Volume 2 (4), 2014, Page-307-315
e-ISSN: 2348-6465

Pharma Health Sciences

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Formulation and Evaluation of Microencapsulated Suspension of Ofloxacin

S Ramu*, D Srinivasa Rao, Naga Surekha, Ramakrishna G

Department of Pharmaceutics, K.C.Reddy Institute of Pharmaceutical Sciences, Jamgamaguntla Pallem, Medikondur, Guntur, India

ARTICLE INFO

Received: 17 July 2014
Accepted: 29 Aug 2014

ABSTRACT

The goal in designing sustained release drug delivery system is to reduce the frequency of the dosing, reducing the dose & providing uniform drug delivery. Sustained release dosage forms provide better control of plasma drug levels, less dosage frequency, less side effect, increased efficacy and constant delivery. The Aim of the study is related to the formulation and evaluation of Ofloxacin 25ml of microencapsulated suspension by solvent evaporation method. The Preparation contains six formulations of suspensions with 2 different polymers with different concentrations as Ofloxacin resinate + HPMC, Ofloxacin resinate + Carbopol 934. The prepared batches of Ofloxacin microencapsulated suspension were evaluated for the pH, viscosity, sedimentation volume; density, drug content and antibacterial activity of all the six formulations were performed. Formulations F-3, F-6 gave better sustained release and antibacterial activity. Comparitive study of F-3, F-6 with marketed product reveal the F-3 is best fitted formulation for preparation of microencapsulated suspension.

Key words: Novel drug delivery system (NDDS), Sustained release dosage form (SR), controlled release drug delivery systems (CRDDS), gastro retentive dosage forms, GRDF)

1. INTRODUCTION

Micro-encapsulation is a process in which tiny particles or droplets are surrounded by a coating to give small capsules of many useful properties. In general, it is used to incorporate food ingredients, enzymes, cells or other materials on a micro metric scale. Microencapsulation can also be used to enclose solids, liquids, or gases inside a micrometric wall made of hard or soft soluble film, in order to reduce dosing

Corresponding author *

S Ramu, K.C.Reddy Institute of Pharmaceutical Sciences, Jamgamaguntla Pallem, Medikondur, Guntur. E Mail: samineni.ramu@gmail.com

frequency and prevent the degradation of pharmaceuticals.[1]

Microcapsule is a small sphere with a uniform wall around it. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell, coating, or membrane.[2]

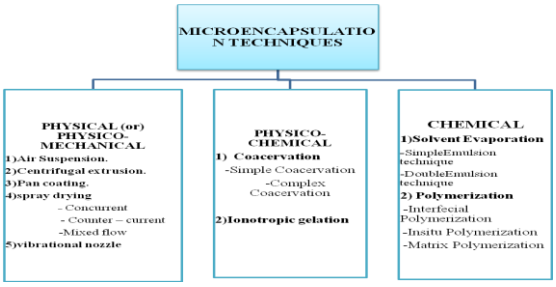
Reasons for microencapsulation

- It is mainly used to increase the stability and life of the product being encapsulated, facilitate the manipulation of the product and control its liberation in an adequate time and space.[3]
- The objective is not to isolate the core completely but to control the rate at which it leaves the microcapsule, as in the controlled release of drugs or pesticides.
- The problem may be as simple as masking the taste or odor of the core, or as complex as increasing the selectivity of an adsorption or extraction process. [4,5]
- Protection of the immediate environment.
- Target release of encapsulated materials
- Separation of incompatible components
- Conversion of liquids to free flowing solids

2. MATERIALS AND METHODS

Ofloxacin was obtained as a gift sample from Bridge pharma pvt ltd, Hyderabad. Indion resin 204 from Hi media labs, Mumbai. HPMC, Carbopol 934, were obtained as a gift sample from Qualigenes fine chemicals.

Techniques used for microencapsulation: [6]



2.1 Solvent Evaporation Technique

Solvent Evaporation Method can be performed by 2 techniques.

1. Single emulsion technique
2. Double emulsion technique

Single emulsion Technique **:** [7]

- The natural polymers are dissolved/ dispersed in aqueous medium followed by dispersion in the non aqu. Medium. Ex: oil.
- In the 2nd step, cross linking of the dispersed globule is carried out either by means of heat or by using chemical cross linkers (Ex. gluteraldehyde, formaldehyde, diacidchloride, etc.)
- Crosslinking by heat is affected by adding the dispersion to previously heated oil. Heat denaturation is not suitable for the thermolabile drugs while the chemical crosslinking suffers isadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation.

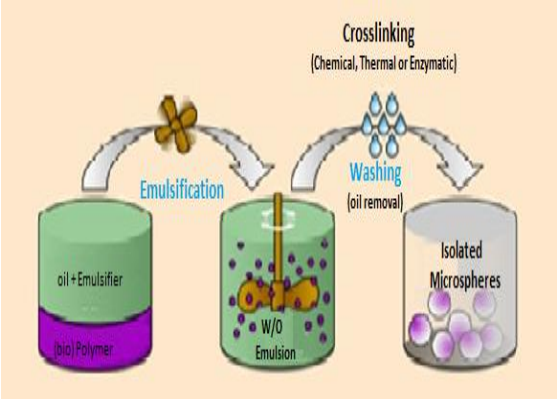


Fig 1: Schematic representation of single emulsion technique.

Double Emulsion Technique**:** [8, 9]

- The formation of the multiple emulsions or the double emulsion of type w/o/w & is best suited to the water soluble drugs, peptides, proteins & the vaccines.
- The aqueous protein solution is dispersed in a lipophilic organic (OIL) continuous phase which is generally consisted of polymer solution that eventually encapsulates protein contained in dispersed aqueous phase.

- The primary emulsion is then subjected to the homogenization before addition to aqueous solution of PVA.
- This results in formation of double emulsion which is then subjected to solvent removal by solvent evaporation maintaining the emulsion at reduced pressure or by stirring so that organic phase evaporates out.

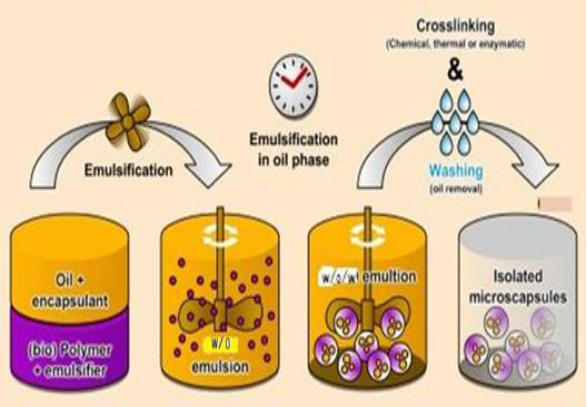


Fig 2: Processing scheme for microsphere-preparation by double emulsion technique

Table 1: Formulation of Microencapsulated Suspension of Ofloxacin:

Ingredients	Quantity of Ingredients (mg)					
	F-1	F-2	F-3	F-4	F-5	F-6
Ofloxacin-Indion204 (1:16)(resonates)	200	200	200	200	200	200
Carbopol 934(5%)	20	25	30	---	---	---
HPMC	---	---	---	20	25	30
Sucrose	15	15	15	15	15	15
Xanthan gum (%w/v)	0.6	0.6	0.6	0.6	0.6	0.6
Sorbitol sol.(70%)(ml)	1.8	1.8	1.8	1.8	1.8	1.8
Glycerin (ml)	0.2	0.2	0.2	0.2	0.2	0.2
Pluronic F68 (5%)	5	5	5	5	5	5
Soyalecithin (1%)	1	1	1	1	1	1
Peppermintoil, sunset yellow(ml)	0.2	0.2	0.2	0.2	0.2	0.2
Methylparaben&propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02

2. 2 Preparation of Drug – Indion 204 resin complex (resinate) [11]

Resinates were prepared by batch process. Pk An accurately weighed amount of drug (100 mg) was dissolved in 100 ml of distilled water. Then ion exchange resin (100 mg) was added and stirred on a magnetic stirrer. Resinate thus formed was filtered and washed with copious amount of deionised water to remove any uncomplexed drug. It was then dried at 50°C and the drug content was determined spectrophotometrically at 293.8 nm.

2.3 Preparation of Suspension Using Resinates [12]

Step1:

Take a dry beaker in that 6 ml water was heated up to 80° C. Sucrose (10 gm) was added under continuous stirring, till the sucrose was completely dissolved. The prepared syrup was cooled properly at room temperature and kept overnight. Syrup was filtered using 120 mesh nylon cloth.

Step2:

5 ml of pure water was taken in a beaker to which 1.8 ml of sorbitol solution and 0.2 ml glycerin were added. The mixture was stirred properly. To this solution, pluronic F 68 (5%), soya lecithin (1%) and HPMC / C934 (5%) in w/w of drug were added with continuous stirring.

Step3:

5 ml of pure water was taken in another beaker to which 200 mg of Ofloxacin – indion 204 complex (resonates) was added. To the resinate suspension, step1 and step2 were added with continuous stirring. Xanthan gum is used as suspending agent. Methyl paraben sodium (0.015%w/v) and Propyl paraben sodium (0.08%w/v) were added as preservatives. The volume was made up to 25 ml by Ultra pure water. The pH was adjusted to 7.2.

2.4 Evaluation

2.4.1 Standard graph of ofloxacin in buffer

The working standard solutions of Ofloxacin were scanned in the UV region and the absorbances were observed against 0.1N HCl solution as blank at 293.8 nm. Finally the calibration curve was plotted between concentration (x-axis) and absorbance (y-axis).

2.4.2 Evaluation of Formulation

The formulation of solid drug: resin complex was evaluated for pH, viscosity, sedimentation volume, density and drug content [10] . The rheological properties of all the formulation like viscosity, type of flow system, shears thinning index (ST index) and thixotropic index (Thix index) were determined by Brook Field viscometer (cone and plate) model.

2.4.3 Sensory Evaluation of Formulation

The sample of each formulation subjected to sensory evaluation by nine members using time intensity method. 10 ml of each formulation held in mouth for about 10 seconds. Bitterness was recorded at the time of 20, 30, 40, 50 and 60 seconds. The evaluation was performed by classifying bitter taste into five levels, level 0: no bitter taste is sensed, 1: acceptable bitterness, 2: slightly bitter, 3: moderately bitter, 4: strongly bitterness. Descriptive Statistics mean and standard deviation were calculated for all variables. Paired t test was applied using INSTAT software. Value $p< 0.05$ has been considered as statistical significant level.

2.4.4 Drug Entrapment Efficiency

Weighed quantity of microspheres were crushed and suspended in distilled water for 24 h to extract the drug from microspheres. The filtrate was then analyzed at 293.8 nm using UV–Vis spectrophotometer (JASCO V630, Japan) for drug content. The encapsulation efficiency was calculated using following equation:

Encapsulation efficiency = (Drug entrapped/Theoretical drug content) × 100

2.4.5 Determination of sedimentation volume

Each suspension (50 ml) was stored in a 50 ml measuring cylinder for 4 days at 350C. Observations were made every 24 hr for 4 days. The sedimentation volume [13] , F (%), was then calculated using the following equation.

$F = 100\ Vu/V$

2.4.6 Measurement of viscosity using brookfield viscometer:

The viscosity (centipoise) of the sample was determined at 250C using Brookfield Synchro-electric viscometer; model LVF (Brookfield Laboratories, Massachusetts) at 100 RPM (spindle #4). All determinations were made in at least triplicate and the results obtained are expressed as the mean values.

Viscosity of suspending agent $\eta 1 = \eta 2\ X\ (\rho 1 t 1\ /\ \rho 2 t 2)$

2.4.7 Determination of flow rate:

The time required for each suspension sample to flow through a 10 ml pipette was determined and the apparent viscosity ($\eta\alpha$ in mls-1) was calculated using the equation:

Volume of pipette (ml)= Flow rate $\eta\alpha$/ Flow time (s)

2.4.8 Drug leaching in to the suspension:

The amount of drug leaching in to the vehicle after the storage of suspension at room temperature for one month was determined by filtering the suspension and

measuring the absorbance at 293.8 nm, using a suspension prepare without microcapsule as a blank. The drugleached in the vehicle was calculated using the calibration curve.

2.4.9 Antibacterial property of microencapsulated suspension

To study invitro antibacterial properties of microencapsulated suspension containing Ofloxacin, six different suspensions such as F-1, F-2, F-3, F-4, F-5, and F-6 were prepared by using three different polymers [14] such as HPMC, Carbopol 934 [15] respectively along with some common ingredients (bases). For the study of antibacterial activities of suspensions agar diffusion method was performed taking Staphylococcus (S) aureus, Bacillus (B) subtilis and Escherichia (E) coli [16]

3. RESULTS AND DISCUSSION

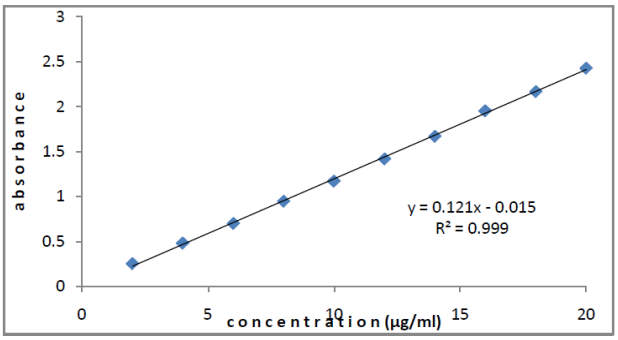


Fig 3: Calibration curve of ofloxacin at 293.8 nm

Table 2: Evaluation of % drug complexation

S.no	Time	% drug Complexation
1	5	71.93±1.04
2	15	94.32±1.52
3	30	92.51±2.27
4	60	94.18±1.92
5	120	92.48±2.28
6	240	91.47±2.27
7	24hrs	93.89±2.11

Table 3: Evaluation of Drug Loading

PH	DRUG LOADING(%wt/wt)	DRUG LOADING AFTER SHAKING(%wt/wt)
3	52.48±2.13	50.79±1.26
3.5	58.93±0.15	52.38±0.38
4	60.39±1.39	56.93±1.36
4.5	59.57±2.18	56.42±1.78
5	58.15±4.43	54.32±3.41
5.5	57.13±1.85	53.27±0.87
6	56.85±0.57	52.13±0.15

Table 4: Evaluation Parameters

EVALUATION PARAMETER	F-1	F-2	F-3	F-4	F-5	F-6
PH	7.2	7.2	7.2	7.2	7.2	7.2
Density	1.184	1.189	1.188	1.246	1.241	1.244
Sedimentation volume	1.3	1.29	1.28	0.99	0.99	1
potency	101%	101%	98%	99%	99%	99%
Redispersibility	+++	+++	+++	+++	+++	+++
shear thinning	1.38	1.38	1.37	1.42	1.43	1.42
thixotropic index	1.38	1.39	1.38	1.48	1.48	1.49
Taste	Sweet, palatable	Sweet, palatable	Sweet, palatable	Sweet, palatable	Sweet, palatable	Sweet, palatable

Table 5: Sensory evaluation of suspension formulations

TIME (SEC)	BEFORE TASTE MASKING MEAN ±SD	AFTER TASTE MASKING WITH FORMULATION (MEAN±SD)					
		F-1	F-2	F-3	F-4	F-5	F-6
10	4.0 ±0.00	0.12± 0.05	0.12± 0.38	0.12± 0.07	0.13± 0.44	0.13± 0.91	0.13± 0.22
20	3.2±0.50	0	0	0	0	0	0
30	2.4±0.52	0	0	0	0	0	0
40	1.8±0.50	0	0	0	0	0	0
50	1.3±0.44	0	0	0	0	0	0
60	0.9±0.44	0	0	0	0	0	0

Table 6: In vitro drug release profile of formulations

Formulation code	Cumulative percent drug release					
	Time (hrs)					
	0.5	1	2	4	6	8
F1	29.63	35.34	52.61	76.54	86.93	97.62
F2	28.12	39.76	52.45	77.31	86.84	96.83
F3	22.91	34.21	49.63	69.78	78.93	91.97
F4	19.53	28.78	39.73	67.35	78.73	89.26
F5	16.72	24.98	36.52	63.09	71.97	82.12
F6	13.34	21.32	32.12	53.47	66.42	77.89

Fig 4: Dissolution profile of microencapsulated formulations

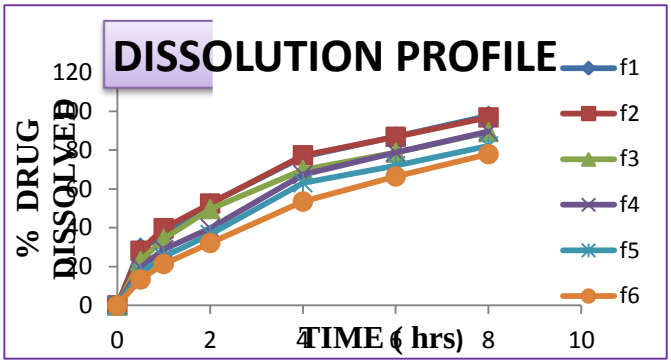


Fig 5: SEM of Ofloxacin – HPMC microencapsulated Suspension

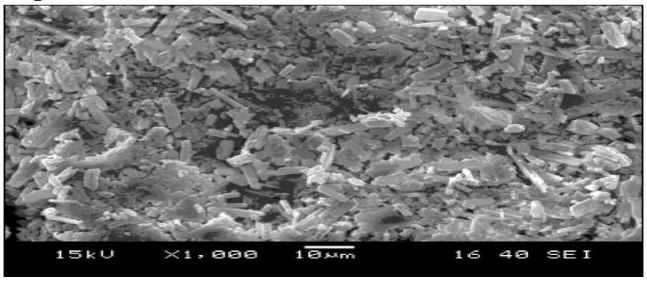
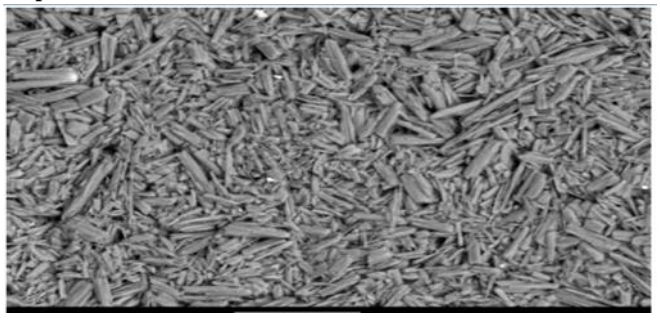


Fig 6: SEM of Ofloxacin – Carbopol 934 microencapsulated suspension



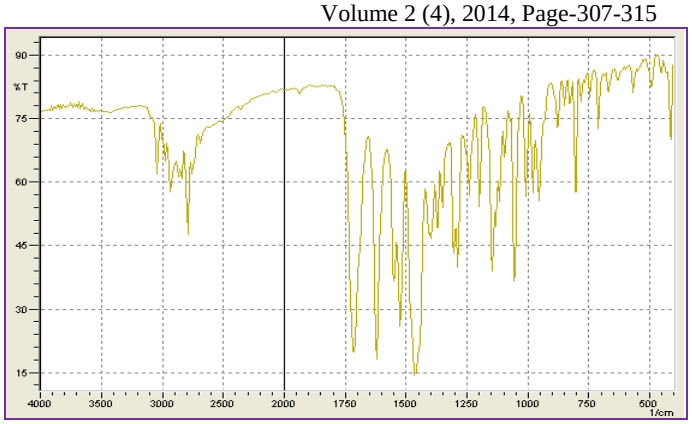


Fig 7: FTIR of pure Ofloxacin

Table 7: Average zone of inhibition of various microorganisms

MICRO ORGANISMS	Average zone of Inhibition					
	F-1	F-2	F-3	F-4	F-5	F-6
S.aureus	45	46.7	49	44	45.1	48.3
B.subtilis	36.5	41	58.2	36.5	40	57.7
E.coli	32.7	34	37.3	31	33.7	36.5

Fig 8: Antimicrobial activity of formulations against microorganisms.

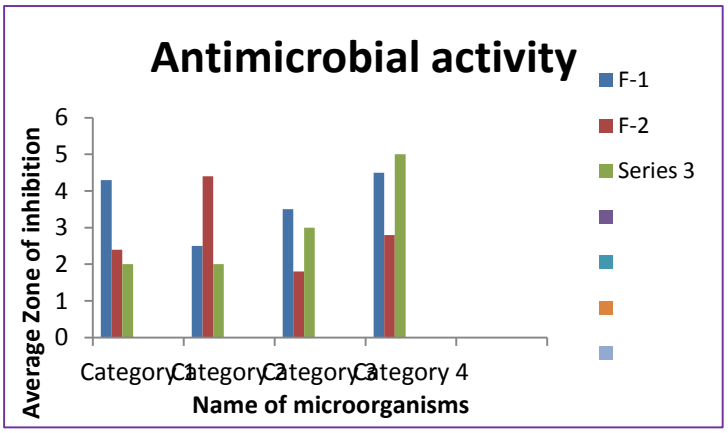


Table 8: Comparitive study of F-3 and F-6 formulations with marketed suspension

Formulations	Microorganisms			Charecteristics of formulations
	S.aureus	*B.subtilis*	*E.coli*	
OS - 1	49	58	36	❖ Half life is 3- 4 hours. ❖ Unpleasent taste.

OS – 2	49	58.2	37.3	❖ Half life is 8 hours. ❖ Taste is masked. ❖ Achieved sustained release. ❖ Zone of inhibition is more compared to Ofloxacin – carbopol 934 formulations.
OS - 3	48.3	57.7	36.5	❖ Half life is 8 hours. ❖ Taste is masked. ❖ Achieved sustained release. ❖ Zone of inhibition is less compared to Ofloxacin – HPMC formulation.

OS – 1 : Ofloxacin marketed suspension
OS - 2 : Ofloxacin – HPMC microencapsulated suspension
OS - 3: Ofloxacin – Carbopol 934 microencapsulated suspension.

Fig 9: Comparitive study of OS-1, OS-2, OS-3 formulations Zone of inhibition against S.aureus.

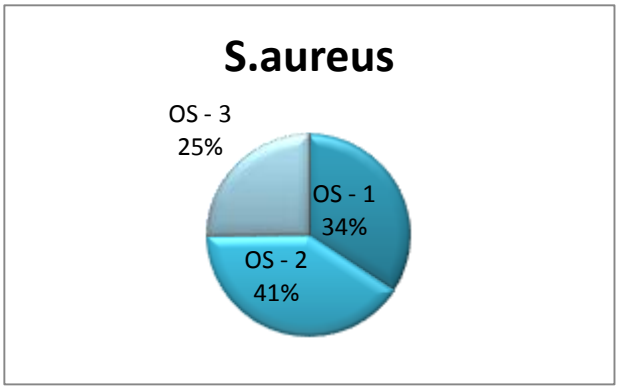


Fig 10: Comparitive study of OS-1, OS-2, OS-3 formulations Zone of inhibition against B.subtilis.

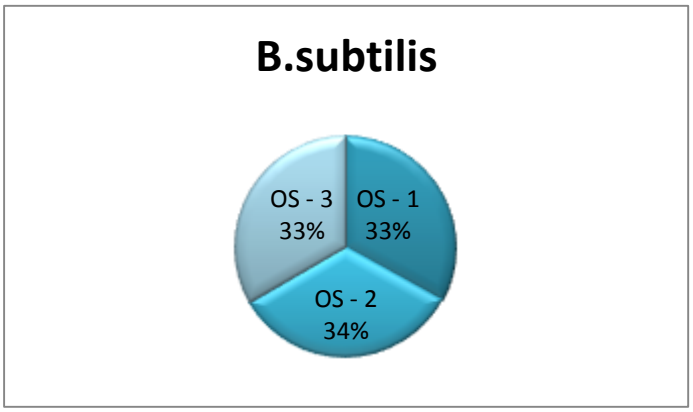
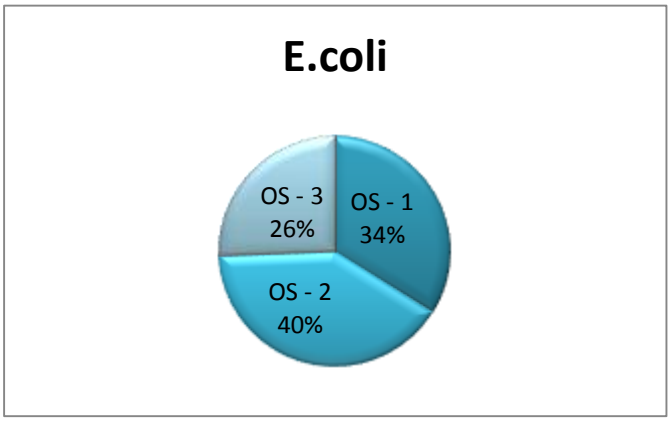


Fig 11: Comparitive study of OS-1, OS-2, OS-3 formulations Zone of inhibition again E.coli



Invitro antibacterial activity reveals the following results in ascending order as follows:

Ofloxacin + HPMC > Ofloxacin + C 934 > Ofloxacin marketed suspension.

The maximum invitro antibacterial activity was found to be with formulation F-3 (OS-2) which is combination of Ofloxacin – HPMC (30mg) and sustained release and taste masking also achieved. F-6 (OS-3) shows good result but F-3 shows best result compared to F-6. OS-1 (marketed suspension) shows antibacterial activity but less half life and unpleasant taste.

4. SUMMARY AND CONCLUSION:

- The results have shown that the dissolution rate of the drug increases with increase in concentration of HPMC. The dissolution rate increase in following order.

- **Ofloxacin marketed suspension < Ofloxacin + C 934 < Ofloxacin + HPMC.**
- *AND*
- **Ofloxacin + HPMC (20mg) < Ofloxacin + HPMC (25mg) < Ofloxacin + HPMC (30mg).**
- Formulations F-3, F-6 gave better sustained release and antibacterial activity.
- Comparitive study of F-3, F-6 with marketed product reveal the F-3 is best fitted formulation for preparation of microencapsulated suspension.
- From the experimental data obtained, it can be concluded that, Ofloxacin + HPMC (30mg) formulation suitable for formulation of microencapsulated suspension of Ofloxacin.

5. ACKNOWLEDGEMENT

The authors are thankful to Principle Prof.D.Srinivasa rao, K.C.Reddy institute of pharmaceutical sciences, Jangamguntlapalem**,** for providing the necessary facilities and help.

6. REFERENCES

1. López C. Ano., Delaino L., Navarro A.S, & Martino M. Encapsulación de compuestos bioactivos con alginatos para laindustria de alimentos. Ciencia yTecnología Alimentaria 2012; 59(1), 18-27
2. Fulzele SV, Satturwar PM, Kasliwal RH, Dorle AK. Preparation and evaluation of microcapsules using polymerized rosin as a novel wall forming material. J Microencapsulation. 2004; 21:83–89.
3. Abhijeet Y patil, Formulation and evaluation of stable oral formulation of bitterless Ofloxacin complexed with ion exchange resin, World Journal of pharmacy & Pharmaceutical Sciences 2012; 6: 12-16.
4. Mundada AS, Bhalekar MR, Avari JG. Formulation and evaluation of dispersible taste masked tablet of roxithromycin. Asian J of Pharm, 2008; 2(2): 32-7.
5. Nanda AR, Kandarapu, Garg S. An update on taste masking technologies for oral pharmaceuticals. Indian J Pharm Sci, 2002; 64 (1): 10-7.
6. R.dubey, "Microencapsulation Technology and Applications" Defence science journal 2009; 59(1): 82-95.
7. Arunachalam A, Rathinaraj BS, Subramanian, Choudhury PK, Reddy AK, Fareedullah MD. Preparation and Evaluation of Ofloxacin Microsphere Using NaturalGelatin Polymer. Int J Appl Biol Pharm Tech, 1(1): 61-67, (2010).
8. Smart Polymer for Controlled Drug Delivery Protein and Peptides: A Review of Patents; Availablefromhttp://www.ingnetaconnect.com/content/ben/pdf/2009/00000003/00000001/art00004, accessed on 24.01.2010.
9. Hosmani AH. Carbopol and its Pharmaceutical Significance: A Review; [cited 2010 Jan 20]. Availablefrom:http://www.pharmainfo.net/reviews/carbopol-and-its-pharmaceutical significance - review.
10. P. K. Bhoyar, D. M. Biyani. Formulation and *In vitro* Evaluation of Sustained Release Dosage Form with Taste Masking of Metformin Hydrochloride. Indian J Pharm Sci 2010; 77(2), 184-90.
11. Subhashree Sahoo, Chandra Kanti Chakraborti, Subash Chandra Mishra. Qualitative analysis of controlled release ciprofloxacin/carbopol 934 mucoadhesive suspension. J Adv Pharm Technol Res. 2011 Jul-Sep; 2(3): 195–204.
12. Bettini R, Colombo P, Peppas NA. Solubility effects on drug transport through pH sensitive, swelling-controlled release systems: Transport of theophylline and metoclopramide monohydrochloride. J Control Release 1995; 37: 105-111.

13. Arunachalam A, Rathinaraj BS, Subramanian, Choudhury PK, Reddy AK, Fareedullah Md. Preparation and Evaluation of Ofloxacin Microsphere Using Natural Gelatin Polymer. Int J Appl Biol Pharm Tech 2010; 1(1): 61-67.
14. MeasurementTechniquesforNanoparticles;Availablefromhttp://www.nanocap.eu/Flex/Site/Download.aspx? ID=3984, accessed on 04.01.2010.
15. Inoue A, Determination of aspect ratios of claysized particles, Clay Science A 1995; 9(5): 259-274.
16. Lich B, SEM-based systems can give researchers a better look at sub-micron Pharmaceutical particles; Available from http://www.dddmag.com/article-SEM-BasedSystems020109.aspx, accessed on 20.01.2010.