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Preparation and Evaluation of Chitosan Microspheres Containing Levofloxacin

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Abstract

Objectives: The objective of present study was to develop chitosan-based sustained release Levofloxacin microspheres to reduce the dosing frequency. ***Materials and Methods:*** The Levofloxacin -loaded microspheres were prepared by emulsification cross-linking method using glutaraldehyde as cross-linking agent. Accurately weighed quantity of Chitosan was dissolved in 1% (v/v) aqueous acetic acid. ***Results:*** The percentage yield of the emulsification cross-linking method was determined to be between 74 and 81.5 percent, and the spherical microspheres had particle sizes ranging from 2 m to 200 m. According to the in vitro dissolution analysis of the improved formulation (F2) (table 7.8), when the medication was enclosed in Chitosan microspheres, 95 percent of the formulation was released after 12 hours, demonstrating that the drug is released from the formulation in a controlled way. ***Conclusions:*** The percentage of entrapment effectiveness, particle size, and percentage of drug release were significantly impacted by the drug: polymer ratio and GA volume. According to research using scanning electron microscopy (SEM), microspheres were round and had a smooth surface. Nicorandil-loaded chitosan microsphere formulations released their drugs via fickian diffusion.

Keywords: Chitosan, emulsion crosslinking method, glutaraldehyde, microspheres, levofloxacin.

INTRODUCTION

A third-generation fluoroquinolone antibiotic with a broad spectrum of activity, levofloxacin is used to treat bacterial infections. The World Health Organization's list of essential medications includes the safe and efficient medication levofloxacin. It was first patented in 1987 and later granted FDA certification for medical use in the US in 1996 [1]. Levofloxacin is FDA approved for the treatment of nosocomial pneumonia, community-acquired pneumonia, acute bacterial rhinosinusitis, acute bacterial exacerbation of chronic bronchitis, prostatitis, acute pyelonephritis, urinary tract infection (uncomplicated or complicated), skin or skin structure infections, prophylaxis and treatment of plague due to Yersinia pestis, and to reduce the incidence of disease progression of inhalational anthrax. Levofloxacin is only used in patients with acute exacerbations of chronic bronchitis, acute bacterial sinusitis, and uncomplicated urinary tract infections who have no other treatment options due to the increased risk of severe side effects (such as tendinitis and tendon rupture, peripheral neuropathy, and CNS effects). Levofloxacin for ophthalmology is also used to treat bacterial conjunctivitis [2]. Numerous polymers have been used to make microspheres, which have then been evaluated for a variety of functions. Microspheres have been thoroughly investigated for their potential utility in the field of medication delivery. One of the many unit dose types is microspheres. Due to the constant maintenance of plasma levels, the total dose and minimal side effects may eventually be decreased. In the area of innovative drug delivery, microspheres are prospective drug delivery carrier systems that are made from a variety of polymers [3-5]. The biological and medicinal uses of chitosan, a deacetylated derivative of (4)2-acetamido-2-deoxy-b-d-glucose or chitin, have been thoroughly investigated. It is appropriate for application in medication delivery and the biomedical field thanks to qualities including biodegradability, low toxicity, and strong biocompatibility. Chitosan has been researched as a medication carrier for the sustained delivery of numerous oral formulations and parenteral formulations [6]. By using emulsion crosslinking, ion-induced

coagulation, and spray-drying techniques, chitosan microspheres have been created. The emulsion chemical crosslinking approach is the one that produces chitosan microspheres most frequently [7]. There are various studies on the synthesis of microspheres using glutaraldehyde (GA) as a crosslinking agent [8-9]. Emulsification is followed by crosslinking with a suitable crosslinking agent in the chemical crosslinking process for making chitosan microspheres (e.g., GA). The purpose of this study was to prepare levofloxacin-filled chitosan microspheres using an emulsion chemical crosslinking technique in order to achieve a controlled drug release profile and to investigate the impact of various formulation factors, such as the drug to polymer ratio and GA, on particle size, encapsulation effectiveness, and in vitro release behaviour.

MATERIALS AND METHODS

Levofloxacin was obtained as a gift sample from Macleods Pharmaceuticals, Mumbai. Chitosan GA was procured from Rankem lab Limited.

Drug Excipient Interaction Study

The drug- excipient interaction study was carried out by FT-IR.

Preparation of Microsphere

Glutaraldehyde was used as a cross-linking agent during the emulsification cross-linking process to create the microspheres. A precisely weighed quantity of chitosan was dissolved in aqueous acetic acid at a concentration of 1 percent (v/v). Levofloxacin was added and well mixed with the polymer solution. A water in oil (w/o) emulsion was created by adding the dispersed phase drop-wise through a disposable syringe (10 ml) to the continuous phase made up of light liquid paraffin and heavy liquid paraffin in a 1:1 ratio with varying volumes of the surfactant (Span 80). Using a stirrer with a three-blade propeller, the stirring was continued at various speeds. A determined amount of aqueous glutaraldehyde (25 percent v/v) was added drop by drop after 20 minutes of stirring. Stirring continued for an additional hour after the final addition of glutaraldehyde. Microspheres that were left over after centrifuging the preparation at 3000 rpm were cleaned four times with petroleum ether. Following the last wash, microspheres were gathered, allowed to air dry at room temperature, and then stored.

Table 1: Composition of Microspheres

Sl No.	Code	Levofloxacin (mg)	Chitosan (mg)	Span80 (% v/v)	Gluter- Aldehyde(ml)
1	F_1	100	250	0.5	0.5
2	F_2	100	200	1.0	0.5
3	F_3	100	300	1.0	1.0
4	F_4	100	345	1.5	0.5
5	F_5	100	400	1.0	1.5

EVALUATION OF MICROSPHERES [10-11]

% Yield

Weighing was done on the prepared microsphere formulations F1 to F5. The measured weight was divided by the sum of all the non-volatile ingredients that went towards making the microspheres.

Drug Entrapment Efficacy

To extract the medication from the microspheres, the weighed amounts of microspheres were crushed and suspended in ethanol. After 24 hours, the filtrate's drug content was measured spectrophotometrically at 292 nm using ethanol as a blank. Using a regression equation constructed from the standard graph, drug concentrations in the samples were determined from the calibration plot.

In-Vitro Drug Release Study

Using a USP dissolution apparatus basket type and phosphate buffer solution (pH 7.4) as the dissolve fluid (900 ml), *in-vitro* drug release research was conducted at 37 0.5 °C and 100 rpm. Microsphere samples that were precisely weighed were introduced to the dissolution media. In the dissolution medium, 5 ml of samples were taken out at intervals of 0–30 min, 60–90 min, 120–150 min, 12–18 hr, and 24 hr. To keep the sink condition, an equal volume of new dissolving fluid was added to the volume every time. By employing a standard curve equation and a UV visible spectrophotometer at 292 nm, these samples were spectrophotometrically examined.

Particle Size Distribution

An optical microscope was used to measure the size of the microspheres. Dry microspheres (10 mg) were manually shaken for 5 min in phosphate buffer saline (pH 7.4) in a test tube. A drop of suspension was deposited on a glass slide, and microspheres were counted at a 100X magnification using a calibrated ocular micrometer.

RESULT & DISCUSSION

Levofloxacin, a fluoroquinolone of the second generation with significant activity against Streptococcus pneumoniae and other gramme positive bacteria, was chosen as the study medication. Fluoroquinolones are the most effective against Mycobacterium TB. By utilising glutaraldehyde as a cross-linking agent during the emulsification cross-linking process, the microspheres were created. Chitosan is a co-polymer of glucosamine and N-

acetylglucosamine and is a naturally occurring polysaccharide. It is a biodegradable, biocompatible, and non-toxic polymer. Due to its inexpensive cost and simple availability, liquid paraffin is employed as an external phase. As an emulsifier, span 80 is employed. It is a surfactant that dissolves in oil and is used to prepare emulsions when the external phase is an oil. Since polymers are insoluble in petroleum ether but liquid paraffin is, it is used to wash microspheres. The resulting microspheres had a very distinct character. Levofloxacin microspheres that had been manufactured were tested on a number of variables; the results are reported below.

The percent yield of microspheres prepared by emulsification cross-linking method were found to be between 74 to 81.5 % as shown in table 2. The entrapment efficiency of all formulations is reported in table 3. Formulation (F_5) having least entrapment efficiency whereas formulation (F_2) showed high entrapment efficiency. This difference occurs due to variation in drug and polymer ratio. The effect of cross-linking agent was also studied and it was found that on increasing amount of cross-linking agent, drug entrapment efficiency was decreased. Finally, formulation F_2 was selected as optimized formulation as it shows highest drug (Levofloxacin) entrapment efficiency among all prepared formulation. *In vitro* drug releases of formulation F_2 was carried out using USP dissolution apparatus type II. The *in vitro* drug release data of F_2 are tabulated in table 4. From the *in vitro* dissolution study of optimized formulation (F_2) (table 4), shows when drug encapsulated in Chitosan microspheres, 95% of drug was released from formulation in 12 hrs. indicating that the drug releases in controlled manner from optimized formulation. The result obtained from *in vitro* drug release study were plotted adopting mathematical model Cumulative Drug Release Vs Time. The result showed that release of F2 followed zero order kinetic (fig 1). The particle size distribution was measured using an optical microscope. The particle size of microspheres ranges from 2 µm to 200 µm, which were spherical in shape. It was observed that when stirring speed was low, larger spherical microspheres were formed(fig.2). The infrared spectra showed stable character of Levofloxacin with polymer and other excipients, and revealed the absence of drug excipient interaction (fig.3-4 & table 5). This occurred due to inadequate stirring speed which was not able to break emulsion droplets. Increasing the surfactant concentration, the mean particle size had diminished. Increasing the amount of cross-linking agent caused a slight increase in size, because of adherence of excess cross-linking agent on surface of microspheres.

Table 2: % Practical Yield of All Formulation

Sl No.	Formulation	Theoretical wt.(mg)	Practical yield(mg)	%Practical yield
1	F1	350	267	76.4
2	F2	300	244	81.5
3	F3	400	296	74.1
4	F4	445	354	79.4
5	F5	500	383	77.1

Table 3: % Drug Entrapment

Sl No.	Formulation Code	Levofloxacin (mg)	Chitosan (mg)	% Drug Entrapment
1	F_1	100	250	92.2
2	F_2	100	200	93.6
3	F_3	100	300	86.2
4	F_4	100	345	90.1

Table 4: *In vitro* drug release study of formulation F_2

S.No.	Time (hrs)	Drug Release (%)
1.	0	0.00
2.	0.5	1.32
3.	1	3.11
4.	1.5	6.22
5.	2	7.34
6.	2.5	8.51
7.	3	9.55
8.	4	11.22
9.	6	16.87
10.	8	24.64
11.	18	56.79

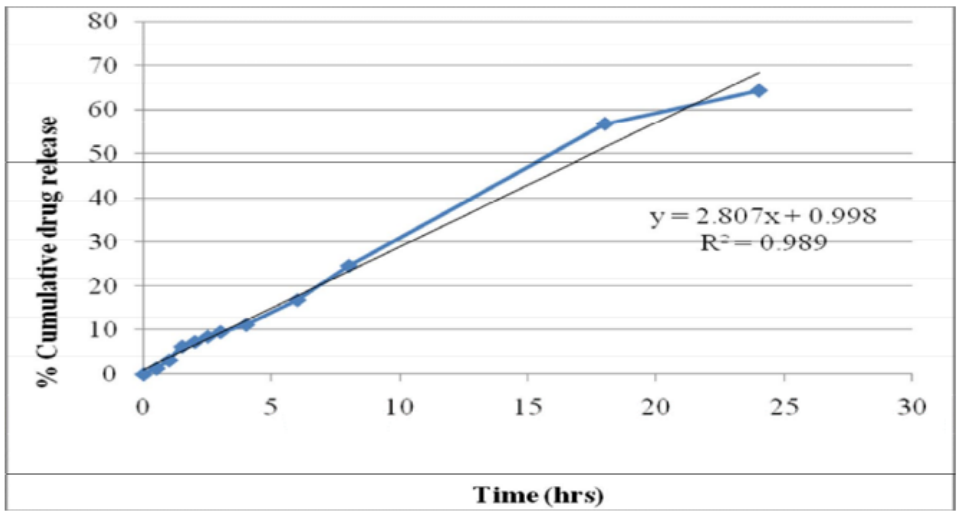

Fig 1: Zero Order Drug Release of F2

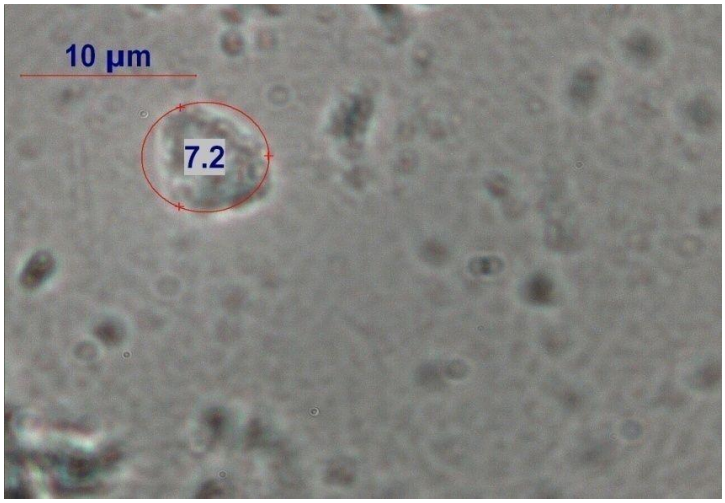

Fig 2: Particle Size Distribution

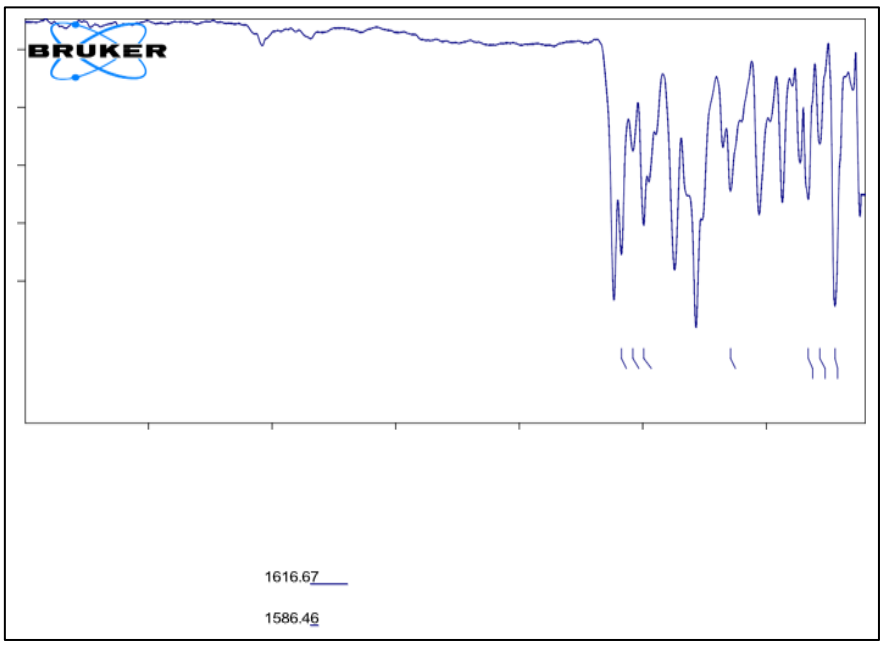

Fig 3: FT-IR (Drug + Excipient)

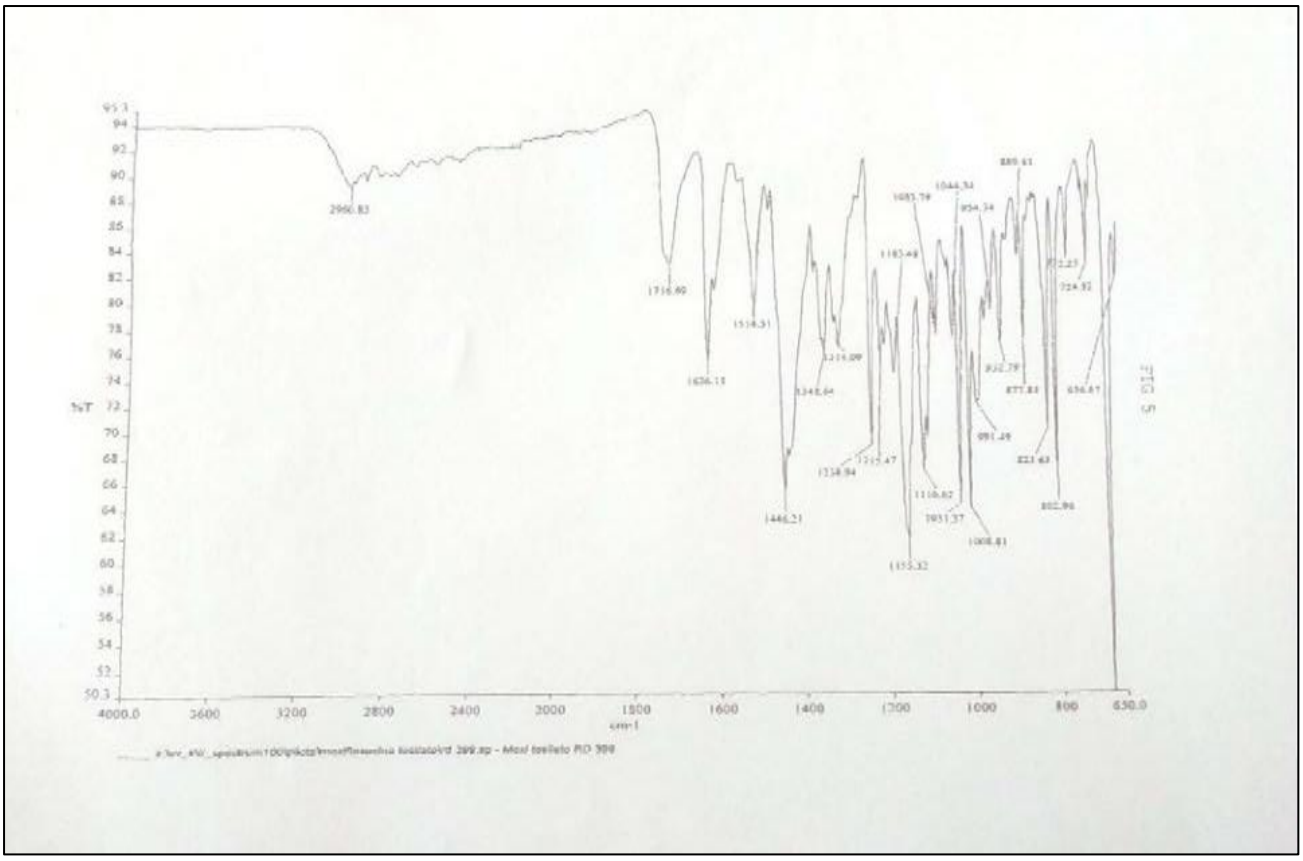

Fig 4: FT-IR Standard Drug

Table 5: IR interpretation of Levofloxacin

Sl No.	Functional Group	Standard (cm^{-1})	Observation (cm^{-1})
1.	COOH group	3265	3265.19
2.	CH_3group	2930	2931
3.	C=O group	1723	1724
4.	C=N group	1292	1293.1
5.	F(halogen) group	1084	1084.92

CONCLUSION

The investigation of optimum formulation showed controlled drug release and could, therefore produce some benefits such as reduction in total dose, frequency of administration and dose related systemic side effects.

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