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Research Article

LUNG TARGETING OF OFLOXACIN THROUGH SIZE SPECIFIC CHITOSAN MICROSPHERES

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Abstract

Pulmonary drug delivery systems offer many advantages over other conventional drug delivery system such as avoidance of first pass metabolism, large surface area suitable for drug absorption and quicker onset of pharmacological activity. One of the drugs, ofloxacin, if formulated into microspheres, could by the mechanical interception of capillary bed, could effectively be targeted to the lungs. The biodegradable and biocompatible polymer chitosan was selected as matrix forming agent for the purpose of microencapsulation. The formulated nanoparticles were then evaluated for various parameters like for particle size, shape and entrapment efficiency etc. The microspheres were evaluated by DSC studies, IR spectroscopy and in vitro drug release study followed by in vivo lung targeting study. The microspheres could be successfully utilized for targeting ofloxacin to the lung tissue.

Keywords: -Ofloxacin, Chitosan, Microspheres, Lung Targettin

Introduction

Pneumonia is one of the widely prevalent diseases of both developed and developing nations particularly in the Asian subcontinent. Ofloxacin has emerged as the gold standard in the treatment of Pneumonia [1]. The oral or parentral administration of the drug does not provide the effective concentration of the drug at the targeted site [2]. Micronization of drugs plays an important role in improving the drug dosage form and therapeutic efficiency. If an antibiotic is formulated into microspheres with suitable particle size, it can be addressed directly to the lungs by the mechanical interception of capillary bed [3]. Many workers have reported for the efficient targeting of drugs through microspheres, the size plays a great role in controlling drug delivery to the target organs and the subsequent uptake of drugs in tissue [4, 5, 6].

Present investigation was taken up in order to develop formulation of microspheres containing ofloxacin followed by its characterization by various techniques as well as evaluation of drug targeting efficiency towards lung.

Materials and methods

Ofloxacin was obtained as gift sample from COAX Bioremadies, India. Chitosan was generously provided by CIFT, Cochin (India) as a gift sample. All other reagents were of suitable analytical grade. Bovine serum albumin was obtained from CDH (P) Ltd and cottonseed oil was purchased from local vendor/ supplier. **Preparation of Ofloxacin Chitosan Microspheres (OCM)**

OCM were prepared by emulsion polymerization method [7, 8]. Chitosan dissolved in 5% acetic acid solution was taken and to it the drug was added with continuous stirring. This solution was dispersed in 80 ml of suspension medium containing cottonseed oil/petroleum ether (60:40) mixture and to it one ml of span 80 was added as an emulsifier. The suspension was stirred with mechanical stirrer (Remi, Mumbai, India) at a constant speed (2800rpm) for 10 minutes and 1ml of glutaraldehyde was added into the

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medium and continued stirring for 3 hours. The microspheres (OCM) thus obtained were washed several times with petroleum ether, filtered and lyophilized. Three batches OCM 1, OCM 2 and OCM 3 were prepared at drug polymer ratios 1:3, 1:5 and 1:10 respectively (Table 1)

Appearance and size distribution measurement

The surface morphology of the microspheres was carried out by using scanning electron microscopy (SEM). Particle size and particle size distribution were studied by using calibrated eye piece micrometer by optical microscopy.

Determination of Drug Entrapment Efficiency

A pre-weighed quantity of microspheres were dissolved in phosphate buffer (pH 7.4). The Ofloxacin content was assayed spectrophotometrically by measuring absorbance at λ_{max} 287 nm. The drug entrapment efficiency was calculated by the method described by Huo et al, 2005 [9].

$$\text{Entrapment Efficiency} = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} * 100$$

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry studies were carried out to study the changes in physical form of the drug, amorphous to crystalline or vice-versa or any polymorphic changes during formulation of microspheres.

***In vitro* dissolution study:**

In vitro drug release was studied using modified dialysis technique. Dialysis bag containing the suspension of OCM was placed in 250 ml of phosphate buffer (pH 7.4)
at 37˚C and stirred at 50 rpm using the USP paddle apparatus. At predetermined time intervals, 5ml of sample was withdrawn and replaced with fresh medium. The samples were filtered and assayed for drug release spectrophotometrically at 287mm.

Analysis of ofloxacin in lung by HPLC

A HPLC method for analysis of Ofloxacin in lung tissue was established. Chromatographic variable were optimized to achieve rapid and precise resolution for Ofloxacin.

The optimized chromatographic parameters were as follows:

Mobile phase: Citro-phosphate buffer (pH-5.0) **:** Acetonitrile :: 60 **:** 40

Column: Bondapak C_{18} 10µ (3.9×300 mm)

Flow rate: 1ml/min

Purge: 10ml/min

Run time: 6 minutes

UV detection: 287nm.

Extraction procedure of lung

The lungs were homogenized by micro crushing in 300µl of purified water until total dilaceration and 300µl of acetonitrile was then added. The mixture was homogenized by micro crushing for 20 minutes. After 10 minutes centrifugation (10000 RPM), 400µl of the supernatant was transferred into a centrifugation tube. Thereafter, 150µl of purified water and 50 µl of 0.5 M hydrochloric acid were added to all samples and the mixture was vortex mixed for 10 seconds. Then 1 ml of dichloromethane was added. The samples were vortex mixed for 10 seconds then the tubes were centrifuged (11000 RPM) for 10 minutes and kept at -20ºC for 30 minutes in a freezer. The aqueous solution was separated and 20 µl of this solution were injected into the chromatograph [10].

***In vivo* study of microspheres**

Twelve adult albino mice between 20 ±2 gm were selected at random and were divided into two groups with six animals in each group to find out the targeting efficacy of ofloxacin in lung tissue. One group was administrated 0.5mg/kg Ofloxacin injection via the tail vein, while the other group received equivalent amount of microspheres (OCM) dispersed in saline through the same route of administration. After 20 minutes of injection, animals were anesthetized and sacrificed [11]. The amount of Ofloxacin in lung tissue was determined by HPLC method [10, 12].

Results and Discussion:

In the morphological studies the microspheres were observed to be mostly discrete particles and spherical in shape (fig-1). The data showing the effect of drug polymer ratio on size of microspheres has been presented in Table 1.

Table 1 – Percent Entrapment and Particle size range of Chitosan Microspheres (OCM)

Batch No	Drug Polymer Ratio	% Entrapment ± SEM (n = 3)	Particle Size Range (µm)					
			0-5	5-10	10-15	15-20	20-25	25-30
OCM 1	1:3	29.6 ±0.004	40	51	63	46	-	-
OCM 2	1:5	39.1 ±0.003	19	91	71	7	6	6
OCM 3	1:10	40.4 ±0.011	30	41	48	72	9	-

It was observed that at drug-polymer ratio 1:5 (OCM 2), maximum particles were in the range of 5-10 µm and this size range was optimum for lung tissue targeting [6]. Scanning electron microscopy (SEM) showed spherical particles with smooth surfaces, which may be attributed to the cross-linking of polymer in stabilized emulsion droplets and hence spherical microspheres

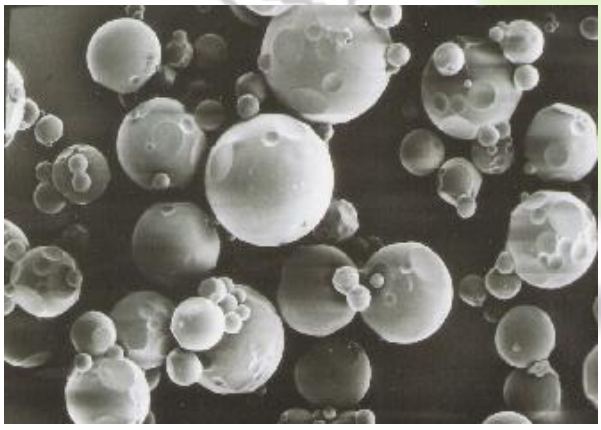
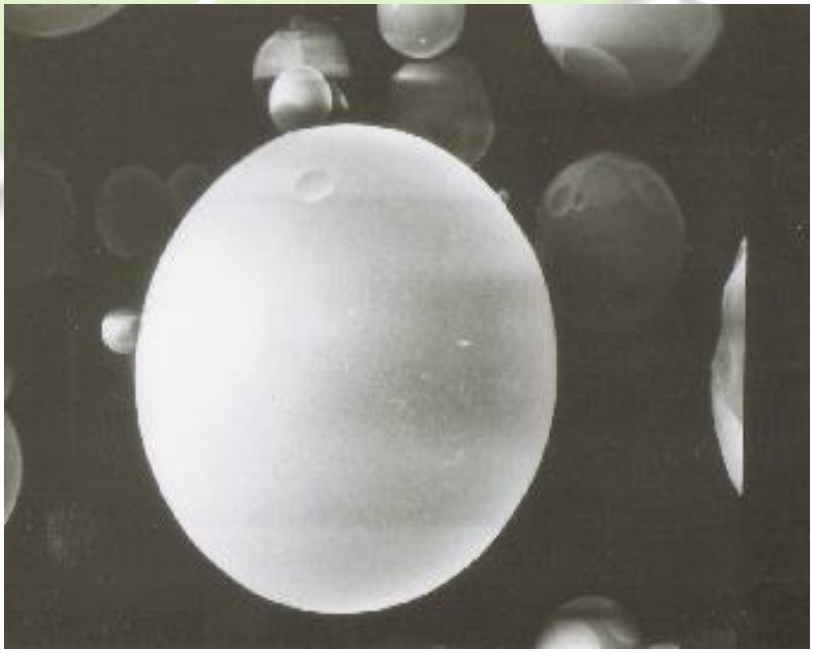


Figure :1 Scanning Electron Microscopy photographs of ofloxacin chitosan microspheres (OCM 2).

(Figure 1). The average drug entrapment efficiency was observed to be around 40 % (Table 1). The particle size distribution is also an important factor since it controls the tissue localization of microspheres after their intra-venous administration. Previous reports had also pointed out that the microspheres with size range 5-15µm have a notable lung targeting [5].

Infra-red spectroscopy

The spectra of albumin and chitosan microspheres showed that there was no change in peaks of drug during preparation of microspheres. The result obtained is depicted in Figure 2 and Figure 3 and in Table 2 and Table 3.



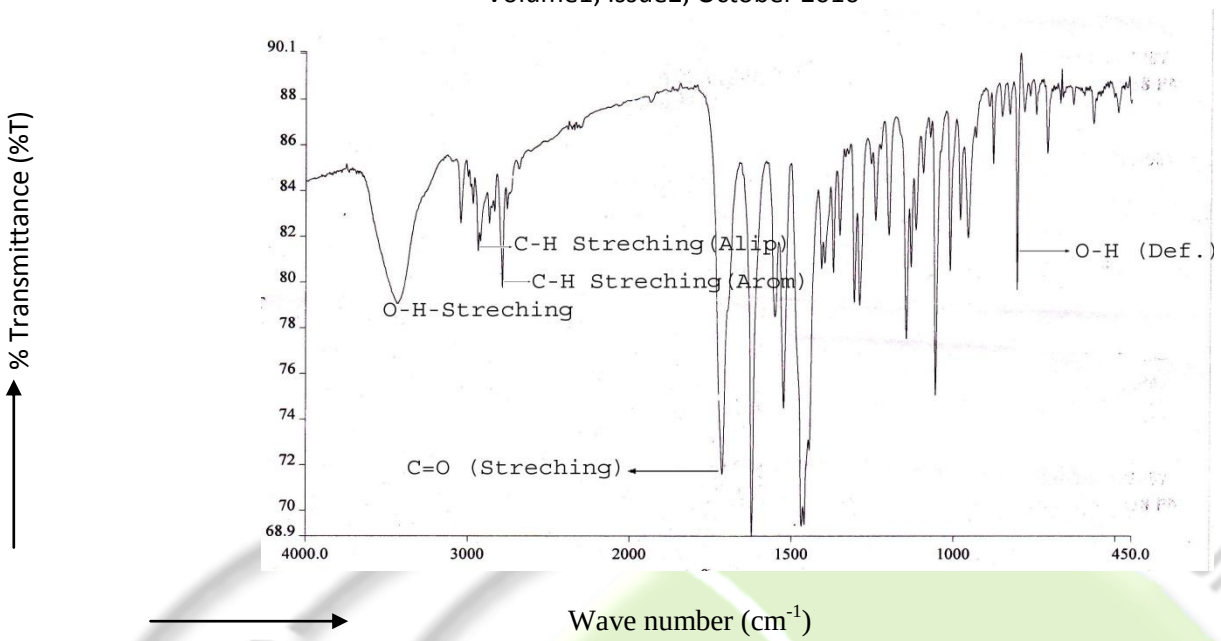


Figure: 2 I. R. Spectra of ofloxacin

Table: 2 Functional groups of Ofloxacin

Wave number	**Remarks**
3300	O-H stretching
3000	C-H stretching(aliphatic)
2800	C-H streching(aromatic)
1750	C=O stretching
710	O-H deflection

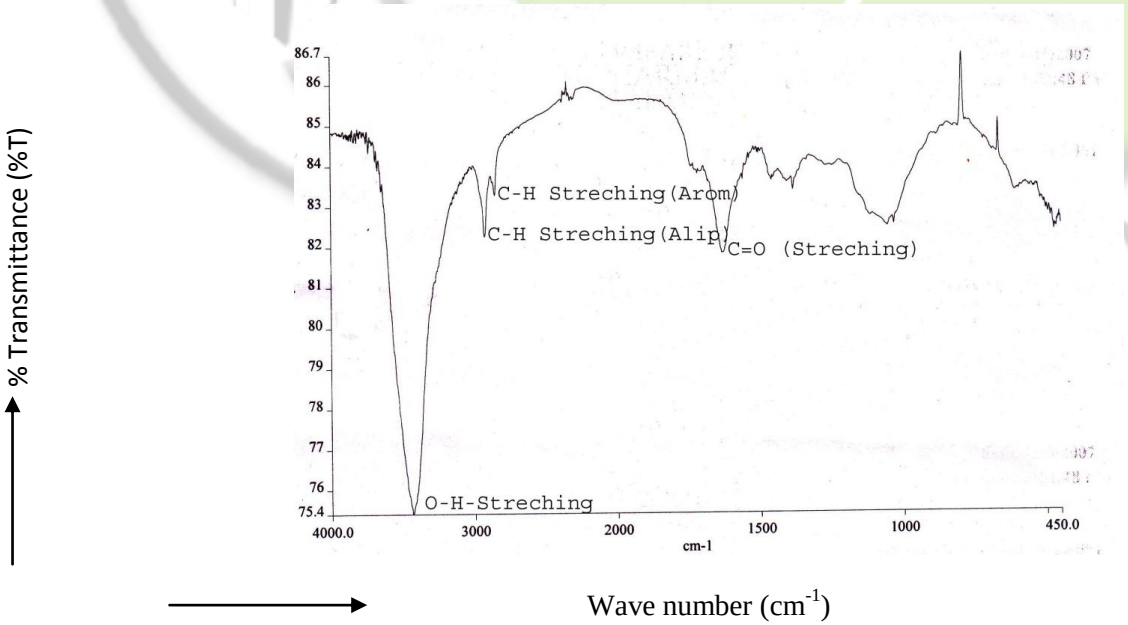


Figure: 3 I. R. Spectra of chitosan microsphere of ofloxacin (OCM2)

Table: 3 Functional groups of Chitosan microspheres (OCM2)

Wave number	Remarks
3400	O-H stretching
2800	C-H stretching(aliphatic)
2750	C-H streching(aromatic)
1750	C=O stretching

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) studies revealed that there were changes in crystalline form of Ofloxacin, which changed into amorphous form although no change was observed with polymer during preparation of microspheres and the peaks are shown in Figure 4 and Figure 5.

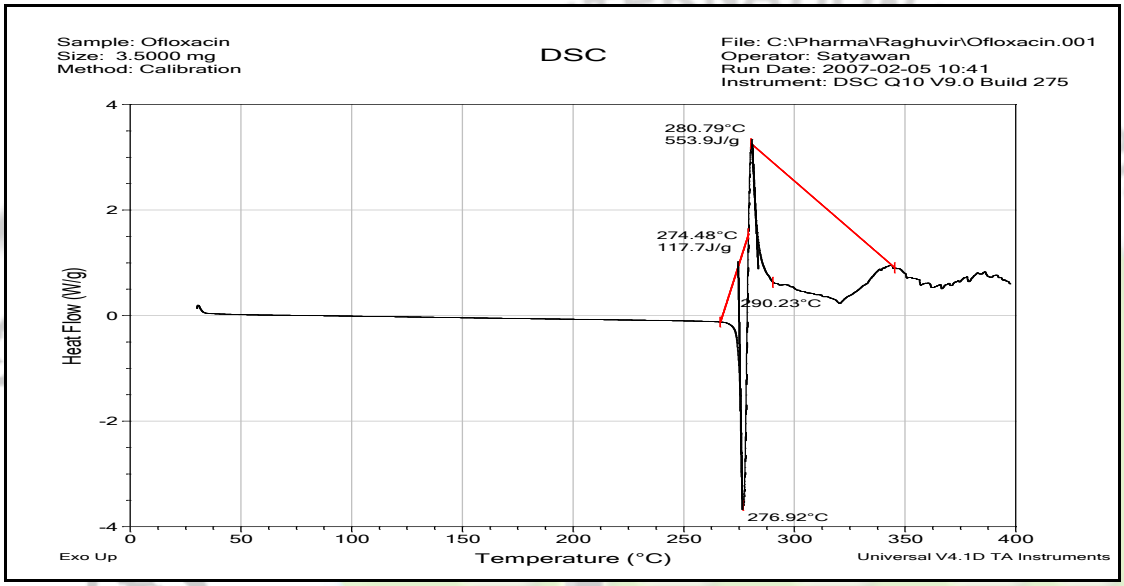


Figure: 4 DSC of Ofloxacin

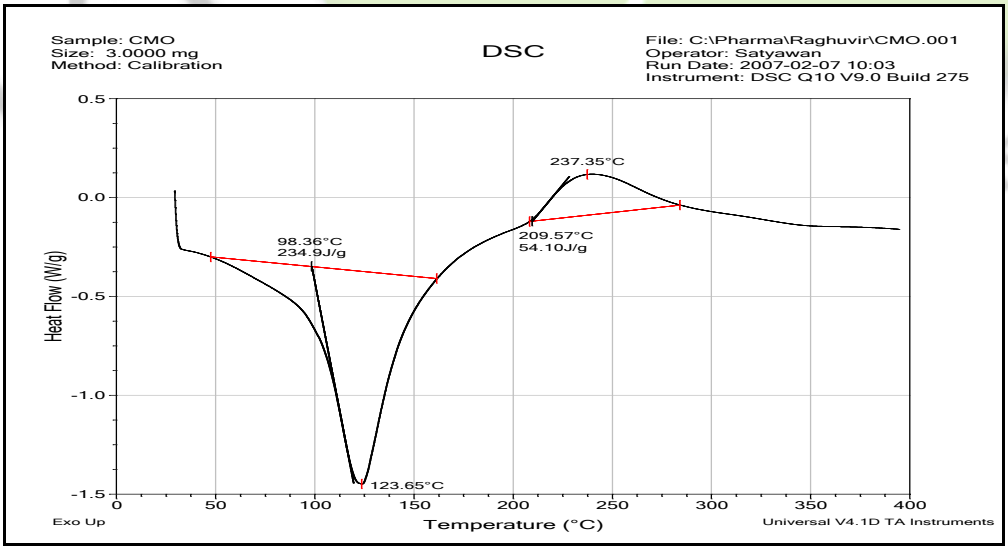


Figure: 5 DSC of chitosan microspheres with Ofloxacin (CMO)

The *in vitro* release study helps us to understand the behavior of these systems in terms of drug release. The pattern of drug release from OCM was observed to be in bi-phasic manner characterized by a burst effect followed by a slow release. The drug release profile of OCM was 88% at 6 hours.

Table 4 shows *in vitro* release for ofloxacin and OCM respectively. About 60% of the total ofloxacin in OCM released in first one hour, which reflected the significant amount of ofloxacin absorbed on or incorporated near the surface of microspheres. In clinical practice this would lead to 'burst effect' which enables the preparation to show fast effect to the patients. However ofloxacin release from chitosan microspheres was completed in 6 hours. During the same period, the released amount was 88% respectively. In comparison with OCM composite, the ofloxacin injection releases the ofloxacin very fast. In approximately 0.5 hour, 89% of ofloxacin has been released. The result indicated that the OCM had a well-controlled release efficacy.

Table: 4 *In vitro* drug release from Ofloxacin microspheres (CMO) in phosphate buffer pH 7.4

Time (hrs)	CMO 1	CMO 2	CMO 3
	% Release ±SEM (n=3)	% Release ±SEM (n=3)	% Release ±SEM (n=3)
0	0	0.0	0.0
0.5	45.02 ±0.03	31.58 ±0.12	25.12 ±0.02
1	60.90 ±0.17	61.37 ±0.10	55.13 ±0.07
2	75 .89±0.21	76.76 ±0.35	82.22 ±0.06
3	78.76 ±0.06	83.85 ±0.40	86.33 ±0.01
4	83.67 ±0.04	90.04 ±0.49	90.76 ±0.16
5	85.55 ±0.04	92.73 ±0.75	93.67 ±0.30
6	88.44 ±0.03	97.01 ±0.57	95.83 ±0.13
24	90.91 ±0.01	98.42 ±0.10	97.72 ±0.12

The drug concentration of lung tissues was determined by HPLC method. The result showed that following I.V. administration of microspheres (OCM) equivalent to 10μg of drug, the concentration of drug in lungs tissues was observed to be 5.715 μg/ml (Table 5). Whereas, negligible drug reached to lung tissues when ofloxacin suspension was injected I.V. Thus it may be concluded that targeted delivery of ofloxacin could be achieved with size specific microspheres.

Table: 5 Concentration of drug in lung after administration of microspheres

Experimental animal no.	Samples	% area	Concentration (µg/ml)	Correction factor	Corrected concentration in lung (µg/ml)
1	Chitosan microspheres (CMO 2)	805680	2.15	0.167	6.4
2	Chitosan microspheres (CMO 2)	725483	2.03	0.167	6.10
3	Chitosan microspheres (CMO 2)	485477	1.69	0.167	5.05
4	Chitosan microspheres (CMO 2)	733006	2.04	0.167	6.10
5	Chitosan microspheres (CMO 2)	651572	1.92	0.167	3.52
6	Chitosan microspheres (CMO 2)	972243	2.39	0.167	7.12
					Mean = 5.715 µg/ml

Conclusion

In the present work, microspheres of Ofloxacin were prepared by emulsion polymerization technique and evaluated for their suitability for pulmonary drug delivery. The microspheres were prepared with chitosan and evaluated for particle size, shape and entrapment efficiency. The microspheres were found to be spherical having size of the microspheres in the range of 10-15 µm and maximum entrapment efficiency of 40.4 ±0.011 was shown by chitosan microspheres (CMO3). The in-vitro drug release studies were carried out for 24 hrs in phosphate buffer pH 7.4. The maximum drug release(98.42 ±0.10% in 24 hrs) was shown by CMO2. DSC and IR study showed no chemical interaction as well as polymorphic changes among the various components of Ofloxacin microspheres. In vivo studies were carried out in Swiss Albino Mice using Ofloxacin microspheres of chitosan. CMO-2 batch was selected for analysis. The results showed that after intra-venous (I.V.) administration (20 minutes), the concentration of drug in lungs tissues was observed to be 5.715 µg/ml while negligible amount of Ofloxacin (pure drug) was observed in lung tissue after IV administration of Ofloxacin solution. Thus it may be concluded that chitosan micospheres could be utilized for the targeting of ofloxacin to lung tissues.

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