EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 6
370-376
Year: 2016

MICROENCAPSULATED CIPROFLOXACIN HYDROCHLORIDE AUGMENTED DRUG DELIVERY AND ANTIBACTERIAL ACTIVITY AGAINST PATHOGENIC *E.COLI* STRAIN

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Article Received on 02/04/2016 **Article Revised on 23/04/2016** **Article Accepted on 13/05/2016**

INTRODUCTION

Ciprofloxacin is a second generation fluoroquinolone having fluorine at position 6 of the quinolone ring having a good activity against most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, including Gram (-) (*Escherichia coli, Haemophillus influenza, Klebsiella pneumonia, Proteus mirabilis*), and Gram(+) (methicillin-sensitive but not methicillin-resistant Streptococcus pneumonia, *Staphyloccccusepidermidis, Enterococcus faecalis, and Streptococcus pyrogens*) bacterial pathogens.[1] Ciprofloxacin is valued for its broad spectrum of activity, its excellent tissue penetration & for their availability in both oral and intravenous formulations. Moreover, ciprofloxacin is used alone or in combination with other antibacterial drugs in the empiric treatment of infections for which for which bacterial pathogen has not identified, including urinary tract infections and abdominal infections &infection caused by specific pathogen known to be sensitive.[2-5] It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops synthesis of DNA and other enzymes. However, due to drug resistance and limited access to bacteria, there is urgent need to develop modified drug delivery systems of ciprofloxacin with desired release profile as well as antibacterial activity. Therefore, in present investigation, gelatine microspheres of ciprofloxacin were prepared by coacervation method. Subsequently, these microspheres were analyzed by using spectral and biological techniques.

MATERIALS AND METHODS

Materials

Ciprofloxacin was obtained as gift sample from Unichem Laboratory, Sonepat, Haryana, India. Gelatin, glutaraldehyde and span 80 were purchased from Himedia, Mumbai, India. All other chemicals used were of highest analytical grade.

Analytical method for Analysis of Ciprofloxacin Drug

Standard curve was prepared using stock solution of 1mg/ml. The standard solution were prepared by proper dilution of the primary stock solution with ultra pure water to obtain standards in the concentration range µg/ml, the absorbance of these solutions was then fitted in the calibration curve to calculate the accuracy. The UV spectrophotometric determinations were prepared at 278 nm using Shimadju-1700UV Visible Spectrophotometer.

Preparation of ciprofloxacin loaded gelatin microspheres by coacervation method[6]

Gelatin was dissolved in 10 ml distilled water previously heated at 50˚C. Ciprofloxacin was dissolved in 5 ml water and dispersed in solution. The aqueous phase was poured drop by drop in oil phase containing span 80, then this was heated to 50˚C, then stirred at 600 rpm for 5 mins (r) to get uniform dispersion followed by cooling at 10˚C which gives phase separation. The dispersion was stirred for 2 hrs. in ice bath at 10˚C after one hr. the gluteraldehyde (25%) 2 ml was added and stirring was continue for the next 1 hr. after 2 hrs. stirring was stopped and beaker was refrigerated at -5˚C for 24 hrs. to get rigid microspheres. The slurry was filtered and microspheres washed with ice cold Isopropyl alcohol & filtered using vaccum filteration assembly and dried for 24 hours.

CHARACTERIZATION OF MICROSPHERES

Characterization based on % yield analysis

The prepared microspheres were collected and weight was divided by total amount of all non-volatile components which were used for the preparation of the microspheres. The % yield was calculated using following formula.

%yield= (Actual weight of product/ Total weight of excipient and drug) *100

Particle Size Analysis

Measurement of the particle size distribution of microspheres was carried out with an optical microscope. Stage micrometer was used to calculate calibration factor. The particle size was calculated by multiplying the number of division of the ocular disc by the particle with calibration factor. 100 randomly chosen spheres were taken to measure their individual size.

%Encapsulation Efficiency

100 mg of Ciprofloxacin loaded microspheres were accurately weighed. They were dissolved in 10 ml of distilled water and kept for 72 hrs. After 72 hr, .filter it and the residue was kept aside and filrerate was diluted suitably and analysed for the drug content spectrophotometrically at 278nm using distilled water as blank. And encapsulation efficiency was calculated using formula given below:

$$E = Qp/Wt*100$$

Where E is the percentage of the encapsulated drug, Qp is the quantity of the drug encapsulated in microspheres in mg and Wt is quantity of the drug added for encapsulation in mg.

Surface Morphology

Scanning electron microscopy (SEM) (FEI Quanta 200E) was performed to characterize the surface of formed microscopes. Microscopes were mounted directly onto the sample stub and processed.

X-Ray Diffraction Study

In order to determine the physical state of the formulation whether amorphous or crystalline X-ray diffraction studies were carried out. The change in the form of drug from crystalline to amorphous is indicated by characteristic peakless patterns. The X-Ray diffraction (XRD) of the final formulation was recorded on the scanning X- ray diffractometer using an X'Pert pro data collector software. The radiation used was generated by a Cu Kα source fitted with Ni filter at 0.15 nm wavelength at 40 mA and 45 KV. Samples were scanned for 2θ value over a range from 5-50 at a scan rate of 1.5/min.

FTIR Analysis

The drug polymer interactions were studied by FTIR Bruker Spectrometer. Two percent (w/w) of the sample, with respect to a potassium bromide (KBR, SD Fine Chem Ltd., Mumbai, India) disc, was mixed with dry KBr. The mixture was ground into a fine powder using mortar and then compressed into KBr discs in a hydraulic press as a pressure of 10000 PSI. Each KBr disc was scanned 10 times at a resolution of 2 cm-l using Happ-Genzel Apodization. The characteristic peaks of both the spectra's resembled each other.

In vitro drug release studies[7-10]

The in vitro release of drug from microspheres were carried out for 12 hours using paddle type Dissolution rate test apparatus USP STD containing 900 ml of dissolution medium maintained at 37±0.5˚C and speed of agitation at 50 rpm. An accurately weighed sample was suspended in dissolution media consisting 900 ml of 7.4 buffer (Shaharyar M.et al., 2006) solution. Dissolution studies were studied for 12 hrs. At prefixed time; 10ml of solution were withdrawn. Sample were assayed spectrophotometrically for the drug content at 278 nm using Shimadzu- 1700 UV Visible spectrophotometer. (Henry A. et.al. 2008) The volume of the dissolution medium was adjusted to 900 at every sampling time by replacing 10ml with the same dissolution medium. The analysis of the drug release mechanism from the pharmaceutical dosage form is an important but complicated process and it is practically evident in the dosage form is an important but complicated process and it is practically evident in the case of matrix systems. As model-dependent approach, the dissolution data are fitted to four popular release model such as zero order, first, Higuchi, and Korsemeyer and Peppas model.

Antimicrobial Activity against E.Coli[11-14]

Antimicrobial activity of the formulation was examined using the microorganism E. Coli strains by using the Nutrient agar media. The test (microspheres) solution was taken of the 10µg/ml concentration. Ciprofloxacin HCL drug was taken as standard for antimicrobial activity at a concentration of 10 µg/ml. Nutrient media was prepared and sterilized by autoclaving at 121˚C for 15 minutes. Petridishes were sterilized in air oven at 140˚C for 3 hrs. Before it could solidify the agar media was mixed with the test organism (one day old subcultered) transferred aseptically to sterile petridishes and allowed to solidify. Then the well of 1ml capacity were made in solidified agar plate with the help of sterile cork borer in such a way that overlapping of zone of inhibition does not occur. Plates were kept at room temperature for half an hour for diffusion of sample into medium. The organism inoculated were then incubated under condition required by microorganism (37˚C), present in them. After the completion of incubation period zone of inhibition by each sample, with organism in different plates, was measured & recorded.

RESULTS AND DISCUSSION

The aim of the present work is to develop and characterize microspheres of ciprofloxacin HCL, which after oral administration could prolong the residence time and increase the bioavailability of the drug. In order to provide the sustained release and to minimize the dose dependent side effects as well as to improve patient compliance microsphere were formulated.

Characterization based on Percentage Yield

The prepared microspheres were collected and weighed. The measured weight was divided by total amount of all nonvolatile components which were used for the preparation of microspheres and percentage yield was calculated. The percentage yield of microspheres was in range of 35-60% for all batches. For the final

formulation A3 percentage yield was found to be 47.5 (Table 1).

Characterization based on particle size analysis

Particle size of microspheres plays an important role in determining the release characteristics. The microspheres were spherical in shape and had a mean particle size. The particle size was taken using projection microscope fitted with an ocular micrometer and calibrated stage micrometer was used to determine mean particle size of each batch. Particle size of the formulation is found to be in ascending order which shows that the particle size increases as the concentration of the polymer increases. Particle size is found to be 39.08, 41.60, 42.08, 44.18, 46.89 of all batches respectively (Figure 1).

Characterization based on Entrapment Efficiency

In the prepared batches different batches showed different entrapment efficiency.A3 batch showed maximum entrapment efficiency. The entrapment efficiency of the batches A1, A2, A3, A4, A5 was calculated as 67.03, 81.5, 99.43, 97.67, 94.18. Thus A3 batch shows maximum entrapment efficiency (Table 1).

Entrapment Efficiency of the formulated Batches.

% Entrapment Efficiency	
Batch Code	**% Yield**
A1	67.03
A2	72.41
A3	80.23
A4	80.74
A5	78.27

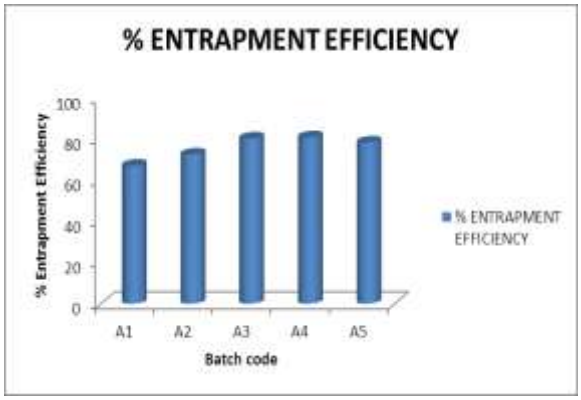


Figure 1 Entrapment efficiency of formulated batches

Entrapment Efficiency of the prepared batches was found to be in range of 67-99% and an increase was observed with increase in polymer ratio. Entrapment Efficiency of Batch A3 was observed to be maximum 99.43 as compared to the other batches having less entrapment efficiency than batch A3. Among all formulation A3 Batch showed best drug release as shown in figure 1.

X-ray Diffraction Study

XRD study showed that the model drug exhibits sharp peaks at 8˚, 9.0˚, 11.9˚, 19˚, 22˚, 24˚,26.8˚, 29˚, 34,˚36˚, 44˚.(2θ) which indicates that the drug is crystalline in nature and model drug with the polymer also shows similar diffraction pattern indicating that crystalline form of model drug was not significantly transformed during microspheres preparation (Figure 2).

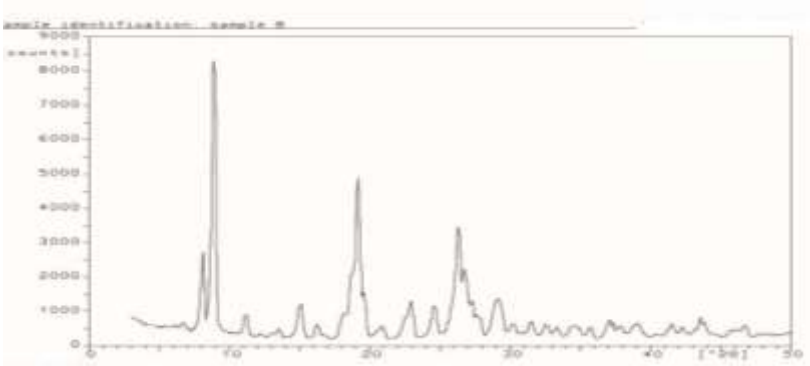


Figure: 2 X-ray Diffraction pattern of Pure Drug.

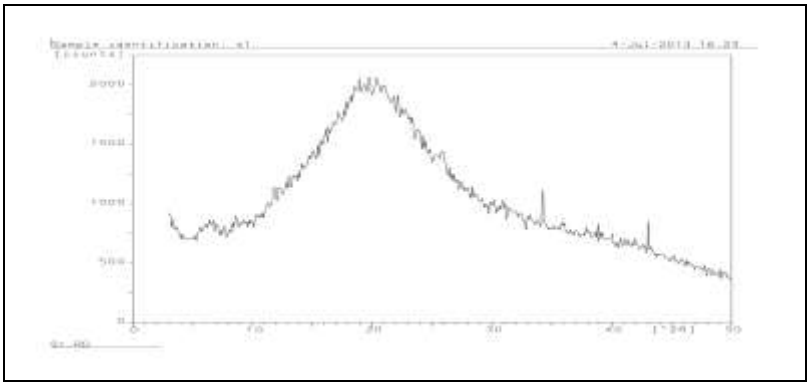
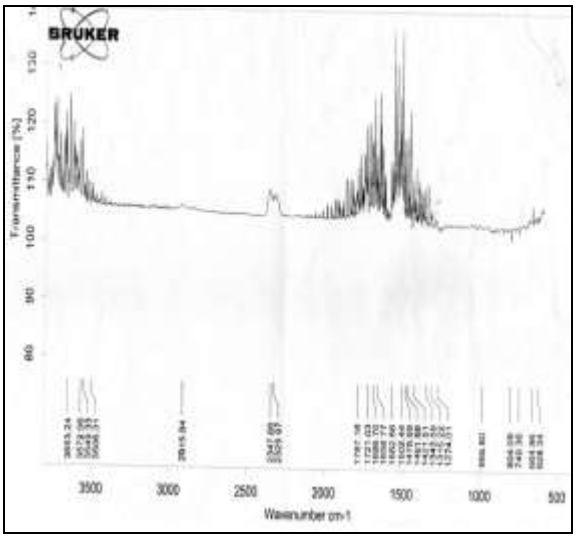


Figure: 2 XRD pattern of Drug loaded Microspheres.

FTIR

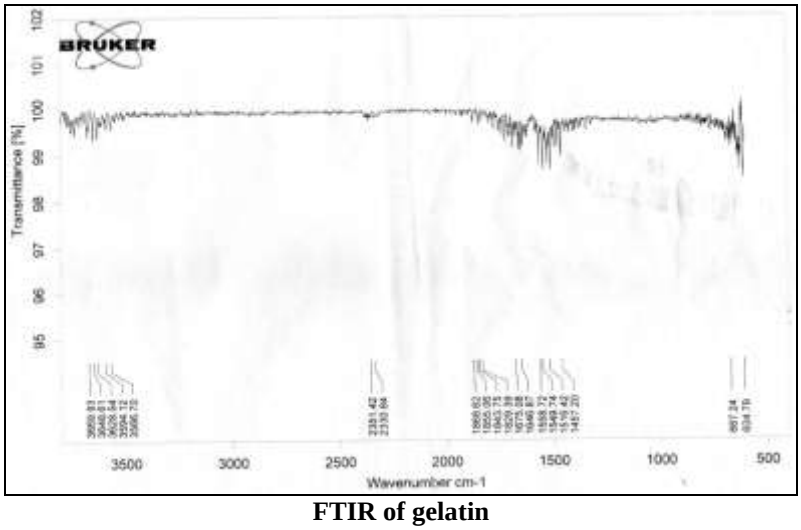
The Ciprofloxacin HCL sample was authenticated using Bruker Spectrum. The spectra produced were compared to the spectra reported in IP. Two percent (w/w) of the sample, with respect to a potassium bromide (KBr). The mixture was ground into fine powder using mortar and then compressed into a KBr discs in a hydraulic press at a pressure of 10000 PSI. Each KBr disc was scanned 10 times at a resolution of 2cm-l. The characteristics peaks were recorded as shown in spectra. The FTIR analysis of drug confirm the authentication of drug as characteristic peaks were observed, IR spectra of pure Ciprofloxacin HCL and its combination with polymer as shown in fig. An FTIR spectra of Ciprofloxacin one prominent characteristic peak was found between 3500-3450 cm-, which was assinged to stretching vibration of OH group and intermolecular hydrogen bonding . The possible peak of imino moiety of the piperazinyl group was less prominent due to intense OH stretching vibration. Another band at 3000-2950 cm-l represented the alkene and aromatic C-H stretching, mainly υ=CH. The peak at 2900 cm was assigned to C-H stretching vibration ofcyclopropyl group. The 1950 to 1450 cm-1 region exhibited FTIR absorption from a wide variety of double-bonded functional groups. The band at 1750 to 1700 cm-1 represented the carbonyl C=O stretching i.e., υ C= . The peak at 1650 to 1600 cm -1 was assigned to quinolones. The band at the 1450 to 1400 cm-1 represented υ CO and at 1300 to 1250 cm-1 suggested bending vibration of O-H group which proved the presence of carboxylic acid. A strong absorption peak between 1050 and 1000 cm-1 was assigned to C-F group. Thus the characteristic peak confirmed the structure of Ciprofloxacin HCl.



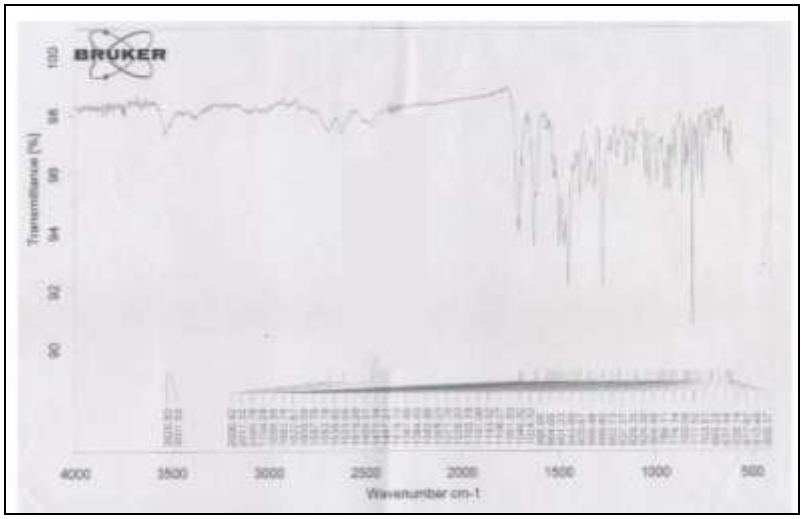
FTIR Spectra of Ciprofloxacin HCl

Characteristic peaks	Reported (cm^{-1})	Observed (cm^{-1})
Hydroxyl group	3500-3450	3506.31
Aromatic cyclic enes	3000-2950	2915.84
CO group of acid	1750-1700	1721.03
Quinolones	1650-1600	1658.77
Carbonyl	1450-1400	1421.51
Hydroxyl(δ O-H bending)	1350-1250	1343.69
Fluorine (C-F stretching	1050-1000	1045.52

Interpretation of spectrum reveals that Ciprofloxacin HCL shows similar peaks as reported in literature. Hence, Ciprofloxacin HCl is an authentic drug.



FTIR of gelatin

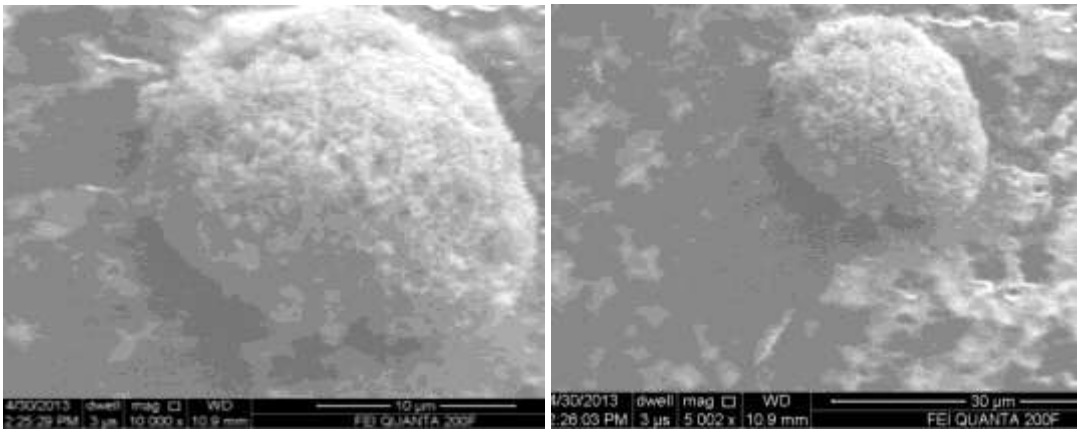


FTIR spectra of drug loaded microspheres

Interpretation of spectrum reveals that formulation of the microsphere shows similar peaks as reported pure drug. Hence, drug has no interaction with polymers.

Surface Morphology

SEM Results showed that optimized batch A3 predominantly spherical in shape with porous surface. The shell of the microspheres also showed some porous structure. It may be caused by the evaporation of the solvent entrapped within the shell of microspheres after forming a smooth and dense skin layer. The porous nature may be responsible for the drug release.



SEM: Images of the Gelatin Microspheres.

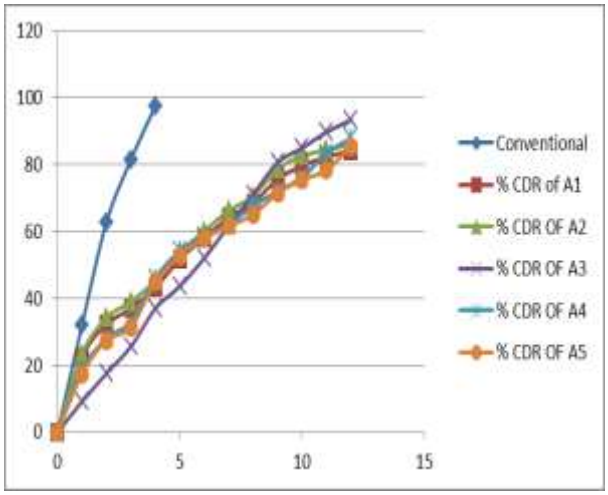
In -Vitro Release study of Drug loaded Microspheres.

The in-vitro drug releasestudies were performed using USP dissolution rate test apparatus (paddle type apparatus, 100 rpm, 37±.5˚c). Microspheres bearing Ciprofloxacin HCl was suspended in 900ml of buffer solution having pH 6.8 and release study were carried out for further 8 hrs. Aliquots of the dissolution medium were withdrawn at specific time interval and amount of the drug was quantified using UV- visible spectrometer.

In- Vitro Release profile for the A1-A5 Batches

Time in Hrs	Cumulative % release of conventional formulation	Cumulative % Drug Release A1(Ratio 1:1)	Cumulative% drug releaseA2 (Ratio1:2)	Cumulative% drug releaseA3 (Ratio1:3)	Cumulative% of drug releaseA4 (Ratio1:4)	Cumulative% of drug releaseA5 (Ratio1:5)
0	0	0	0	0	0	0
1	32.035±0.76	22.42	23.42	9.143±1.04	18.56	17.35
2	62.757±0.65	32.18	34.18	17.54±1.03	28.05	27.44
3	81.524±0.45	37.05	39.05	25.57±0.63	33.05	31.37
4	97.566±0.98	43.25	45.25	36.98±1.44	46.09	44.86
5		51.79	53.74	43.64±0.71	54.22	52.83

6		58.12	60.12	52.05±2.60	58.60	57.96
7		64.24	64.24	61.75±.094	62.10	61.61
8		68.57	70.58	71.13±0.61	67.73	65.12
9		75.65	78.65	80.64±1.67	72.03	71.53
10		79.65	82.65	84.98±2.07	76.21	75.54
11		82.36	84.36	89.76±0.87	83.43	78.37
12		84.12	86.12	93.34±2.57	87.96	85.45



Cumulative percentage release of All batches vs time.

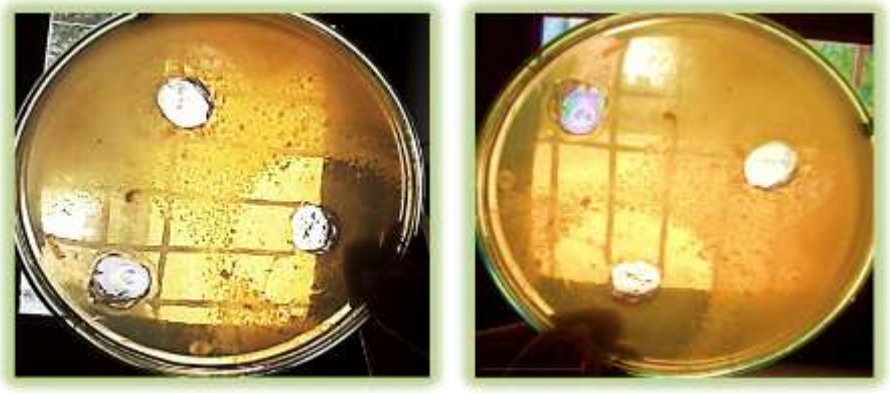
For Microspheres with core: coat ratio of 1:3 (ciprofloxacin: gelatin) has given(Table 5.2) the best sustained effect over a period of 12 hours and 93.34 % of the drugwas released at the end of 12 hours. So it is selected as the best optimized batch for sustained release delivery of the Ciprofloxacin.

Antimicrobial Activity against E.Coli

Antimicrobial activity of the microspheres of the selected batches was observed usingthe cup-plate method taking 10µg ml of Ciprofloxacin HCl as a standard for antibacterial activity. By measuring the zone of inhibition of the Standard drug and the microspheres as test drug we determine the antimicrobial activity of Microspheres.

Antimicrobial Activity against E.Coli

	Microorganism used: E.Coli Zone of Inhibition(Diameter in mm)	**Avg.**
Std.	36.236.537.3	36.66mm
Test	35.935.436	35.7mm



Photographs for the Antimicrobial activity with E. Coli.

The comparision of the test with standard shows that the microspheres have an antimicrobial activityapproximetely similar to the standard drug Ciprofloxacin HCl. So Microspheres passes the Antimicrobial activity.

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