	7.3± 0.11	8.4± 0.1	11.5± 0.57	11.6± 0.57
SF3	1.5± 0.15	3.3± 0.15	4.2± 0.05	5.5± 0.05	7.4± 0.05	7.3± 0.05
SF4	2.5± 0.1	4.3± 0.15	8.6± 0.05	9.2± 0.05	11.6± 0.05	11.6± 0.05
SF5	3.5 ±0.1	5.6± 0.05	8.5± 0.05	10.5± 0.05	13.6 ± 0.05	13.6± 0.05
SF6	3.3 ±0.1	4.6± 0.05	7.5± 0.05	9.5± 0.05	11.5 ± 0.05	11.6± 0.05

All values are expressed in SD (n=3).

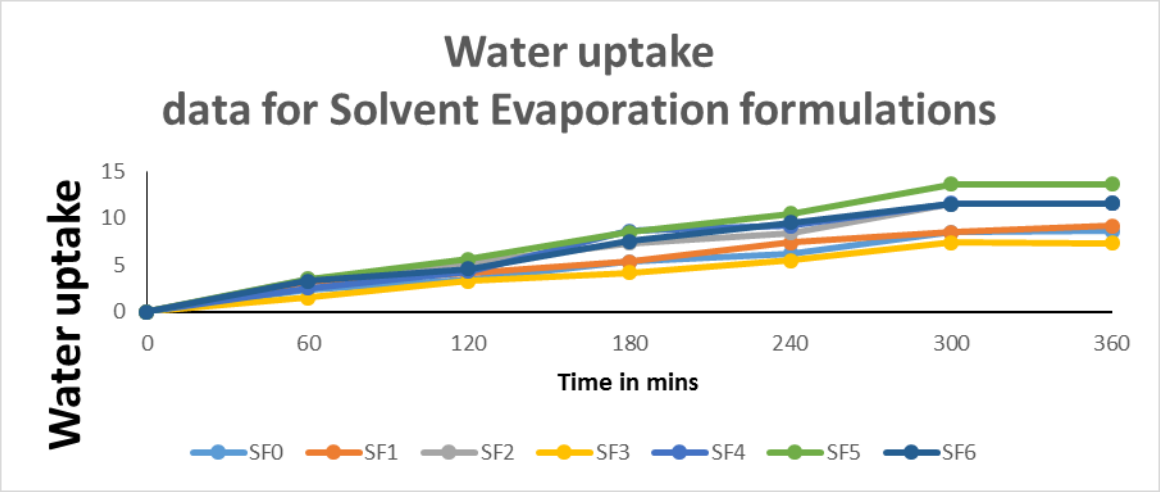

Figure No. 2: Water uptake data for Solvent Evaporation formulations

Gel fraction is the amount of insoluble parts of polymer obtained after formulation. The obtained results were in the range of **98.3±0.1 to 99.94±0.05.**

Size analysis results were in the range of **1.22±0.11 to 1.93±0.09** for dried beads and **2.30±0 .17 to 2.97± 0.19** for wet beads.

Percentage yield obtained was in the range of **92.5±1 to 99.2±0.57.**

Drug content mainly gives the amount of drug present in the formulation. The drug content was in the range of **82.4± 0.03 to 95.3± 0.03 *mg*.**

Drug entrapment efficiency gives the amount of drug being entrapped in the polymer during formulation of mucoadhesive microspheres. The entrapment efficiency was in the range of **85.4± 0.57 to 96± 1.** The maximum value obtained for the formulation containing carbopol 934P because of the high viscosity nature of the polymers makes them to hold on drug particles in the polymer matrix.

Table No. 4: Physicochemical evaluations of Ciprofloxacin HCl Solvent Evaporation formulation

Formulation Code	% Yield	Drug content (mg)	Drug entrapment efficiency (%)	Gel Fraction	Size analysis	
					Dried beads	Wet Beads
SF0	94.5±1.52	82.4± 0.03	85.4± 0.57	98.3±0.1	1.24±0.32	2.32±0.01
SF1	94.2±1.53	86.2± 0.01	87.3± 1	99.2±0.1	1.35±0.12	2.45±0 .12
SF2	93.2±1.15	91.3±0.03	92.5± 1.15	99.6±0.05	1.44±0.02	2.62±0 .32
SF3	95.2±2.08	90.2± 0.03	91.4± 1.52	98.2±0.1	1.22±0.11	2.30±0 .17
SF4	92.5±1	88.6± 0.23	89.6± 1.15	99.56±0.01	1.65±0.04	2.52± 0.02
SF5	92.8±0.57	94.3± 0.03	95± 1	99.83±0.05	1.83±0.09	2.93± 0.19
SF6	99.2±0.57	95.3± 0.03	96± 1	99.94±0.05	1.93±0.09	2.97± 0.19

All values are expressed in SD (n=3)

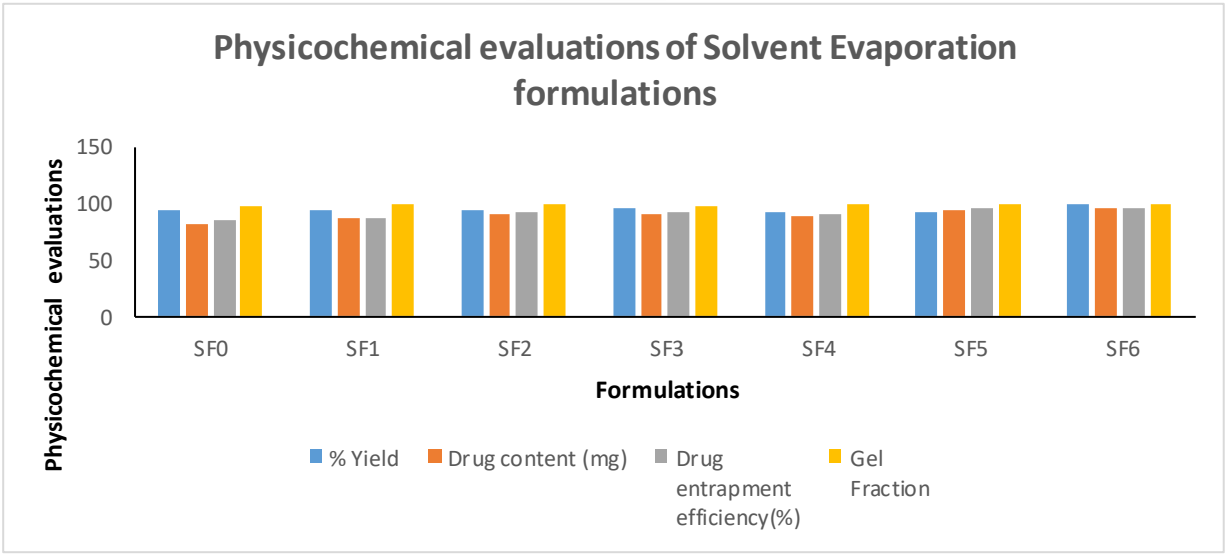

Figure No. 3: Physicochemical evaluations of Solvent Evaporation formulations

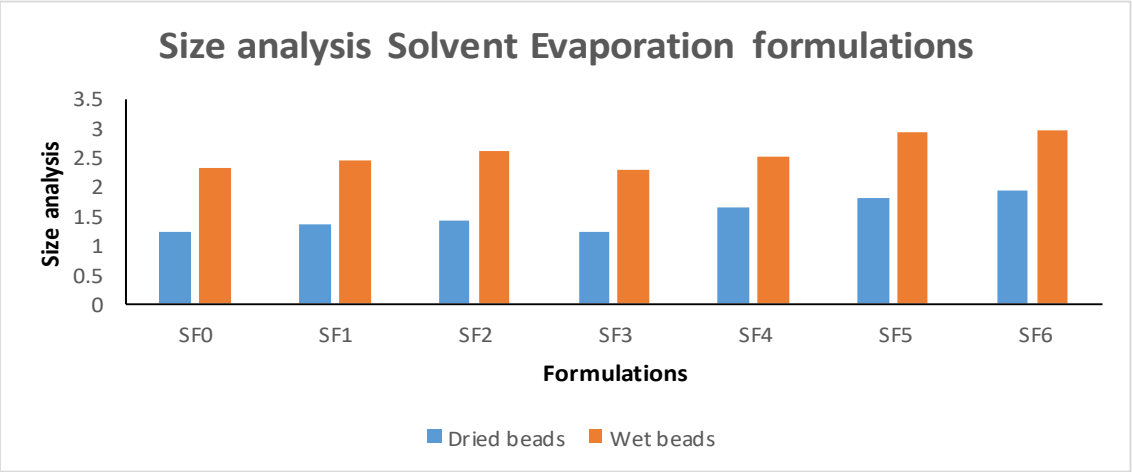

Figure No. 4: Size analysis Solvent Evaporation formulations

The SEM reports obtained showed good spherical shape and also surface morphological characters.

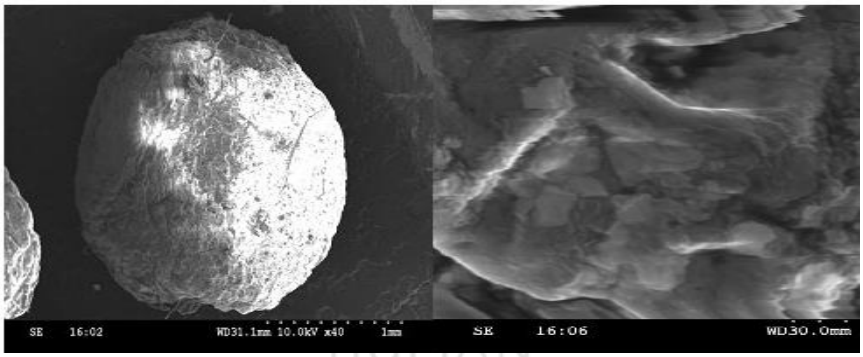

Figure No. 5: SEM photograph of SF6 Formulation

***In vitro* drug release studies:**

Table No. 5: Cumulative % *in-vitro* drug release studies formulations SF0– SF6

Time (Hrs)	SF_0	SF_1	SF_2	SF_3	SF_4	SF_5	SF_6
1	6.75±0.02	9.14±0.07	18.82±0.04	7.84±0.06	10.38±0.03	12.04±0.05	**11.83**±0.05
2	10.32±0.02	16.54±0.07	29.24±0.04	16.56±0.06	18.27±0.03	19.25±0.05	**18.64**±0.05
3	19.44±0.02	28.38±0.07	31.37±0.04	24.28±0.06	27.16±0.03	30.96±0.05	**29.72**±0.05
4	25.23±0.02	37.39±0.07	44.46±0.04	35.74±0.06	39.83±0.03	45.86±0.05	**36.47**±0.05
5	32.23±0.02	45.56±0.07	55.57±0.04	44.28±0.06	48.14±0.03	59.84±0.05	**45.75**±0.05
6	39.34±0.02	58.18±0.07	68.94±0.04	57.16±0.06	60.02±0.03	71.45±0.05	**57.82**±0.05
7	46.33±0.02	64.74±0.07	69.26±0.04	65.27±0.06	68.93±0.03	80.58±0.05	**69.96**±0.05
8	49.56±0.02	76.26±0.07	72.17±0.04	76.96±0.06	79.51±0.03	88.94±0.05	**76.03**±0.05
9	52.12±0.02	84.35±0.07	75.17±0.04	80.43±0.06	82.63±0.03	90.25±0.05	**89.42**±0.05
10	60.11±0.02	85.12±0.07	78.17±0.04	84.65±0.06	89.24±0.03	91.67±0.05	**99.12**±0.05

All values are expressed in SD (n=3)

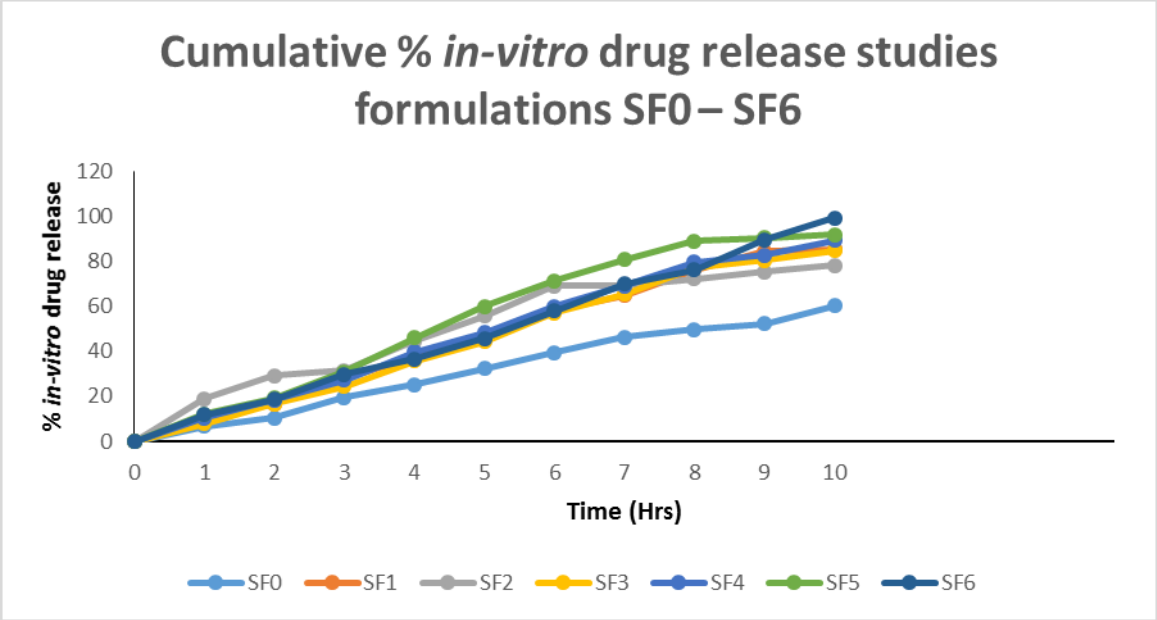

Figure No. 6: Cumulative % *in-vitro* drug release studies formulations SF0– SF6

In-vitro mucoadhesion test was performed by using *in vitro* Wash- Off test. The % Adherence was in the range of ***72 % to 90%.***

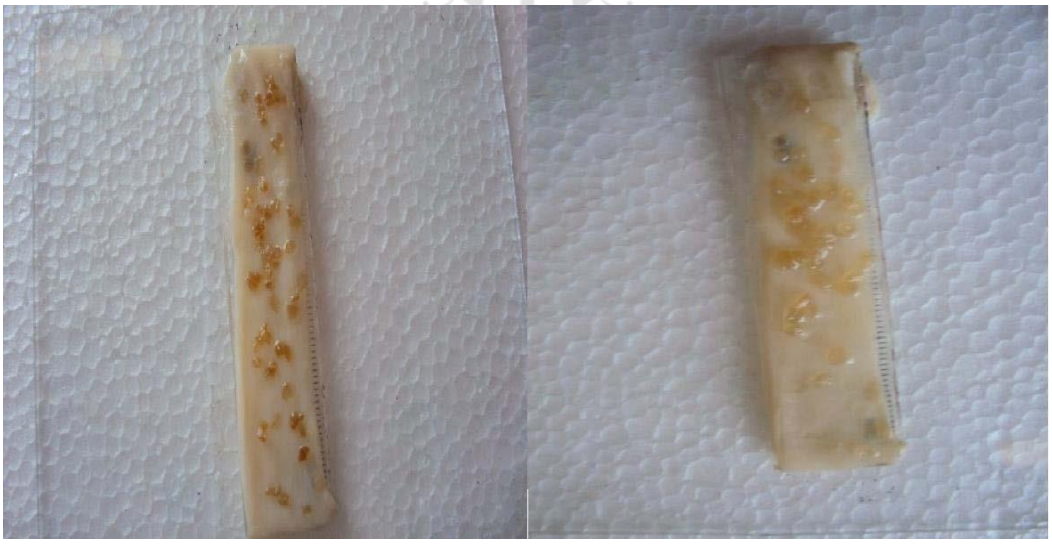

Figure No. 7: Glass side with mucoadhesive microspheres before test and after test

Table No. 6: *In-vitro* mucoadhesion test for solvent evaporation method

Formulation code	% Adherence in time (hrs)				
	0	2	4	6	8
SF0	44.1%	44.5%	45.1%	46.5%	47.1%
SF1	80.2%	80.5%	81.1%	81.8%	82.1%
SF2	81.8%	82.3%	83.5%	83.5%	84.4%
SF3	83.2%	83.9%	84.2%	84.9%	85.3%
SF4	70.2%	70.9%	71.3%	71.9%	72.2%
SF5	70.5%	70.8%	71.3%	72.5%	73.5%
SF6	88.2%	88.7%	89.5%	89.9%	90.1%

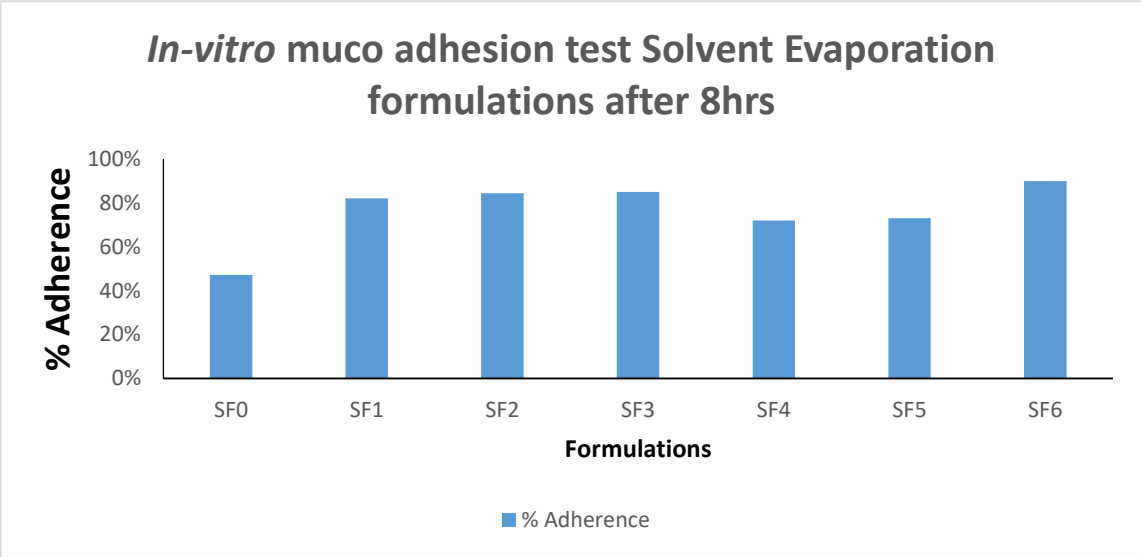

Figure No. 8: *In-vitro* muco adhesion test Solvent Evaporation formulations

DSC method for drug-excipient interaction analysis:

Dsc studies reveal that there is no interaction between the drug and the excipients.

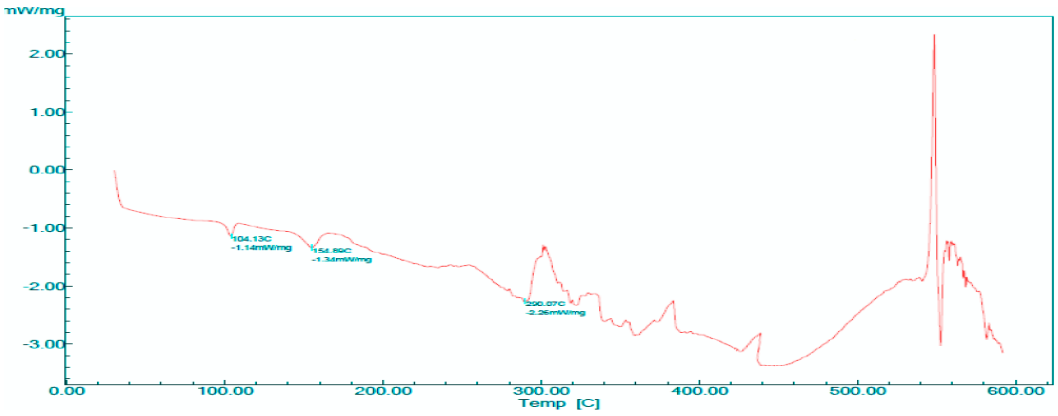

Figure No. 9: DSC of drug

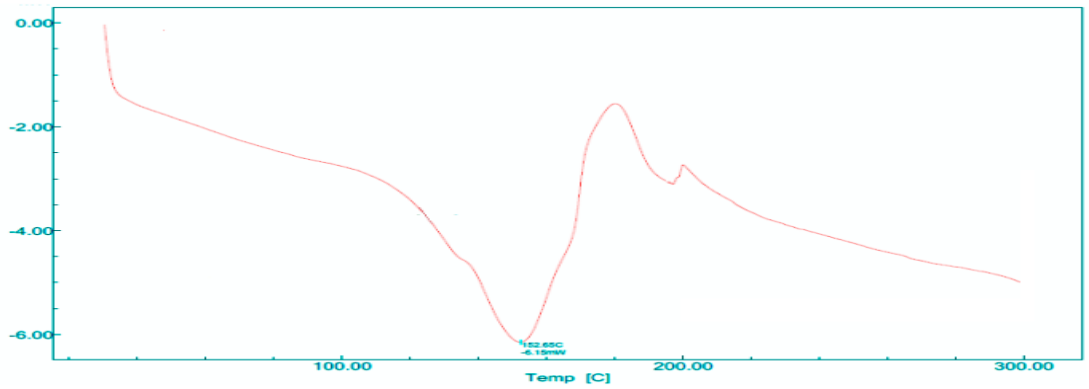

Figure No. 10: DSC of optimized formulation of solvent evaporation (SF6)

Table No. 7: DSC studies

Concentration µg/ml	Peak area		
	Ciprofloxacin HCl	SF6	IF6
20	1373684	2168213	2403243
25	1555545	2486654	2819254
30	1708280	2766713	3197738
35	1863167	3101485	3558368
40	2024329	3383824	3889324

Stability studies: There is no change in drug content and % drug release for the period of 3 months, so it is continued for the next three months as per ICH guidelines for stability studies.

Table No. 8: Stability studies

Formulations	Time	% drug content	Cumulative % drug release	
			25±2°C / 65±5%RH	40±2°C / 75±5%RH
SF6	First day	95.3±0.03	99.68±2.15	99.67±2.98
	30 days	95.2±0.02	99.64±2.10	99.63±2.59
	90 days	95.01±0.02	99.64±2.08	99.63±2.98

CONCLUSION

In our research work, we have prepared formulations with solvent evaporation method. In both optimized formulations, SF6 is more suitable than other prepared formulations because of slow release than other formulations. The slow release of these formulation is because of high swelling and water uptake.

The formulation SF6- showed 99.12% drug release in 10 hrs. Particle size analysis was done by scanning electron microscopy (SEM). The Particle size of SF6 was 41.87 respectively. The swelling ratio of optimised formulation SF6 was found to be 9.6±0.05 in 360 mins. The

water uptake of SF6 was found to be11.6± 0.05 in 360 mins. Percentage yield obtained was in the range of 92.5±1 to 99.2±0.57. Drug content mainly gives the amount of drug present in the formulation. The drug content was in the range of 82.4± 0.03 to 95.3± 0.03 *mg*. The % Adherence was in the range of 72 % to 90%. Dsc studies reveal that there is no interaction between the drug and the excipients. Stability studies reveal that there is no change in drug content and % drug release for the period of 3 months, so it is continued for the next three months as per ICH guidelines for stability studies.

Conflict of interest: The authors declares no conflict of interest.

Acknowledgement: The author is thankful to the Deccan School of pharmacy, Nampally Aghrapura, Hyderabad, for providing necessary facilities to carry out this work.

REFERENCES

1. A.Amperiadou, M.Georgarakis, Controlled release Salbutamol sulphate Microcapsules prepared by Emulsion Solvent Evaporation technique, International Journal of Pharmaceutics 115 (1995) 1-8.
2. Kumari Navita, Aggarwal Geeta, Harikumar SL"Mucoadhesive Microspheres as A Review" Journal of Drug Delivery & Therapeutics; 2014, 4(5), 48-54.
3. Mohsin khan, Vaseem Ansari, Poonam Khuswaha, Arunkumar, Juberakthar "Mucoadhesive Microspheres as a controlled drug delivery system" Asian J Pharm ClinRes, 2015 8 (4), 1-20.
4. British Pharmacopoeia 2009:2;1381-1382.
5. United States Pharmacopeia 2013:36(1);1290.
6. Nallagundla H. S. Reddy Srinivas Patnala, and Isadore Kanfe, Investigation of Biowaivers for Immediate Release Formulations Containing BCS III Drugs, Acyclovir, Atenolol, and Ciprofloxacin Hydrochloride, Using Dissolution Testing, American Association of Pharmaceutical Scientists 2016:1-8.
7. S.A. Breda , Alvaro F. Jimenez-Kairuz, Ruben H. Manzo, María E. Olivera , Solubility behavior and biopharmaceutical classification of novel high-solubility ciprofloxacin and norfloxacin pharmaceutical derivatives, International Journal of Pharmaceutics 371 (2009) 106–113.
8. Raymond C Rowe, Paul J Sheskey, Paul J Weller, Handbook of pharmaceutical Excipients published by pharmaceutical press, fourth edition, 2003;159-162, 656-658 & 111-115.
9. Wang S, Guo S, Cheng L. Disodium norcantharidate loaded poly (*e*-caprolactone) microspheres. *Int J Pharm.* 2008; 350: 130-7.
10. Huiqunyu, choboxiao, synthesis and properties of novel hydrogels from oxidised konjac glucomannan crosslinked gelatine for *in vitro* drug delivery – Carbohydrate polymers 2008; 72:479 – 489.
11. Clement l. Higginbotham, Luke M. Geever, Ciaran C. Cooney, John g.lyons, James e. Kennedy, Michaelj.d.nugent, Sineaddevery, Characterisation and controlled drug release from novel drug – loaded hydrogels – European journal of pharmaceutics and biopharmaceutics 2009; 69:1147 – 1159.
12. A. Srinatha, J.KPandit, and S. Singh, ionic cross-linked chitosan beads for extended release of ciprofloxacin, Indian J. Pharm. Sci 2008; 70(1):16-21.
13. Qurrat-ul-Ain1, SadhnaSharma1, G. K. Khuller and S. K. Garg, Alginate-based oral drug delivery system for tuberculosis: pharmacokinetics and therapeutic effects Journal of Antimicrobial Chemotherapy 2003; 51:931–938.
14. Sanjay K. Jain, Anekant Jain, Yashwant Gupta, and Manisha Ahirwar, Design and Development of Hydrogel Beads for Targeted Drug Delivery to the Colon, AAPS Pharm Sci Tech 2007; 8 (3) Article 56.
15. K.P.R. Chowdary, and Y. Srinivasa Rao Design and *In Vitro* and *In Vivo* Evaluation of Mucoadhesive Microcapsules of Glipizide for Oral Controlled Release: A Technica Note *AAPS Pharm Sci Tech*. 2003; 4(3):

article 39.
16. M.A. Altaf, Sreedharan, N. Charyulu, ionic gelation controlled drug delivery systems for gastric mucoadhesive microcapsules of captopril, Indian J. Pharm. Sci 2008; 70(5):655-658.
17. K. Makino, E.J. Mack, T. Okano, S.W. Kim, Self-regulated delivery of insulin from microcapsules, Biomater. Artif. Cells Immobiliz. Biotechnol 1991; 19:219– 228.
18. Maincent P, Kim Y, Fluckiger L, Hoffman M, Lartaud-Idjouadiene I and Atkinson J. The antihypertensive effect of orally administered nifedipine-loaded nanoparticles in spontaneously hypertensive rats, British Journal of Pharmacology, 120: 1997, 399 – 404.