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"PREPARATION AND EVALUATION OF CLOXACILLIN GASTRO RETENTIVE MICROSPHERES"

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ABSTRACT

The objective of the present study was to prepare, characterize and evaluation of gastroretentive microspheres of Cloxacillin. To achieve these objective nine formulations of microspheres were prepared by emulsion solvent evaporation method using Eudragit RS100 and Kollicoat MAE 100 P polymer. Prepared microspheres were evaluated for Particle size analysis, Surface morphology, Fourier transform infrared spectroscopy analysis, Drug encapsulation efficiency, Percent buoyancy, *In-vitro* drug release study and Stability study. The microspheres formed have smooth surface and spherical in shape as observed in scanning electron microscopy. The drug entrapment efficiency of the formulation is in the range of 76.46% to 93.41%. Particle size increases with increasing concentration of polymer, particle size ranging from 3 to 20 µm. The percent buoyancy was more than 70% up to 12 hours. The percent buoyancy was increased significantly with increase in polymer is in the range of 72.40% to 83.49%. The Drug-polymer compatibility was studied by using FTIR spectroscopy. The study revealed that there is no interaction between the drug and selected polymers. The drug release study was found to be controlled release manner and release kinetics follows peppa's model and non-fickian in nature. The stability studies data was found that there was no such difference in drug entrapment efficiency and *in-vitro* drug release. So it indicates that biocompatible and cost effective polymers like Eudragit RS 100 and Kollicoat MAE 100 P polymer can be used to formulate efficient microspheres with good percentage entrapment efficiency and controlled release up to 24 hr in phosphate buffer pH 7.4. Hence these microspheres of Cloxacillin can be targeted to gastroretension.

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INTRODUCTION

Oral drug administration has been the predominant route for drug delivery to ease the convenience of administration, patient convenience and adaptability in formulations. However, it's a well-accepted reality nowadays that drug absorption throughout the gastrointestinal canal isn't uniform. Victimization presently utilized unleashes technology, oral drug delivery for twelve or maybe twenty four hours is feasible for several medications that are absorbed uniformly from gastrointestinal canal [1].

Recent scientific advancement in viscus retentive systems shows accumulated interest in teachers and industrial analysis teams concerning the novel indefinite quantity forms which will be maintained within the abdomen for a protracted and sure amount of your time. One among the foremost possible approaches for achieving a protracted and sure drug delivery profiles within the gastrointestinal canal is to regulate the viscus duration, using gastroretentive dosage forms that will provide us with new and important therapeutic option. Gastroretentive systems will stay within the viscus region for many hours and thence considerably prolong the viscus duration of medication. Prolonged viscus retention improves bioavailability, reduces drug waste, and improves solubility for medicine that square measure less soluble in an exceedingly high pH scale surroundings. Gastro retention helps to supply higher handiness of latest product with new therapeutic prospects and substantial advantages for patients [2-4].

Microspheres are well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of the drug [5]. These are the carriers for drug is one such approach which can be used in a controlled release fashion [6].

Cloxacillin is in the penicillin family of medications, an antibiotic useful for the treatment of a number of bacterial infections. This includes impetigo, cellulitis, pneumonia, septic arthritis, and otitis externa. It is not effective for methicillin-resistant Staphylococcus aureus .Cloxacillin is incompletely but dependably absorbed from oral route, especially taken in empty stomach. It is >90% plasma protein binding elimination occurs primarily by kidney, also partly by liver. Plasma $t_{1/2}$ is about 1hour [7].

Hence Cloxacillin is a widely prescribed antibiotic antibacterial agent having low bioavailability(less than 37%), short plasma half-life(less than 1hour), and more adverse effect. To overcome these problems this drug has been selected to formulate as a gastro retentive microspheres.

MATERIALS AND METHODS:

Cloxacillin was gifted sample from Medreich Pharmaceuticals Limited Bangalore. Eudragit RS100 obtained from Yarrow Chem Products, Mumbai. Kollicoat MAE 100P was obtained from Sigma-aldrich. All reagents and chemical used were of analytical grade.

Estimation of drug

Cloxacillin was dissolved in pH7.4 phosphate buffer solution and further diluted with the same and scanned for maximum absorbance in UV double beam spectrophotometer (Shimadzu 1700)in the range from 200 to 400 nm, using phosphate buffer pH 7.4 as blank. The ƛ max of the drug was found to be 217 nm.

Preparation of Cloxacillin Gastroretentive Microspheres by Emulsion Solvent Evaporation Technique.

Cloxacillin microspheres were prepared using Eudragit RS 100, Kollicoat, Polyvinyl alcohol and Tween-80 as continuous phase by emulsion solvent evaporation technique. Initially dichloromethane was mixed uniformly at room temperature, then Eudragit RS 100 and Kollicoat in 1:1 ratio was dissolved in the above solution. To this mixture, 250mg of Cloxacillin was added; mixed thoroughly and injected drop wise in to aqueous solution of Polyvinyl alcohol (0.5% w/v, 200ml), Tween-80(0.01% w/v,200ml) through a 22G needle at 30-40˚C and the resultant o/w type emulsion was stirred on a mechanical stirrer at 1200 rpm for 1hr. The microspheres obtained was washed for 2-3 times with distilled water and dried at room temperature [8, 9]. Different concentrations and ratios of polymers used in the formulation of microspheres are mentioned in Table No: 1.

Table No: 1. Different ratios of polymers used in the formulation of Cloxacillin microspheres.

Formulation	Cloxacillin	Eudragit RS 100	Kollicoat	Dichloromethane
F1	250	0.5	-	30
F2	250	1.0	-	30
F3	250	1.5	-	30
F4	250	2.0	-	30
F5	250	-	0.5	30
F6	250	-	1.0	30
F7	250	-	1.5	30
F8	250	-	2.0	30
F9	250	1.0	1.0	30

Evaluation of Microspheres:

Fourier Transformed Infrared spectroscopic analysis:

The Fourier Transformed Infrared spectroscopic analysis (FT-IR) is done to study the polymer- drug interactions and the physical state of drug in the microsphere. The FT-IR of pure drug and the drug loaded microspheres is done separately to judge accurately and scanned in the spectral region between 4000 cm-1 and 600 cm-1.

Surface morphology:

The surface morphology is most commonly measured by Scanning Electron microscopy. The surface morphology has been studied by using JEOL JSMT- 330A.Scanning electron microscopy (SEM).

Particle Size and zeta potential analysis:

The prepared Gastro retentive microspheres were evaluated for particle size and zeta potential were determined by photon correlation spectroscopy using Horiba scientific SZ-100. The samples were prepared by applying suitable dilution. The size measurement was performed in triplicate at room temperature

Drug Entrapment Efficiency:

Weighed amount of microspheres (100mg) with phosphate buffer pH 7.4 (10ml) was added in a vial. The solution was stirred vigorously for 24 hours with mechanical stirrer. Supernatant was collected by centrifugation and drug content in supernatant was determined by using UV spectrophotometer at wavelength 217.nm. Efficiency of drug entrapment is calculated by the following formula.

$$\text{Drug entrapment efficiency}(\%) = \frac{\text{Amount of drug releazed}}{\text{Amount of drug initially taken}} X100$$

***In-vitro* buoyancy:**

An *in-vitro* floating study was carried out using 0.1N Hcl containing 0.02% tween-80 as a dispersing medium. Microspheres were spread over the surface of 900 ml of dispersing medium at 37±0.5ºC. A paddle rotating at 100 rpm agitated the medium. Each fraction of microspheres floating on the surface and those settled down were collected at a predetermined time point. The collected samples were weighed after drying.

$$\text{Floating microspheres } (\%) = \frac{\text{Weight of floating microspheres}}{\text{Initial weight of floating microspheres}} X100$$

***In-vitro* drug release study:**

The *in-vitro* release of drug from floating microspheres was carried out using Electrolab TDT-08L Dissolution apparatus by using basket method. Microspheres (250 mg) were placed in a basket to avoid the floating of microspheres. The dissolution test was performed using 900 ml of dissolution medium . The content was rotated at 100 rpm at 37±0.5°C. Using phosphate buffer pH 7.4.5-ml sample was withdrawn from the dissolution medium at various time intervals using a pipette and analyzed drug release using a UV–Visible spectrophotometer at 217nm. The receptor volume was maintained constant by replacing with equivalent volume of buffer after each withdrawal.

Stability studies:

The Cloxacillin loaded microspheres formulation was filled in tightly closed glass vials and subjected to short term stability testing according to the international conference on Harmonization (ICH) guidelines for zone 3 and 4. The packed containers of microspheres were kept at room temperature (25±2ºc) and acceleration condition (40±2ºc/75±5% RH) in a stability chamber for a period for one month. The sample (F=9) were analyzed at 45 days and evaluated for drug content.

RESULTS AND DISCUSSION:

Calibration Curve of Cloxacillin

Table No: 2. Calibration curve of Cloxacillin.

SI.No	Concentration(µm/ml)	Absorbance
1	0	0
2	2	0.159
3	4	0.331
4	6	0.481
5	8	0.642
6	10	0.768

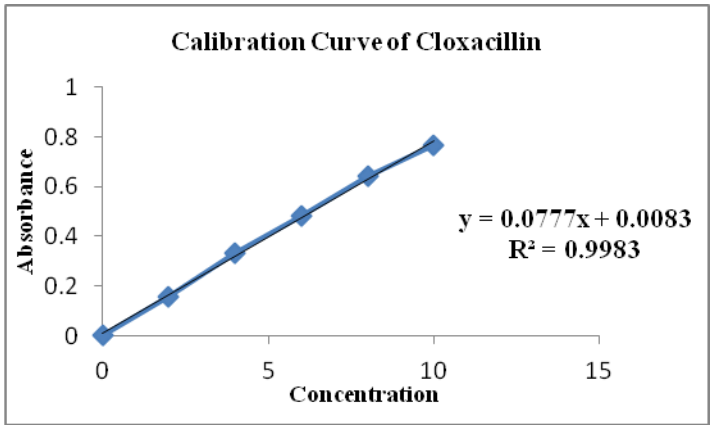


Fig No: 1. Calibration curve of Cloxacillin.

Results the R2 value of standard graph of drug not more than 0.99 i.e. it obeys beer`s law.

Drug-polymer interaction study by FT-IR spectrophotometer:

The IR spectra shows peak at 3387cm^{-1} is shown due to O-H stretching.3196cm^{-1} is shown due to N-H stretching.3070cm^{-1} is shown due to C-H stretching.1841cm^{-1} is shown due to C-O stretching.1724cm^{-1} is shown due to C-C stretching.1445cm^{-1} is shown due to C-N stretching.755cm^{-1} is shown due to C-C stretching. These similar peaks are obtained in IR spectra of pure drug (Cloxacillin) and F9 formulation. It indicates that there is no interaction between drug and polymer used for the preparation of nanoparticles and the results are showed in Figure No: 2 and 3.

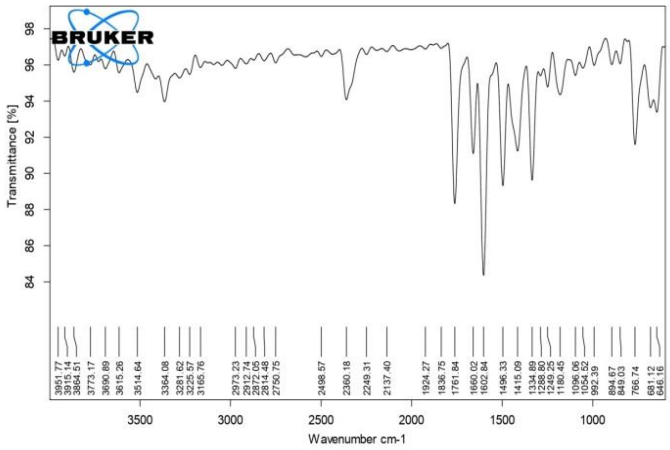


Fig No: 2. IR spectrum of Cloxacillin.

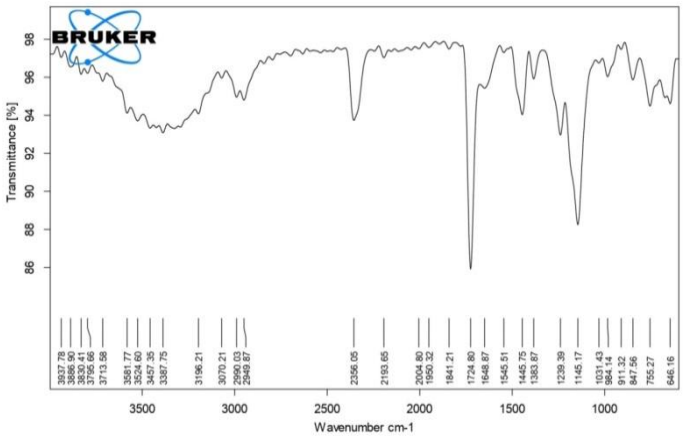


Fig No: 3. IR spectrum of formulation F9.

Surface Morphology:

The surface morphology of the prepared microspheres was characterized by Scanning Electron microscopy (SEM) studies. Figure No: 4, 5 and 6 shows the SEM images of gastroretentive microspheres containing the drug Cloxacillin. The SEM reveals that the prepared microspheres was spherical shape, smooth surface and free flowing in nature.

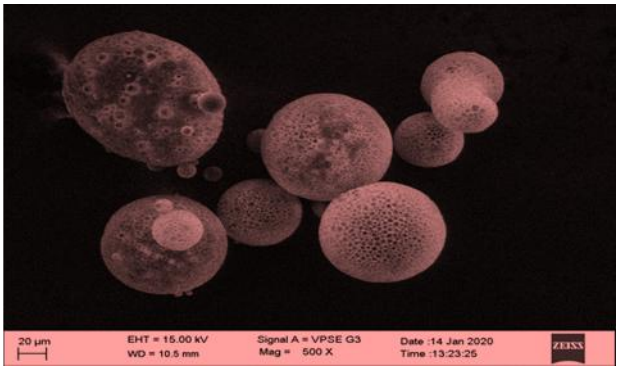


Fig No: 4. SEM images of formulation F2

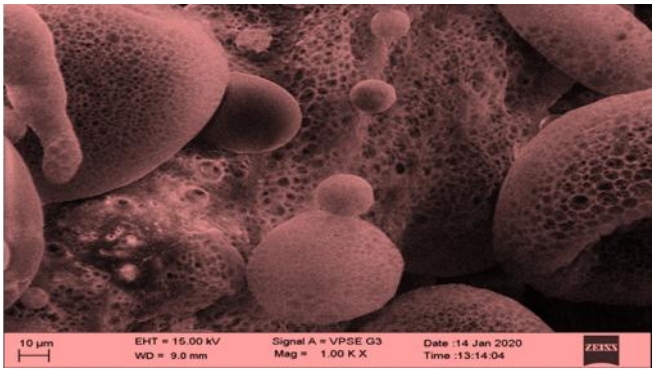


Fig No: 5. SEM images of formulation F7

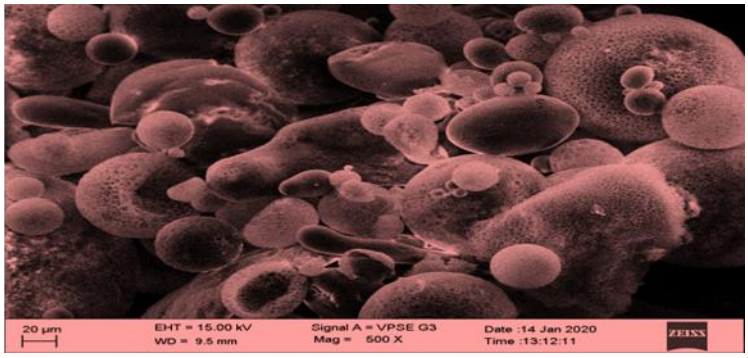


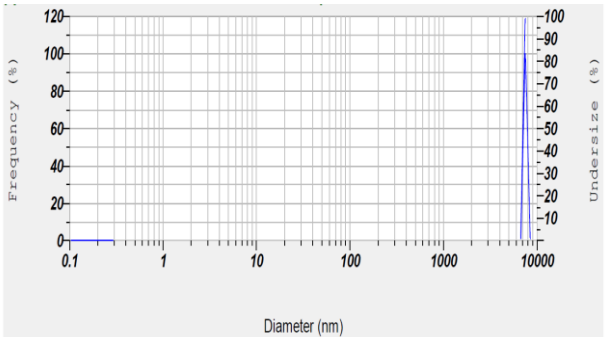
Fig No: 6. SEM images of formulation F9.

Particle size, zeta Potential, Drug Entrapment Efficiency analysis and Buoyancy study:

The particle size determinations of prepared microsphere were carried out by using particle size analyzer (Horiba Scientific SZ-100). By Zeta potential distribution and size distribution by intensity, the average diameter of the prepared microsphere were found to be in the range of 3 µm to 20 µm. Here the particle size of the prepared microsphere was increased on increasing in the polymer ratio content. The results are shown in Table No:3 and Figure No:7 and 8.

Table No: 3. Particle size Drug Entrapment Efficiency analysis and Buoyancy study of prepared microspheres Formulation.

SI.no	Formulation code	Particle size (µm)	Drug EE (%) ($\bar{X}$ ± SD) (n=3)	*In-vitro* buoyancy ($\bar{X}$ ± SD)(n=3)
01	F1	05	76.76±0.012	72.40±0.02
02	F2	20	91.96±0.028	52.45±0.04
03	F3	08	93.41±0.026	67.30±0.05
04	F4	03	80.91±0.022	82.15±0.05
05	F5	13	77.21±0.018	62.50±0.02
06	F6	17	84.21±0.015	68.20±0.06
07	F7	10	91.24±0.022	74.32±0.04
08	F8	09	91.56±0.001	79.30±0.05
09	F9	20	84.92±0.011	83.49±0.05



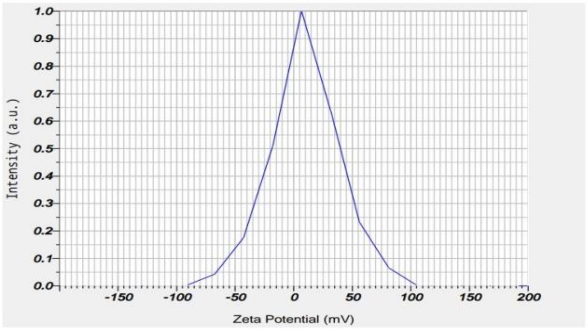


Fig No: 7. Particle size analysis by size distribution by intensity.

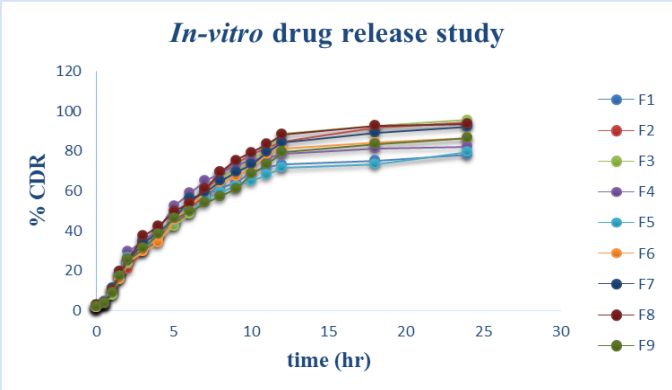
Fig No: 8. Particle size analysis by Zeta potential distribution.

***In-vitro* drug release study:**

The *in-vitro* dissolution study of microspheres was performed by using dissolution test apparatus in phosphate buffer pH 7.4 (gastrointestinal pH). The cumulative percentage of drug release from F1 to F9 formulations ranges from 78.14% to 95.47%. The combination of Eudragit RS100 and Kollicoat MAE100 P in the formulation which gives the good results because the polymers are being soluble around pH 7.0 which leads to the formation of pores in the coating layer which allows medium to release the drug, ruptures the outer coat and prolonged drug release. The results are shown in Table No: 4 and Figure No: 9-12.

Table No: 4. *In-vitro* drug release kinetics of microspheres formulation F1 to F9.

Time (hr)	% Drug Release ($\bar{X}$ ± SD) (n=3)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	2.24	1.63	1.5	2.12	1.56	1.64	2.15	2.91	2.15
0.5	4.45	3.82	3.33	4.54	3.25	4.4	4.12	3.24	3.95
1	10.23	8.51	7.64	11.12	11.45	9.64	11.26	10.16	9.16
1.5	18.48	16.6	17.24	19.56	18.26	15.47	19.45	19.45	17.45
2	24.81	21.04	23.45	29.44	26.45	25.13	25.46	25.64	25.63
3	34.69	30.84	31.65	35.46	30.21	29.64	34.26	37.56	31.24
4	38.26	36.27	35.78	41.23	39.39	34.15	39.46	42.26	38.56
5	45.12	43.26	42.16	52.34	45.15	45.46	46.24	49.54	46.24
6	53.24	50.18	48.24	59.16	50.14	52.78	56.23	53.85	49.56
7	58.16	59.48	57.73	65.16	56.14	59.45	59.34	61.24	54.26
8	61.31	69.24	66.21	69.18	59.26	64.15	65.24	69.28	57.35
9	64.26	73.45	70.47	73.15	62.32	67.15	69.27	75.35	61.26
10	69.21	78.24	75.46	75.64	65.14	71.46	73.26	79.26	68.56
11	72.14	81.45	81.24	76.45	68.14	78.41	79.64	83.46	73.25
12	73.16	84.47	88.46	78.54	71.45	81.27	84.25	88.26	79.56
18	75.16	91.46	92.46	81.46	73.46	84.26	89.26	92.46	83.46
24	78.14	94.15	95.47	82.16	79.46	86.42	92.18	93.41	86.45



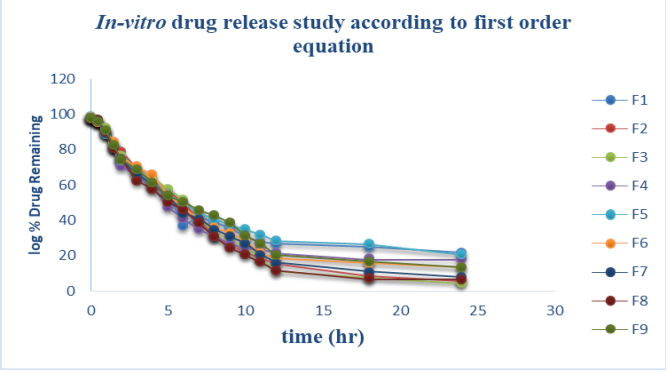
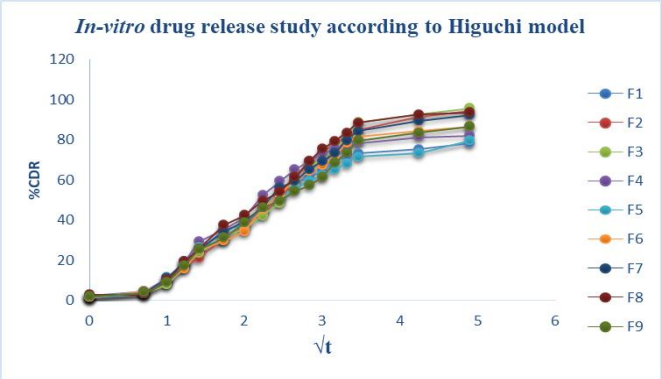


Fig No: 9. *In-vitro* drug release study.
Fig No: 10. *In-vitro* drug release study according to first order equation.



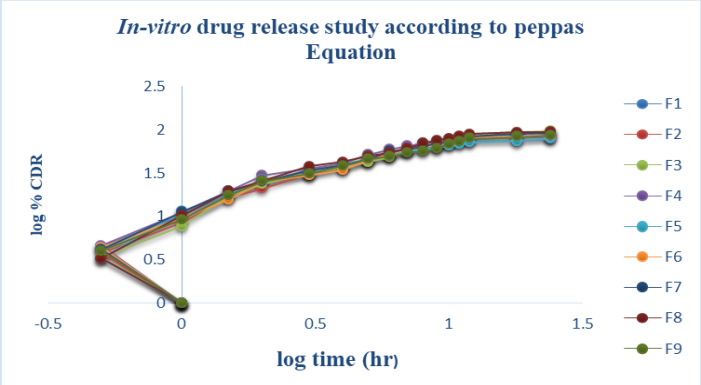


Fig No: 11. *In-vitro* drug release study according to Higuchi model.

Fig No: 12. *In-vitro* drug release study according to peppas equation

***In-vitro* release kinetics:**

The release kinetics were evaluated by making use of Zero order, First order, Higuchi's and Korsmeyer-Peppa's equations. The drug release through the microspheres of Cloxacillin follows Peppas kinetics with controlled release mechanism, by fitting in the Korsmeyer-Peppa's equation, the release kinetics follows non-Fickian kinetics. If the 'n' values of Korsmeyer-Peppa's equation is below 0.5, which indicates Fickian kinetics. If the 'n' value of Korsmeyer-Peppa's equation is in between 0.5 to 1, this indicates non-Fickian kinetics. The prepared microspheres of Cloxacillin release kinetics fitted in Korsmeyer-Peppa's equation gives the 'n' values are in between 0.5 to 1, so the release is following non-Fickian, controlled release mechanism. The results are shown in the Table No: 5.

Table No: 5. Regression Co-efficient analysis of the *in-vitro* drug release data of cloxacillin microspheres according to release kinetic models.

Formulation	Zero order		First order		Higuchi	Peppas	
	n	R^2	n	R^2	n	n	R^2
F1	4.6955	0.8436	0.091	0.9210	0.9554	0.7862	0.9447
F2	5.8700	0.9069	0.148	0.9855	0.9601	0.9022	0.9713
F3	5.8776	0.9185	0.158	0.9698	0.9622	0.9210	0.9595
F4	5.1015	0.8262	0.111	0.9218	0.9476	0.7978	0.9358
F5	4.5204	0.8470	0.085	0.9286	0.9611	0.8074	0.9180
F6	5.3693	0.8893	0.118	0.9616	0.9614	0.8414	0.9672
F7	5.4789	0.8991	0.133	0.9833	0.9722	0.8256	0.9545
F8	5.7863	0.8917	0.156	0.9799	0.9649	0.8896	1.9385
F9	5.0654	0.9021	0.108	0.9727	0.9718	0.8288	0.9518

Stability study:

The Prepared microsphere was packed in screw capped HDPE bottles and was stored at 40± 20 C and 75 % RH for 45 days. After storage for 45 days, the products were tested for drug entrapment efficiency and drug release study. The results are given in Table No.6 and 7.

Table No: 6. Drug entrapment efficiency of formulation F9.

Formulation Code	Drug entrapment efficiency	
	Before stability test	After stability test
F9	**84.92±0.01**	**83.78±0.012**

Table No: 7. Drug release study after Stability studies of formulation F9.

Formulation Code	Percentage of drug release	
	Before stability test	**After stability test**
F9	86.45±0.01	85.53±0.031

CONCLUSION

The Preformulation studies involving description of solubility, melting point, of the drug and evaluated for various parameters. The gastroretentive microspheres of Cloxacillin were prepared by emulsion solvent evaporation method by using polymers, such as Eudragit RS100, tween 80 and Kollicoat MAE100 P. From the above studies, it can be concluded that the particle size analysis indicated that the particle were in the size range of 5 to 20 µm. The percent buoyancy was more than 70% up to 12 hours. The percent buoyancy was increased significantly with increase in polymer The microspheres were smooth surface and spherical in nature, as shown by the SEM studies. However by the pharmacokinetic studies it indicates that *in-vitro* drug release of the formulation F9 follows kinetics of Peppa's model and the mechanism followed non-Fickian kinetics. The formulations were found to be stable in short term stability studies.

From the results it indicates that biocompatible and cost effective polymers like Eudragit RS 100 and Kollicoat MAE 100 P can be used to formulate efficient microspheres with good percentage entrapment efficiency and controlled release up to 24 hr in phosphate buffer pH 7.4. Hence these microspheres of Cloxacillin can be targeted to gastroretension, so it can be suggested that there is further scope for *in-vivo* and pharmacokinetic study and Recommend for future Research.

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List of Abbreviations:

FT-IR	Fourier Transformed Infrared spectroscopic
SEM	Scanning Electron microscopy
µm	Micro Meter

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