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RESEARCH ARTICLE
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PREPARATION OF EVALUATION OF LIPID BASED NANO CARRIERS FOR DIABETIC DRUG

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Abstract:
Wound healing is a complex process crucial for tissue restoration and function recovery. In this study, Mupirocin-loaded liposomal hydrogels, incorporating oxidized alginate and gelatin, were developed and evaluated for wound healing applications. Liposomal hydrogels combine the advantages of liposomes, such as targeted drug delivery and stability, with hydrogels' moisture retention and ease of application on wounds.
Using a 32 full factorial design, the concentrations of oxidized alginate and gelatin were varied, and critical parameters were studied, including gelling time, swelling index, water vapor transmission rate (WVTR), and in vitro drug release. Fourier-transform infrared (FT-IR) analysis confirmed the successful oxidation of sodium alginate. The optimized liposomal hydrogel displayed desirable properties, including a gelling time of 43 seconds, swelling index of 71.43%, and WVTR of 98.85 g/m2/h.
In vitro release studies demonstrated sustained drug release, and SEM and XRD studies provided insights into the hydrogel's structure and amorphous state of the drug. Stability studies confirmed the hydrogel's stability over time.

Keywords: wound healing, liposomal hydrogels, oxidized alginate, gelatin, drug delivery, in vitro release, SEM, XRD, stability, in vivo studies.

Introduction:
A wound is a type of injury in which skin is torn, cut, or punctured, that results in the loss of epithelial integrity accompanied by disruption of normal structure and function of the skin and its underlying tissues [1] .A wound can be described as a defect or a break in the skin, resulting from physical orthermal damage or as a result of the presence of an underlying medical or physiological condition. According to the Wound Healing Society, a wound is the result of disruption of normal anatomic structure and function of a tissue 2 . Wounds can be classified as acute or chronic wounds based on the nature of repair process. Acute wounds are usually tissue injuries that heal completely, with minimal scarring, within the expected time frame, usually 8-12 weeks.The primary causes of acute wounds are mechanical injuries due to external factors such as abrasions which are caused by frictional contact

between the skin and hard surfaces[3]. Mechanical injuries also include penetrating wounds caused by knives, gun shots and surgical wounds caused by surgical incisions. Liposomal hydrogels have advantage over other conventional formulation such as creams, ointments and gels[4]. They enhance the skin retention of drugs, a higher drug concentrations in the skin and at the same time slow down the systemic absorption of drugs. They also act as a drug depot and provide a sustained localized drug delivery and liposomal hydrogels deliver adequate amount of drugs for their therapeutic activity[5] .

Evaluation

Gelling time

The experiment involved the reaction of approximately 1 ml of oxidized alginate dissolved in 0.1M borax with 1 ml of gelatin solution in 15 ml glass vials with a diameter of 20mm. This reaction was carried out under magnetic stirring, utilizing a teflon-coated stir bar measuring 5mm in diameter and 10mm in length. The entire process was performed at a controlled temperature of 37°C. The gelling time, which indicates the moment the stir bar stopped moving, was accurately recorded. The concentrations of oxidized alginate and gelatin were systematically varied to study their effects on the gelling time [6].

Swelling index

The fluid uptake ability of hydrogels was determined by a conventional gravimetric method, hydrogels were partially hydrated, then equilibrated in air at room temperature, after which, each hydrogel formulation was immersed in distilled water at room temperature (37 ºC) and its increase in weight with time was noted. The water content (WC) was calculated[7]

$$\text{Equilibrium fluid content } (\%) = \frac{w_s - w_d}{ws} \times 100$$

Where, W_s and W_d represent the weight of swollen and dry sample, respectively.

Water vapour transmission rate (WVTR)

When the hydrogels have reached equilibrium fluid uptake, the hydrogel was removed from the distilled water and re-equilibrated in air at room temperature to constant weight. The water retention of the hydrogel was calculated[8].

$$\text{WVTR} = \left({}^{G}/_{t}\right) A$$

Where,

WVTR = water vapour transmission rate (g/m^2.h)

G = weight loss (g)

t = time (h)

A = test area (cm^2)

***In vitro* release studies**

The in vitro release studies were carried out using a modified Franz diffusion cell setup. A dialysis membrane with a molecular weight cutoff of 5000 (Hi Media) was positioned between the donor and receptor compartments. The liposomal hydrogel was placed in the donor compartment, while the receptor compartment was filled with 25 ml of phosphate buffer (pH 7.4), maintained at a constant temperature of 37±0.5°C with continuous stirring. At specific time intervals, samples were withdrawn from the receptor compartment through a side tube, and the medium was replenished with fresh PBS to maintain a constant volume. The amount of released drug was quantified using a UV-Visible Spectrophotometer (Shimadzu 1800, Japan)[9].

Viscosity
Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Inc, Middleboro) was used to determine the viscosity of different formulations at 25 ± 1.0°C. The Rheocalc V2.6. software was used for the calculations[10,11].

Spreadability
Spreadability was determined by wooden block and glass slide apparatus. A ground glass slide was fixed on the block and an excess formulation (2 g) was placed between two glass slides and 100 g weight was placed on the upper glass slide for 5 min to compress the formulation to uniform thickness. Weight (100 g) was added to the pan. The time in seconds required to separate the two slides was taken as a measure of spreadability[12].

$$S = \frac{m \times l}{t}$$

Where,
S = Spreadability
m = Weight tide to upper slide
l = Length of glass slide
t = Time taken to separate the slide completely from each other

Percentage drug content
A quantity of liposomal hydrogel (1 g) was carefully placed in a 50 ml volumetric flask, and the volume was adjusted to 50 ml using methanol. Subsequently, 5 ml of this prepared solution was diluted to 25 ml with methanol[13]. The drug content was then determined by measuring the solution's absorbance at 228 nm using a UV-Vis spectrophotometer.

pH
The pH of each liposomal hydrogel was measured using previously calibrated pH meter. The measurements were made in triplicate.

Scanning electron microscopy (SEM)
Scanning electron microscope (SEM) images were acquired using a Hitachi S3400 instrument located in Tokyo, Japan. The liposomal hydrogels were positioned on a glass disc, which was then carefully attached to a metallic stub. Subsequently, the samples underwent an evaporation process under a vacuum condition overnight. To enable SEM analysis, the samples were metallized in an argon atmosphere, ensuring a 10-nm gold palladium thickness [14,15].

Stability studies
The optimized formulation was packed in a screw capped bottle and studies were carried out for 12 months by keeping at:
- 25± 2 °C and 60 ± 5% RH
- 30 ± 2°C and 65 ± 5% RH

And for 6 months for accelerated storage condition at

- 40 ± 2°C and 75 ± 5% RH

Samples were withdrawn on 0, 3, 6 and 12 months for long term storage condition and 0, 3 and 6 months for accelerated storage condition and checked for changes in physical appearance and drug content[16].

RESULT AND DISCUSSION

FT-IR analysis

The FT-IR spectra (Figure 1) of the oxidized alginate displayed a prominent peak at 1631 cm-1, corresponding to the vibrational symmetric stretching of the carbonyl function of the aldehyde groups. This peak was not observed in the sodium alginate spectra, confirming the successful oxidation of sodium alginate. Additionally, the absence of the hydroxyl stretching vibration at 3250-3350 cm-1 indicated the conversion of the hydroxyl group to an aldehyde group, further validating the oxidation process.

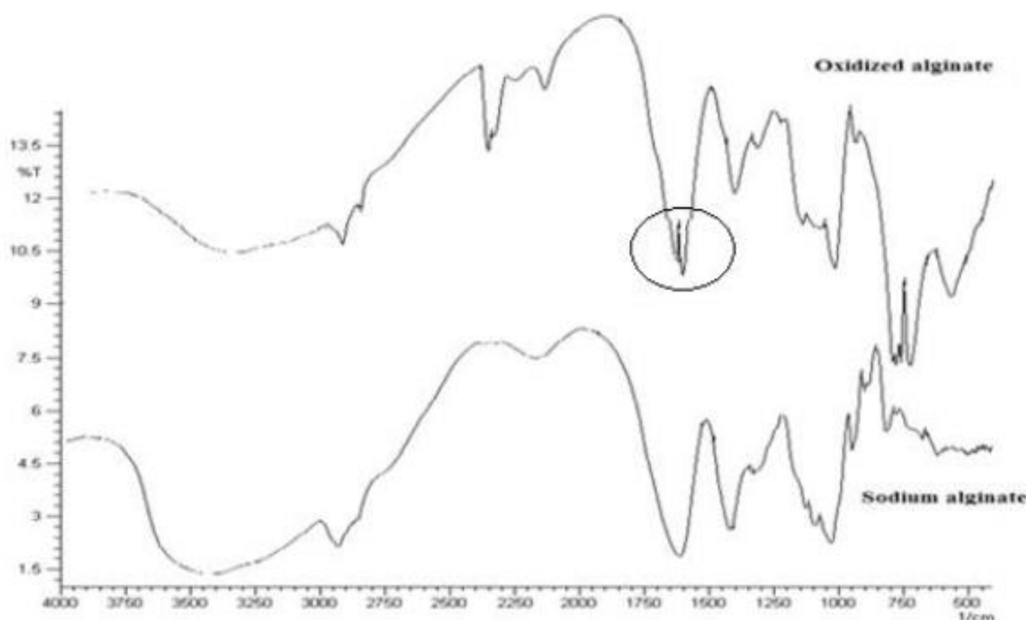

Figure 1. FT-IR spectra of sodium alginate and oxidized alginate

Preparation of Mupirocin liposomal hydrogels

The research study utilized a 32 randomized full factorial design to investigate the influence of independent variables, namely the amount of oxidized alginate (A) and gelatin (B), on various dependent variables such as gelling time (Y1), swelling index (Y2), water vapor transmission rate (Y3), and in-vitro drug release (Y4). The factors were examined at three different levels denoted as -1, 0, and +1, representing low, medium, and high values, respectively (refer to Table 1).

Table 1. Variables in 3^2 factorial designs of Mupirocin liposomal hydrogels.

Independent variable	Levels		
	Low(mg)	**Medium (mg)**	**High(mg)**
A: Oxidized alginate	10.00	20.00	30.00
B: Gelatin	05.00	10.00	15.00
Dependent variable			
Y1 Gelling time (sec)			
Y2 Swelling index (%)			
Y3 WVTR ($g/m^2/h$)			
Y4 *In vitro* drug release (%)			

Table 3. Matrix of 3^2 factorial designs for Mupirocin liposomal hydrogels

Run	Factor 1 *A: Oxidized alginate* *%*	Factor 2 *B: Gelatin* *%*
1	30.00	05.00
2	10.00	05.00
3	30.00	05.00
4	20.00	15.00
5	10.00	10.00
6	30.00	10.00
7	20.00	15.00
8	30.00	15.00
9	20.00	10.00

Experiment was designed for 9 runs (Table 2) varying oxidized alginate and gelatin compositions and analyzed for the responses.

Gelling time

The gelling time of the hydrogel was investigated using a 32 randomized full factorial design with independent variables, oxidized alginate (A) and gelatin (B), examined at three different levels (-1, 0, and +1) . The gelling time ranged from 31±1.52 to 44±1.0 seconds, and it was observed that an increase in the concentrations of oxidized alginate and gelatin resulted in a decrease in gelling time. This gelation phenomenon is attributed to the reaction between the aldehyde groups of oxidized alginate and the amino groups of gelatin, forming Schiff-base linkages. The periodate oxidation process cleaves the carbon-carbon bonds of the cis-diol groups present in the molecular chain of alginate, generating reactive aldehyde functionalities. These aldehyde groups then engage in chemical crosslinking with amino functionalities through Schiff-base connections.

Table 4. Gelling time, swelling index and water vapour transmission rate of Mupirocin liposomal hydrogel formulations

Formulations	Gelling time* (sec)	Swelling index* (%)	WVTR* ($g/m^2/h$)
MLH1	45±1.0	68.35±0.01	99.47±0.55
MLH2	38±1.52	78.43±0.05	113.43±0.76

MLH3	38±0.57	73.64±0.39	107.37±1.74
MLH4	32±1.52	89.29±0.02	129.51±1.64
MLH5	35±0.57	84.57±0.02	122.29±0.42
MLH6	28±1.52	78.19±0.04	113.32±0.69
MLH7	38±1.0	77.92±0.085	112.39±0.35
MLH8	28±0.57	83.76±0.05	118.83±0.19
MLH9	42±1.52	71.42±0.59	103.74±0.25

***Standard deviation, n=3**

Drug content

The drug content was determined to ascertain that the drug loaded liposomes is uniformly loaded in the formulation. The drug content ranged between 96.55±0.48% - 98.76±0.23%. The results obtained are shown in Table 5.

Summary of regression analysis

The obtained "p" values for the corresponding "F" values were consistently below 0.05, indicating the significance of the model terms in all cases. Both the Predicted R-Squared and Adjusted R-Squared values showed reasonable agreement, suggesting the model's reliability in predicting outcomes . To measure the signal-to-noise ratio, Adequate Precision was utilized, and it is desirable to have a ratio greater than 4. In our case, the calculated value significantly exceeded this threshold, indicating a high level of precision and reliability in the experimental results .

Table 5. Encapsulation efficiency and drug content of Mupirocin liposomes.

S. No	Formulations	Encapsulation efficiency* (%)	Drug content*(%)
1	LM1	55.21±0.52	97.61±0.56
2	LM2	65.85±0.34	96.55±0.48
3	LM3	62.25±0.52	98.11±0.13
4	LM4	59.89±0.34	98.36±0.66
5	LM5	57.46±0.73	98.52±0.16
6	LM6	69.14±0.35	97.23±0.32
7	LM7	52.73±0.66	98.14±0.28
8	LM8	57.53±0.19	98.76±0.23
9	LM9	62.33±0.36	97.45±0.33

Scanning electron microscopy (SEM)

Liposomes surface morphology and shape were investigated by SEM analysis (Figure 2).
The Mupirocin loaded liposomes have vesicular structure and were spherical in shape.

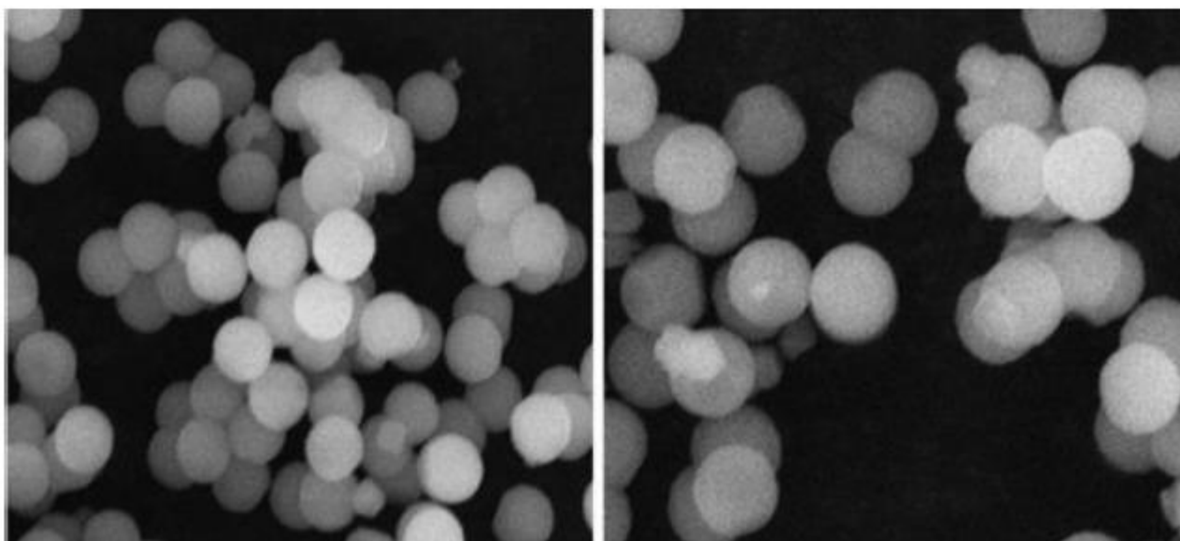

Fig 2: SEM images of optimized Mupirocin liposomes

***In vitro* dissolution data of optimized Mupirocin liposomes (OLM) in phosphate buffer pH 7.4**

Time (h)	**Cumulative drug release* (%)**
	OLM
0	0
1	10.65±0.24
2	19.89±0.81
3	26.75±0.52
4	35.59±0.41
5	44.85±0.78
6	53.78±0.51
7	61.97±0.45
8	72.84±0.79
9	81.75±0.51
10	87.59±0.23
11	92.84±0.78
12	95.43±0.22

***Standard deviation, n=3**

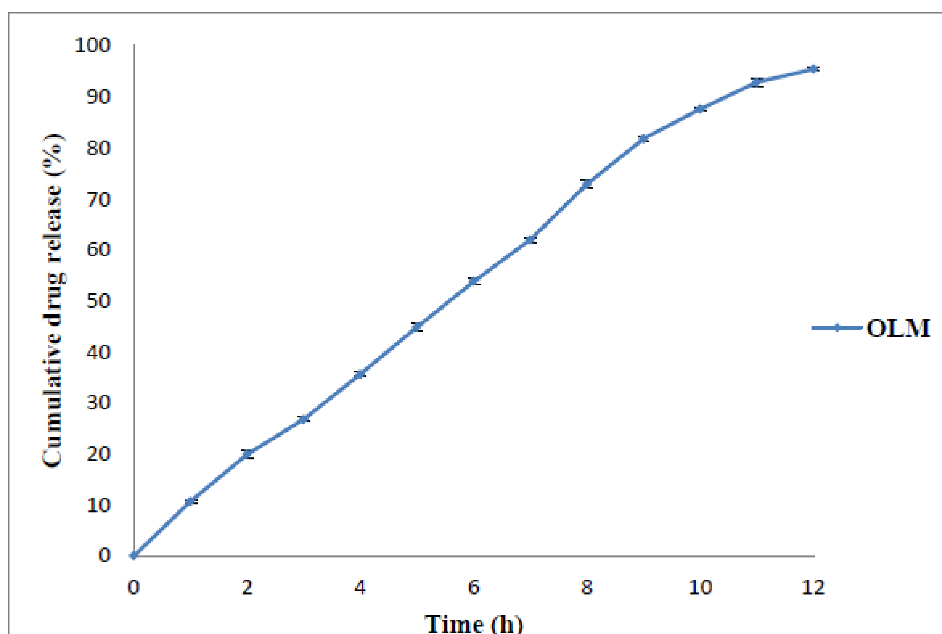

Figure 3. ***In vitro*** **drug release profile of optimized Mupirocin liposomes (OLM) in phosphate buffer pH 7.4**

Summary and Conclusion

The optimized Mupirocin liposomal hydrogel exhibited a gelling time of 43 seconds, a swelling index of 71.43%, and a water vapor transmission rate of 98.85 g/m2/h..In vitro drug release from the optimized Mupirocin liposomal hydrogel was 95.43±0.22% at the end of 12 hours, following Higuchi release kinetics, indicating a diffusion-controlled release.

The spreadability of Mupirocin liposomal hydrogel formulations was optimum, although it decreased with an increase in the concentrations of oxidized alginate and gelatin, due to increased hydrogel viscosity.

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