ORIGINAL ARTICLE

Formulation and *In Vitro* Evaluation of Mupirocin-loaded Alginate Microspheres

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Abstract

Introduction: Mupirocin (pseudomonic acid A) is a topical antibiotic used for the treatment of impetigo a superficial infection of the skin. **Aim:** The goal of this study was to evaluate the technological feasibility of delivering antibiotic using alginate microspheres. **Materials and Methods:** Microspheres were prepared by emulsification method using sodium alginate as a polymer and calcium chloride ($CaCl_2$) as a cross-linking agent. In this investigation, a novel method was used to investigate the joint influence concentration of alginate and $CaCl_2$ in a blend of liquid paraffin in the dispersion medium. Potential variables such as the concentration of drug and sodium alginate: Drug ratios were kept constant in the experimental design. Different concentrations of the cross-linking agent and different time periods of the cross-linking process were used to change the extent of cross-linking. The cross-linked microspheres were characterized by evaluating the extent of cross-linking, the morphology, and drug loading and *in vitro* studies of drug release. **Results and Discussion:** The particle size of the drug loaded microspheres was 75 µm, and they have narrow size distribution. Total drug loading was determined to be 15.10 ± 0.01. Preparation process gave a yield production of nearly 80% for the prepared batches. The microspheres released 71.3% of mupirocin at 72 h. **Conclusion:** The study revealed that for obtaining controlled delivery of mupirocin; the microspheres should be prepared using relatively an optimum concentration of sodium alginate and $CaCl_2$ and in the dispersion medium.

Key words: Calcium chloride cross linking agent, microspheres, mupirocin, sodium alginate

INTRODUCTION

Mupirocin is a mixture of pseudomonic acids as a model drug was chosen due to its antibiotic activity as well as for the presence of hydroxyl groups. It is used topically and effective against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* used to treat *Staphylococcus* and *Streptococcus* skin infections such as impetigo.[1] Mupirocin exerts a bacteriostatic effect at the minimal inhibitory concentration (MIC) with bactericidal concentrations usually 8-32 times greater than the MIC. Mupirocin acts by different mechanisms than other available antibiotics because it reversibly binds to bacterial isoleucyl transfer-RNA synthetase.[2,3] Wound healing is imparted in infected tissues, and the clinical effectiveness of mupirocin is undoubtedly the result of its antibacterial action.[4,5] Sodium alginate has been used in the management of hemostatic agent in surgical dressings. The cross-linking of alginate is a function of both the alginate composition and the length of the molecule. The affinity of the crosslinking cation for the alginate is also of great importance in the crosslinking reaction. The preparation of alginate particles described here is based on the cross-linking properties of calcium ion is believed to interact with five different oxygen atoms of two adjacent guluronate units in intra-chain bindings.[6,7] Microspheres are known to swell in aqueous environments due to hydration. As a new polymeric structure is formed by introducing bridges between polymeric chains during the cross-linking procedure, the extent of the swelling process depends on the degree of cross-linking.

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Therefore, the denser the cross-linking bridges between the polymer molecules, the more packed is the structure.[8-10] The concept behind the current work is to use the microparticles with different concentrations of alginate which could give good support to proliferate the cells and could control the delivery of the drug in a controlled manner.

MATERIALS AND METHODS

Mupirocin was received as a gift sample from Fourrts India Laboratories Pvt. Ltd. Sodium Alginate (Loba Chemie Pvt. Ltd., Bombay), calcium chloride ($CaCl_2$) (RFCL. Ranbaxy, Mumbai), and all other chemicals used are of analytical grade. The viscosity of aqueous sodium alginate solution (2% w/v) was found to be 3500 cPs. The main objective of this present work is to prepare mupirocin loaded sodium alginate microspheres and to investigate for surface morphology, particle size, drug loading, and release characteristics.

Preparation of mupirocin loaded sodium alginate microspheres[11-13]

The mupirocin loaded sodium alginate microspheres were prepared by emulsification method followed by cross-linking with $CaCl_2$. Mupirocin was dispersed in aqueous solution of sodium alginate (2% w/v) and homogenized uniformly for 1 h under mechanical stirrer at 2000 rpm. The homogeneous aqueous solution was dropped into liquid paraffin oil (light and heavy [1:1 ratio]) which is previously added with 2% (v/v) Span 80 and continued stirring for 2 h at 2000 rpm. The formed emulsion was cross-linked with 5 ml of 0.2 M $CaCl_2$ for stabilization of microspheres. The solidified microspheres were recovered by centrifugation, washed with petroleum ether and air-dried at room temperature [Table 1].

Table 1: Formulation of mupirocin microspheres

Contents	Quantity			
	MM I	MM II	MM III	MM IV
Mupirocin (mg)	500	500	500	500
Sodium alginate (g)	2	3	4	5
Heavy liquid paraffin (ml)	250	250	250	250
Light liquid paraffin (ml)	250	250	250	250
Span 80 (ml)	2	2	2	2
Calcium chloride (ml)	25	25	25	25
Petroleum ether	Q.S	Q.S	Q.S	Q.S
Water	Q.S	Q.S	Q.S	Q.S

In vitro evaluation studies

Surface morphology[14]

The prepared microspheres were observed both by using confocal laser microscopy and scanning electron microscopy (SEM) to study surface morphology. The external morphology of microspheres was visualized using SEM (SEM, Hitachi, Japan). The sample for the SEM analysis was prepared by sprinkling the microspheres one side of the double adhesive stubs. The stub was then coated with gold using ion sputter coater. The microspheres were viewed at an accelerating voltage of 0.3-30 kV and identified by using SE, BSE detectors.

Particle size determination[15]

The size analysis of microparticles was performed by laser diffraction using a Microtrac S3500, USA particle size analyzer. The dried microsphere was dispersed in Isopropyl alcohol and vortexed for 10 s. The particulate dispersion obtained was added to the sample dispersion unit containing stirrer and stirred in order to minimize the interparticle interactions; the laser obscuration range was maintained between 10% and 20%. The instrument was set to measure the samples 3 times at a rate of 50 counts per second.

Percentage yield of microspheres[16]

The percentage yield of microspheres was calculated by using the following formula:

$$\% \text{ Yield of microspheres} = \frac{\text{Practical weight}}{\text{Theoretical weight}} \times 100$$

Drug loading efficiency[17]

Drug-loaded microspheres equivalent to 10 mg of mupirocin were digested with 50 ml of pH 7.4 phosphate-buffered saline (PBS) at room temperature for 12 h. The solution was filtered and analyzed at 228 nm using UV spectroscopy to determine the amount of mupirocin present in the microspheres. The drug loading in microspheres was estimated by using the formula:

$$L = Q_m / W_m \times 100$$

Where L is the percentage loading of microspheres, Q_m the quantity of mupirocin present in W_m g of microspheres.

Release study[18]

The drug release rates from the microspheres were studied in 100 ml of pH 7.4 PBS as dissolution medium for 72 h using magnetic stirrer under sink conditions. Accurately weighed quantity of mupirocin microspheres equivalent to 10 mg of mupirocin and added to dissolution medium and kept at 37°C ± 5°C. At present time intervals aliquots were drawn and replaced by an equal volume of dissolution medium to maintain a constant volume. After suitable dilutions, the samples absorbance was analyzed spectrophotometrically at 228 nm and compared with the stock solution. The cumulative percentage drug release was calculated and shown in the Table 2.

Table 2: Cumulative percentage drug release data

Time (h)	Drug release (%)			
	MM IV	MM II	MM III	MM IV
1	18.46±0.41	16.2±0.2	13.2±0.2	10.36±0.20
2	26.96±0.55	22.36±0.35	17.36±0.35	14.26±0.30
4	38.36±0.40	31.33±0.41	28.33±0.41	22.30±0.2
6	48.9±0.45	45.53±0.25	40.53±0.25	29.73±0.30
12	62.33±0.25	51.13±0.15	54.13±0.15	37.43±0.35
24	82.43±0.35	68.3±0.17	70.3±0.17	50.46±0.31
48	88.2±0.2	76.6±0.2	77.6±0.2	63.5±0.3
72	97±0.34	89.4±0.2	84.4±0.2	71.3±0.2

Table 3: Percentage yield and DLE data

Formulations	Yield %	DLE %
MM I	94.66±0.47	10.51±0.12
MM II	98.31±0.08	8.67±0.25
MM III	97.45±0.13	7.46±0.04
MM IV	98.03±0.15	15.10±0.01

DLE: Drug loading efficiency

RESULTS AND DISCUSSION

Physicochemical characterization of mupirocin microspheres

The microspheres prepared by emulsification method were spherical with a regular shape. The size range is 74-100 µm in all cases. The photographs [Figures 1 and 2] of microspheres were taken by confocal microscopy and SEM. In this case, the $CaCl_2$ has reacted with the sodium alginate before the formation of the spheres. Uniform particle size independent of the $CaCl_2$ concentrations can be explained in this way. The procedure followed to prepare the mupirocin microspheres produced a good yield of microspheres. As shown in Table 3, this indicated a low loss of microspheres during preparation and recovery. The estimation of percentage drug loading was done by UV partially as given in Table 3. The formulated microspheres were with free flowing yellow colored powder in nature. All the drug loaded microspheres are cross-linked with suitable reagents to stabilize the formulation. The stirring speed and polymer/drug ratio were optimized by observing the particle size under a microscope. A higher percentage of drug loading was observed in MM IV due its better physical entrapment within the polymer at higher concentration and the results were tabulated in Table 3.

All the formulations showed a burst effect in the release study of the first hour attributed by diffusion of dissolved drug. From this point of view, drug particles could have migrated at the surface during preparation and drying of microspheres. The drug release in next hours followed a

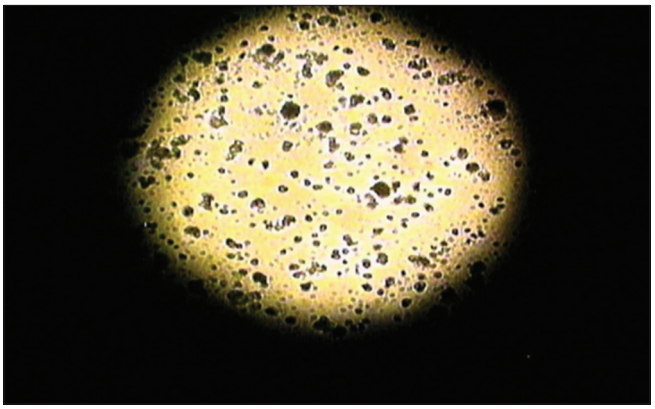

Figure 1: Confocal laser microscopy view

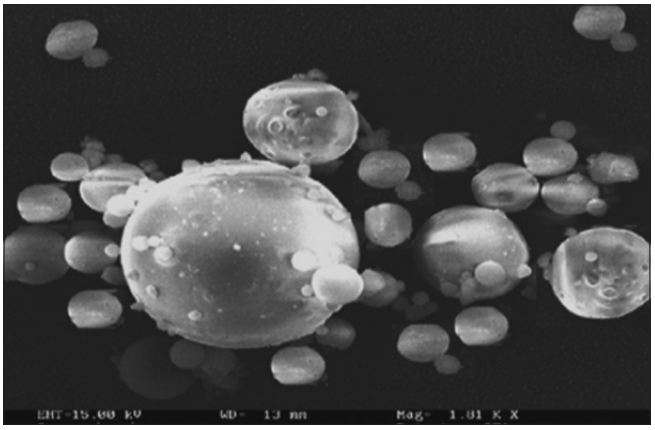

Figure 2: Scanning electron microscopy view

line with slight slope was observed. By observing these values at this condition, the kinetics might be predictive for release in topical applications of mupirocin-alginate particles for wound healing. In 72 h, almost 97% of the entrapped mupirocin was released from MM I microspheres but only 71% was released from MM IV microspheres. Hence, it was revealed that a gradual decrease in the rate and extent of release can be attributed to the increase in the density of the polymer matrix with increased polymer concentration.

CONCLUSION

The results from our study indicate that sodium alginate microspheres containing mupirocin were well suited for the controlled release of mupirocin and were promising for the antibiotic activity. If one desire to achieve an immediate release of a lipophilic drug from a lipophilic polymer, our findings suggest that the addition of a hydrophilic salt could be used to manipulate the release profile. This minimizes the restrictions on the choice of polymer for drug delivery; for instance, a burst release of a drug can be obtained from both hydrophilic and lipophilic polymers. This could alter the kinetics, allowing drugs like mupirocin to be eluted in a linear, rather than logarithmic profile.

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