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Development and Evaluation of Mupirocin Loaded Liposomal Hydrogels for Diabetic Wound Healing Properties

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ABSTRACT
The aim of the present study was to develop and evaluate liposomal hydrogel of mupirocin as a diabetic wound dressing. Liposomal hydrogel as a wound dressing provides a barrier that effectively prevents the contamination of the wound and further progression of infection to deeper tissues. Hydrogels combine the features of moist wound healing with good fluid absorbance. Liposomes were developed by using different ratios of Phospholipon 90H and cholesterol and the hydrogels was developed by using oxidized alginate and gelatin. Liposomes prepared were then incorporated into the hydrogels. The formulations were characterized by FT-IR and DSC for drug and polymer compatibility and surface morphology was studied by SEM. Liposomal hydrogel were evaluated for their size, shape, encapsulation efficiency and for in vitro studies. The FT-IR and DSC confirmed the absence of any drug polymer interaction. The average size of liposomes was found to be in range of 708.21-802.33 nm and shape was found to be spherical. The maximum encapsulation efficiency was found to be 69.13%. The in vitro release profile of liposomal hydrogel formulation LH6 was found to give sustained release of drug. The stability study confirmed that the formulation prepared were stable.

Keywords: *Diabetic wound dressing, Mupirocin, Phospholipon 90H, Cholesterol, Oxidized alginate, Gelatin*

1. INTRODUCTION

Diabetes has become a global health concern with the increase in complications such as diabetic wounds [1]. The principal functions of wound dressings are to remove wound exudates, to prevent the entry of harmful bacteria into the wound, and to promote natural healing [2]. Liposomal hydrogel as a wound dressing provides a barrier that effectively prevents the contamination of the wound. Mupirocin a topical antibiotic drug encapsulated within the liposomes increases the drug concentration locally, decreases systemic drug concentration and also prevents further progression of infection to deeper tissue and hydrogels of oxidized alginate and gelatin combine the features of both moist wound healing with good fluid absorbance [3].

2. EXPERIMENTAL

2.1. Material and method

Mupirocin was provided as a gift sample by Fourrts (India) Laboratories Pvt. Ltd. Phospholipon 90H was provided as a gift sample by Lipoid, Germany and Cholesterol, Sodium alginate, Gelatin and chloroform were purchased from Lobachemie.

2.2. Preparation of liposomes

Liposomes were prepared by solvent stripping technique in a rotary flask evaporator. Phospholipon 90H and cholesterol were dissolved in chloroform. Organic solvent was slowly removed under reduced pressure so that a thin film of dry lipid was deposited on the inner wall of the flask. This film was further hydrated dispersed in 10ml saline phosphate buffer pH 7.4 at 25° C for a required period of time along with mupirocin [4].

2.3. Preparation of hydrogels and incorporation of liposomes

Oxidized alginate (58% oxidized) was made to react with gelatin to form the cross-linked gel in the presence of 0.1M borax. Gels were prepared by using a syringe, in which one syringe was filled with the solution of ADA in 0.1M borax and the other with equal volume of gelatin in water. The mixing of the polymer solutions along with the optimized liposomal formulation led to gelation and cross-linking in a few seconds leading to the

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formation of the liposomal hydrogel [5]. The formulation chart shown in table 1 represents the different amounts of polymers used.

2.3. EVALUATION OF LIPOSOMAL HYDROGELS

2.3.1. Particle size measurement

The mean particle size of liposomes were studied by dynamic light scattering (DLS) using Malvern Mastersizer (Hydro 2000 S units)[4].

Table 1. Formulation chart of Liposomal Hydrogels

Formulations	Drug (%)	Cholesterol (mg)	Lipid (mg)
F1	2	50	200
F2	2	100	300
F3	2	150	400
F4	2	50	400
F5	2	100	300
F6	2	150	200
	Optimized Liposomal formulation incorporated	**Oxidized alginate (%)**	**Gelatin (%)**
LH1		10	5
LH2		20	10
LH3		30	15
LH4	**F3**	10	15
LH5		20	5
LH6		30	10
LH7		10	10

2.3.2. Encapsulation Efficiency

The entrapped drug concentrations were determined after the lysis of prepared liposomes with absolute alcohol and sonication for 10 min. The concentration of the drug in absolute alcohol was determined spectrophotometrically. The entrapment efficiency is the ratio of expressed as the amount of drug in supernant liquid to the amount of drug added [6].

2.3.3. Scanning electron microscopy

SEM photographs of the liposomes were taken with a scanning electron microscope Model Joel- LV-5600, USA, at the required magnification at room temperature. Prepared liposomes were deposited on a glass disc applied on a metallic stub and evaporated under a vacuum overnight. Before the SEM analysis, the samples were metallized under an argon atmosphere with a 10-nm gold palladium thickness (EMITECH-K550 Sputter Coater, Houston, TX) [7].

2.3.4. Measurement of the equilibrium fluid uptake

The equilibrium degree of swelling was determined by a conventional gravimetric method dry samples were immersed in 200 mL distilled water at room temperature for 3 h to reach swelling equilibrium. The swollen samples were then filtered over unabsorbed water by a 100-mesh sieve and drained for 10 min to remove redundant water. The equilibrium fluid content was determined according to equation given below [7,8].

$$\mathrm{EFC}\ (\%) = \frac{w_s - w_d}{ws} \times 100 \qquad (1)$$

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

2.3.5. Water vapour transmission rate (WVTR)

When the equilibrium water content (EWC) had been reached, the hydrogel was removed from the distilled water and re-equilibrated in air at room temperature to constant weight. The water retention (WR) of the hydrogel was calculated using the equation and similarly plotted as a function of time [7,8].

$$\mathrm{WVTR} = \frac{(G/t)}{A} \qquad (2)$$

Where,
WVTR = water vapour transmission rate ($g/m^2.h$)
G = weight loss (g), t = time (h), A = test area

2.3.6. In vitro drug release

Franz diffusion cell was used to determine the release profile of drug from liposomal hydrogel. The cells consisted of donor and receptor chambers between which a cellophane diffusion membrane (cut-off 12,000 Da; Himedia, Mumbai, India), was positioned and stuck with adhesive (Franz, 1975). Capacity of receptor chamber was 24 ml. A magnetic bead was placed in the receptor chamber. The diffusion medium consisted of phosphate buffer saline (PBS) pH 7.4. Whole assembly was put on magnetic stirrer with slow stirring and temperature was maintained 37.0 ± 0.5 ºC. The formulations were kept over the membrane in donor compartment and stirred. Samples were withdrawn from the receptor compartment at predetermined time intervals, and the volume was replenished with same volume of diffusion medium. Addition of diffusion medium to the receptor compartment was performed with great care to avoid trapping air beneath the diffusion membrane. The samples were analyzed spectrophotometrically at 223 nm after appropriate dilutions. The % drug release was calculated and graph of % drug release vs time was plotted. For each formulation, the release studies were performed in triplicate [9].

2.3.7. Stability Studies of the Formulations

The physical stability of the liposomal hydrogel formulations were evaluated after storage for 6

months. Samples were stored at room temperature (20 ºC) or at 4 °C. After every 1 month, formulations were evaluated for encapsulation efficiency and *in vitro* drug release.

3. RESULTS AND DISCUSSION

3.1. Particle size measurement

The particle size carried out my zeta-sizer varied from the 708.21-802.33 nm, the particle size increased with the increase in quantity of cholesterol.

3.2. Encapsulation Efficiency

The percentage of the drug entrapped into liposomes was found to be in the range of 47.86-69.13 %. The high drug entrapment efficiency further confirmed the high association of drug with liposomes.

Table 2. Particle size and percentage Entrapment efficiency of liposomal formulations

Formulations	Particle Size (nm)	Entrapment efficiency (%)
F1	708.21 ± 1.1	52.21 ± 1.44
F2	715.43 ± 1.8	63.13 ± 1.32
F3	723.56 ± 1.6	69.13 ± 1.02
F4	743.24 ± 2.3	59.02 ± 1.13
F5	756.16 ± 1.1	51.64 ± 1.16
F6	802.33 ± 1.4	47.86 ± 1.32

3.3. Scanning electron microscopy

The shape and surface of the liposomes were analyzed by SEM and it revealed that the liposomes were almost spherical in shape with smooth surface as shown in Figure 1.

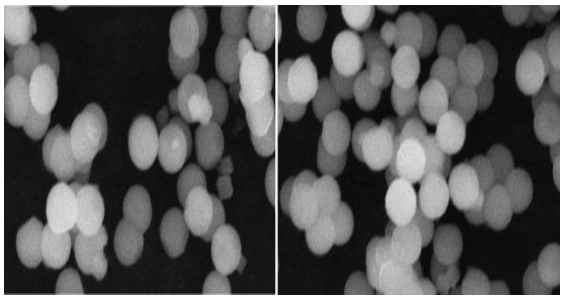

Figure 1: SEM images of liposomal formulation F3.

3.4. Measurement of the equilibrium fluid uptake

The equilibrium fluid uptake **(**degree of swelling) is important swelling characteristics of hydrogels, the degree of swelling ranged from 55.44-85.04 %. These characteristics indicated the ability of wound dressing in absorption of wound exudates.

3.5. Water vapour transmission rate (WVTR)

Hydrogels are used as wound dressing materials to prevent or reduce the body liquid loss through controlling absorption and transmission as well as maintaining the minimum humidity in the wound area to accelerate the process of healing. WVTR is an important factor that shows the potential of the hydrogel in transmission of wound exudates. Increase in the transmission rate also fastens drying of the wound. Decreased WVTR shows the accumulation of exudates which may cause delay in the healing process and leads to bacterial growth.

3.6. In vitro drug release

The *in vitro* release profiles of mupirocin from the liposomal hydrogels were depicted in Figure 2. The release of mupirocin ranged from 52.43-77.56% indicating a sustained release, there was a decrease in drug release with increase in cholesterol ratio due to increased rigidity of the liposomal membrane which acts a barrier for drug diffusion along with hydrogel as a barrier.

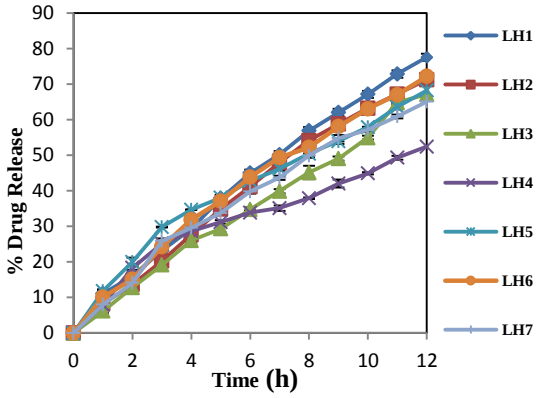

Figure 2: Graphs showing the in-vitro drug release of liposomal hydrogel formulations.

3.7. Stability Studies

The formulations were subjected to stability studies. It was found that there were no significant changes in encapsulation efficiency and *in vitro* drug release indicating the prepared liposomal hydrogels were stable.

4. CONCLUSION

This study of mupirocin loaded liposomal hydrogels on diabetic wound dressing combines the beneficial properties of both sustained release of drug in preventing infection and moist wound dressing with good fluid absorbance.

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Table 3. Percentage Equilibrium fluid uptake and water vapour transmission rate of liposomal hydrogel formulations.

Formula-tions	% Equilibrium fluid uptake	% Water vapour transmission rate
LH1	60.41 ± 0.23	26.87 ± 0.45
LH2	82.65 ± 0.56	47.71 ± 0.61
LH3	73.18 ± 0.13	38.24 ± 0.52
LH4	55.44 ± 0.22	26.78 ± 0.12
LH5	89.06 ± 0.76	49.18 ± 0.32
LH6	83.23 ± 0.55	43.22 ± 0.88
LH7	85.04 ± 0.91	39.03 ± 0.43

5. REFERENCES

[1]. W. Wang, S. Lin, Y. Xiao, Y. Huang, YiTan, L. Cai, X. Li, (2008) Acceleration of diabetic wound healing with chitosan-crosslinked collagen sponge containing recombinant human acidic fibroblast growth factor in healing-impaired STZ diabetic rats, ***Life Sciences***, **82:** 190-204.

[2]. Y. Lee, J. Chang, M. Yanga, C. Chien, W. Lai, (2012) Acceleration of wound healing in diabetic rats by layered hydrogel dressing, ***Carbohydrate Polymers,*** **88:** 809-819.

[3]. S. Thirumaleshwar, P. K. Kulkarni, D. V. Gowda, (2012) Liposomal Hydrogels: A novel drug delivery system for wound dressing. ***Current Drug Therapy,*** **7:** 212-218.

[4]. S. Suresh, C. Narendra, D. Maheshwari, A. J. Kundawala, (2010) Formulation and evaluation of liposomes containing antitubercular drugs by Taguchi's orthogonal array design. ***Acta Pharmaceutica Sciencia,*** **52:** 79-88.

[5]. B. Balakrishnan, M. Mohanty, P.R. Umashankar, A. Jayakrishnan, (2005) Evaluation of an *in situ* forming hydrogel wound dressing based on oxidized alginate and gelatin. ***Biomaterials,*** **26:** 6335-6342.

[6]. S. L. Law, C. L. Shih, (2001) Characterization of calcitonin-containing liposomes formulations for intranasal delivery. ***Journal of Microencapsulation,*** **18:** 211-221.

[7]. H. Vaghasiya, A. Kumar, K. Sawant, (2013) Development of solid lipid nanoparticles based controlled release system for topical delivery of terbinafine hydrochloride. ***European Journal of Pharmaceutical Sciences,*** **49:** 311-322.

[8]. M. Kokabi, M. Sirousazar, Z. M. Hassan, (2007) PVA-clay nanocomposite hydrogels for wound dressing. ***European Polymer Journal,*** **43:** 773-781.

[9]. X. Shi, W. Wang, A. Wang, (2011) Effect of surfactant on porosity and swelling behaviors of guar gum-g-poly(sodium acrylate-co-styrene)/attapulgite super absorbent hydrogels. ***Colloids and Surfaces B: Biointerfaces,*** **88:** 279-286.