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PREPARATION OF LIPID BASED NANO CARRIERS AS DRUG DELIVERY SYSTEM FOR DIABETIC WOUND HEALING

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Abstract:
Wound healing is a complex process involving various cellular events and molecules. Liposomal hydrogels containing therapeutic agents have emerged as a promising approach for wound healing due to their controlled drug release, improved stability, and targeted delivery. In this study, liposomal hydrogels loaded with Mupirocin and Glucophage were developed and evaluated for wound healing properties. Mupirocin prevents bacterial infections, while Glucophage promotes angiogenesis and tissue repair.
Compatibility studies using Fourier transform infrared spectroscopy and differential scanning calorimetry confirmed the compatibility of Mupirocin with excipients. A 32-factorial design was employed to optimize the liposomal formulation, resulting in formulations with a yield of 75.87%, particle size of 264.30 nm, and polydispersity index of 0.233. The formulations showed controlled drug release over 12 hours.
The liposomal hydrogels were evaluated for drug content, viscosity, water vapor transmission, fluid uptake, and rate of evaporation. In vivo studies demonstrated significant improvements in wound healing with the optimized liposomal hydrogels. Histopathological examination revealed well-structured skin and subcutaneous tissue, demonstrating their potential as effective wound dressings.
The integration of Mupirocin and Glucophage in liposomal hydrogels presents a novel therapeutic strategy for wound management. These liposomal hydrogels offer controlled drug delivery and enhanced wound healing, benefiting patients with chronic or non-healing wounds.

Keywords: Mupirocin, Phospholipon 90H, Cholesterol, Oxidized alginate, Gelatin

Introduction:

Wound healing is a complex biological process involving various cellular events and molecular mechanisms to restore tissue integrity[1]. Over the years, advancements in wound management have sought innovative approaches to enhance the healing process. Among these, liposomal hydrogels containing therapeutic agents have emerged as a promising strategy for wound healing[2.] Liposomes, lipid-based vesicles, offer several advantages, including controlled drug release, improved drug stability, and targeted delivery to wound sites, making them an ideal platform for wound management [3].Liposomal hydrogels have advantage over other conventional formulation such as creams,ointments

and gels. 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 [4] . The composition and concentration of lipid in liposomes, incorporated into hydrogels, are the two major factors which play an important role in the rheological properties of hydrogels. In the case of hydrophilic drugs, used in the formulation, the drug release is not affected by the quantity of lipid loaded in hydrogels, but can be affected by the quantity of rigid membrane which is used in liposomes[5].In the case of lipophilic drugs used in the formulation, the concentration of lipid added in the liposome in the hydrogel has a strong effect on the release of drug and the rigidity of membranes is not important [6] . The release of drug is controlled by degradation of hydrogel matrix and the controlled release rate of the drug is attained by designing hydrogel.

Oxidized alginate and Gelatin Liposomal Hydrogels of Glucophage

Preparation of liposomal hydrogel

Experimental design

A randomized, 3^2 full factorial design with two factors at three levels was employed to systematically study the formulation of hydrogels. A total of 9 formulations were prepared. Oxidized alginate (A) and Gelatin (B) were selected as independent variables and % gelling time, % fluid uptake and % water vapour transmission rate are fixed as dependent variables. In case of 3^2 factorial designs, a full-model polynomial equation were established by subjecting the transformed values of independent variables to multiple regression analysis, and contour plots were drawn using the equation. Design-Expert 9 software (Stat-Ease Inc., USA) was used for generation and evaluation of the statistical experimental design[8,9]

Preparation of hydrogels and incorporation of optimized Glucophage liposomes

Oxidized alginate (51.44% oxidized) was reacted with gelatin to form the cross-linked gel in the presence of 0.1M borax. Gels were prepared by using a double syringe fibrin glue applicator, in which one syringe was filled with the solution of oxidized alginate in 0.1M borax and the other with equal volume of gelatin in water along with optimized Glucophage liposomal hydrogels. The applicator was fitted with a 20G needle. The mixing of the polymer solutions inside the hypodermic needle on pushing the plunger in the applicator led to gelation and cross-linking in a few seconds with the incorporation of optimized liposomes and subsequent formation of the liposomal hydrogel. The liposomes added into the hydrogel contained 2% w/w Glucophage [10,11]

Kinetics of drug release

To investigate the drug release mechanism from the optimized formulation, the release data were analyzed using different mathematical models. This analysis involved determining specific parameters such as 'n', which represents the time exponent, 'k', the release rate constant, and 'R', the regression coefficient. These parameters allowed us to gain insights into the release mechanisms of the drug from the formulation[12].

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[13].

In vivo Studies of Liposomal Hydrogels

In vivo studies

The study was approved by Institutional animal ethical committee. The rats were anesthetized by intramuscular injection of ketamine at a dose of 40 mg/kg body weight. The dorsal hair of each animal was shaved and disinfected using 70% (v/v) ethanol. Full-thickness skin excision wounds of 1.5 cm × 1.5 cm and a depth of about 1 mm on the dorsal aspect of the thoracolumbar region of the rats were created. These wounds were treated with different formulations of six animal groups as shown in Table 1. The dressings were changed every 12 h.

Table 1. Wounds treated with different formulations

Groups	Treatment
Group 1	Treated with normal saline (Control) solution and remained uncovered throughout the experiment (S1).
Group 2	Treated with Mupirocin (T-bact Ointment) marketed product (S2).
Group 3	Treated with Glucophage (Fucidin Ointment) marketed product (S3).
Group 4	Treated with hydrogel without drug (S4).
Group 5	Treated with Mupirocin loaded liposomal hydrogel (S5).
Group 6	Treated with Glucophage loaded liposomal hydrogel (S6).

All rats were separately housed in individual cages and wound size was measured at predetermined intervals, the relative wound size reduction was calculated[9].

$$\text{Relative wound size reduction (\%)} = \{(A_0 - A_t)|A_0\} \times 100$$

Where, A_0 and A_t are the wound size at initial time and time "t", respectively.

Results and Discussions

Encapsulation efficiency

It was observed that encapsulation efficiency of Glucophage varied with the lipid composition and the entrapment ranged from 51.22±0.23 - 64.34±0.48% (Table 2).Increase in the amount of cholesterol in the liposomes has increased the encapsulation of the Glucophage to a certain extent. Cholesterol increases the rigidity of the liposomal membrane. Thus high concentration of cholesterol produce more rigid liposomes and may reduce the bilayer permeability, enhance the stability of liposomes and resulting in high encapsulation efficiency, at the same time beyond a certain concentration, cholesterol may disrupt the regular structure of the liposomal membrane, result in lower encapsulation efficiency[100]. Increase in phospholipon ratio increased the entrapment efficiency and increase in cholesterol ratio decreased the entrapment efficiency this may be attributed to the saturation of lipid domains with reference to drug where lower concentration of phospholipon limits entrapment capacity.

It was also noted that the encapsulation efficiency of Glucophage was much lower compared to the encapsulation of Mupirocin.

Drug content

The drug content of the Glucophage liposomal formulations was in the range of 96.45±0.76 - 98.67±0.56% indicating the Glucophage was uniformly distributed in the liposomes. The results obtained are tabulated in Table 2.

Table 2. Encapsulation efficiency and drug content of Glucophage liposomes

Formulations	Encapsulation efficiency* (%)	Drug content*(%)
LS1	52.64±0.57	96.45±0.76
LS2	60.62 ±0.17	98.04±0.14
LS3	64.34 ±0.48	98.67±0.56
LS4	55.93 ±0.17	97.45±0.33
LS5	54.06±0.23	97.34±0.33
LS6	59.21±0.23	98.22±0.28
LS7	51.22±0.35	98.52±0.73
LS8	54.53±0.34	96.88±0.89
LS9	57.76±0.42	97.17±0.64

***Standard deviation, n=3**

***In vitro* drug release studies of the optimized liposome formulation (OLS)**

The *in vitro* studies were carried using phosphate buffer pH 7.4. The release of Glucophage from the optimized liposomes was 90.53±0.72% at the end of 12 h (Table 3). It was observed that the inclusion of higher proportion of cholesterol in liposomal formulation results in prolonged drug retention (Figure 1). These results correlated with earlier reports in which inclusion of higher proportion of cholesterol in liposomes resulted in prolonged drug release.

The release kinetics studies reveals that the Glucophage was released from liposomes by a diffusion-controlled mechanism and a linear correlation occurs when the percent of drug released was plotted against the square root of time according to Higuchi equation (Table 4). These results are in agreement with many researchers who found that the drugs were released from liposomes by a diffusion-controlled mechanism.

Table 3. *In vitro* dissolution data of optimized Glucophage liposomes (OLS) in phosphate buffer pH 7.4

Time (h)	Cumulative drug release* (%)
	OLS
0	0
1	12.46±0.44
2	21.91±0.61
3	32.35±0.22

4	39.57±0.52
5	46.95±0.31
6	53.88±0.78
7	60.37±0.55
8	67.93±0.91
9	74.67±0.86
10	81.13±0.34
11	86.78±0.81
12	90.53±0.72

***Standard deviation, n=3**

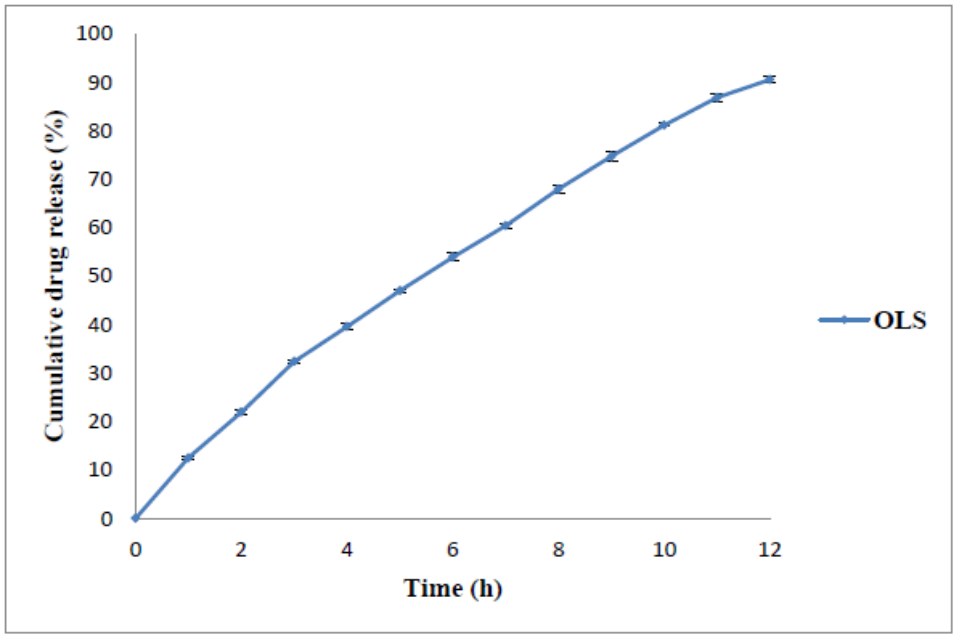

Figure 1. *In vitro* drug release profile of optimized Glucophage liposomes (OLS) in phosphate buffer pH 7.4

Table 4. Release kinetic data of optimized Glucophage liposomal formulation (OLS)

Formulation	Zero order	First order	Higuchi	Korsemeyer-Peppas	
	R^2	R^2	R^2	R^2	**n**
OLS	0.9877	0.8116	0.9037	0.8116	1.5883

***In-vivo* studies**

The wound area was observed and measured at different time intervals of 5, 10, and 15 days post-operation. Reduction in percentage wound area was calculated (Figure 50 - 53). At 5^{th}, 10^{th} and 15^{th} day, there was an increased reduction in percentage wound area treated with Glucophage and Mupirocin loaded liposomal hydrogels compared to the wounds treated with standard (marketed product), hydrogel without drug and control (Table 5 and Figure 2)

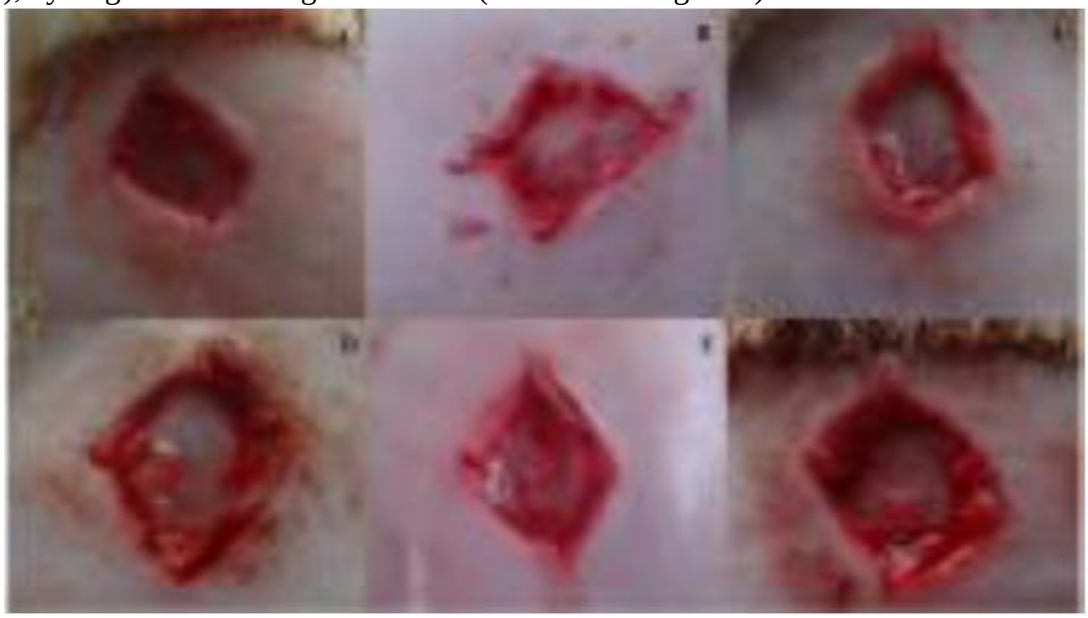

Figure 2 . Photographs of macroscopic appearance of wounds on 1^{th} day: (A) control, (B) Glucophage ointment (marketed product), (C) hydrogel without drug, (D) Glucophage liposomal hydrogel

The results indicate that the percentage wound reduction rate was higher in the order of the Glucophage liposomal hydrogel (96.89±1.26%) > Glucophage ointment (marketed product) (85.11±1.34%) > hydrogel without drug (82.56±1.27%) > > control (71.13±1.68%).

Wound Healing Activity

Treatment	Day 1	Day 5		Day 10		Day 15	
	Wound* (cm)	Wound* (cm)	% Wound contraction*	Wound (cm)	% Wound contraction*	Wound (cm)	% Wound contraction*
Control	1.5±0.62	1.14± 0.12	24.12± 1.87	0.85± 0.09	43.35± 1.63	0.44± 0.05	71.13± 1.68
Standard (T-bact Ointment)	1.5±0.49	0.99± 0.11	33.76± 2.56	0.58± 0.11	61.41± 1.53	0.26± 0.03	82.34± 1.17
Standard (Fucidin Ointment)	1.5±0.38	0.97± 0.37	35.54± 3.20	0.50± 0.14	66.59± 1.33	0.22± 0.16	85.11± 1.34
Hydrogel (without drug)	1.5±0.78	0.98± 0.08	34.16± 2.14	0.57± 0.27	62.18± 1.19	0.26± 0.09	82.56± 1.27

SUMMARY AND CONCLUSION

Aim of the research work was to develop and evaluate the oxidized alginate and gelatin liposomal hydrogels of Mupirocin and Glucophage for wound healing properties.The prepared liposomal were evaluated for percentage yield, particle size, polydispersity index, drug content, and in vitro drug release studies. The liposomal hydrogels were evaluated for drug content, viscosity, water vapour transmission, fluid uptake study, and rate of evaporation and in vitro drug release studies. The formulations were prepared by applying design of experiments (3 2 factorial design) and evaluated. In vivo and stability studies were performed on optimized formulations. Optimized liposomal hydrogels showed enhanced percentage reduction in wound
area. Thus, the study objectives envisaged are achieved. The liposomal hydrogels of Mupirocin and Glucophage significantly improved the wound healing. Thus, liposomal hydrogels have proved to be a potential wound dressing with excellent wound healing properties.

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