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RESEARCH ARTICLE

FORMULATION AND EVALUATION OF LIPOSOMAL LOADED HYDROGEL OF DOXORUBICIN HYDROCHLORIDE

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Abstract- Prepared gel formulations were found to be effective formulation . The results obtained from various studies like in-vitro study, antimicrobial study and Transmission electron microscopy study. It was concluded that F2 gel formulation was having best having best anti-microbial activity when compared to other prepared gel formulations. (F1, F3. F4). For future prospective these gel formulations can be good for the treatment of mouth ulcer and the effect of dosage form can be further studied by carried out *in-vivo* studies.

INTRODUCTION

Cancer is the second leading cause of mortality worldwide. Overall, the prevalence of cancer has actually increased; just in the United States alone, approximately 1,665,540 people suffered from cancer, and 585,720 of them died due to this disease by 2014.[1] Therefore, cancer is a serious problem affecting the health of all human societies.

Skin

The human body has two system that protect it from the harmful organisms existing in the environment (i) The internal defense system destroys microorganisms and bacteria that have already attacked the body.(ii) The external defense system prevents microbial microorganisms to enter into the body.[2]

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Advantages and Disadvantages of topical drug delivery[3,4]

Advantages	Disadvantages
Avoidance of first pass metabolism. Convenient and easy toapply. Ability to deliver drugs more selectively to a specificsite. Provide suitability of self-medication.	Skin irritation occur due to drug orexcipients. Poor permeability of some drugs throughskin. Drugs having large particle size can't easily absorbed through theskin. Can be used only for drugs those having very small plasma concentration for suitableaction.

PREPARATION OF DOXORUBICINLIPOSOMES

Preparation of Liposomes

Preparation of Liposomes

Method used for the preparation of liposomes is Lipid film hydration by hand shaking method.Initially, cholestrol and soya lecithin were weighed and accurately dissolved in proposed ratio of chloroform and methanol(2:1) stirred for two minutes.100 mg of ketoconazole was added to above solution and continue the stirred for 2 mins. Now, the above solution is subjected for hydration with 10 ml distilled water and added 0.25 ml of Tween 80 and continue the stirring for about one hour for the formation of liposomal vesicles. Finally, the product obtained is collected and stored in a hermetically sealed container for further evaluation studies

Table: Different Formulations of Liposome

S.No.	INGREDIENTS	F1	F2	F3	F4
1.	doxorubicin hydrochloride(mg)	100	100	100	100
2	Soya lecithin(mg)	100	200	250	100
3.	Cholestrol(mg)	20	30	40	50
4.	Chloroform(ml)	20	20	20	20
5.	Methanol(ml)	10	10	10	10
6.	Distilled water(ml)	10	10	10	10
7.	Tween 80(ml)	0.25	0.25	0.25	0.25

EVALUATION OF LIPOSOMES

Determination of percentage drug entrapment efficiency:

Drug entrapment efficiency was calculated by using centrifugation method. The liposomal suspension of 1 ml was taken and centrifuged at 3500 rpm for 15 min. The sediment obtained from the centrifugation was suspended in 100 ml of phosphate buffer pH 7.4, and the absorbance was taken at 294 nm. From that, the amount present in 1 ml of suspension was obtained.[5]

Morphology analysis:

Prepared liposomes for all the formulations were viewed under for observing the vesicle formation and discreteness of dispersed vesicles.A slide was prepared by placing over it and this slide was viewed under optical microscope at 40x magnification photographs were taken to prepared slides using digital camera[6-8]

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***In-vitro* drug release study:**

The *in vitro* release for all formulated ketoconazole liposomes were carried out for 8 hours using in phosphate buffer pH 6.8. The studies were carried in USP dissolution apparatus at $37^0C \pm 0.5^0C$ and 50 rpm speed.900 ml of phosphate buffer pH 6.8 was used as a dissolution medium.1ml of samples were withdrawn at every 30 mins upto 480 mins and make upto 10 ml with pH 6.8 and analyzed for ketoconazole content at 294nm with pH 6.8 as blank using UV- Spectrophotometer[12]

Percentage Yield of Liposomes:

The prepared liposomes were collected and weighed.The measured weight was divided by the total amount of drug and excipients which were used for the preparation of liposomes[13]

Determination of drug content

Drug entrapped multilamellar liposomes (100 mg) were suspended in 100 ml solution of chloroform: methanol (2:1). The resultant dispersion was kept for 20 min for complete mixing with continuous agitation and filtered

through a 0.45 µm membrane filter. The drug content was determined spectrophotometrically at 294 nm using a regression equation derived from the standard graph. Results were based on triplicate determination[14]

pH Determination

The pH were determined by using digital pH meter. The glass electrode was calibrated with the solutions determined for the equipment (pH of 4.00 and 7.00).The preparation was left for about 15 min for attaining while measuring. The analysis of formulation were done in triplicate, and average values were calculated[15-16]

In vitro drug release

The apparatus consists of a glass cylinder open at both ends. A dialysis membrane soaked in distilled water(24 h before use)is fixed to the one end of the cylinder with the aid of an adhesive. Gels equivalent to 10 mg of Ketoconazole is taken inside the cell(donor compartment)and the cell is immersed in a beaker containing 800 ml of phosphate buffer pH 7.4 containing 10%v/v methanol (to maintain sink condition), act as receptor compartment. The whole assembly is fixed in such a way that the lower end of the cell containing gel is just above the surface of diffusion medium (1-2mm deep) and the medium was agitated using a magnetic stirrer at the temperature 37± 0.5^0 C. Aliquots (5ml) are withdrawn from the receptor compartment periodically and replaced with same volume with fresh buffer. The samples were analysed by using UV-visible spectrophotometer at 294nm[17].

Release kinetics

To analyze the *in vitro* release data various kinetic models were use to describe the release kinetics. The zero order rate Eq. (2) describes the systems where the drug release rate is independent of its concentration. The first order Eq. (3) describes the release from system where release rate is concentration dependent. Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion.[18]

Determination of Particle Size and Poly dispersity Index by Zeta Sizer

The Ketoconazole loaded liposomes was characterized for their size and Poly dispersity index were also analysed by Zetasizer [Beckmen Coulter ™ Delsa Nano Version 3.73/2.30] at 25^0C at an angle of 90^0C, after appropriate dilution using double distilled water.[19]

Stability Studies

The drug loaded liposomes were subjected to stability studies for a period of 3 months at room temperature (30±2^0C,refrigerator condition i.e.[4±2^0C] and at accelerated condition[40±2^0C,75%RH].After 1month,2 months and 3 months of storage ,the formulations was subjected to test for physical stability and pH[20]

LT AND DISCUSSION

Evaluation of Liposomes

Following parameters were used for the evaluation of liposomes which were listed below:

Physical examination

Liposomes formulation has smooth texture and appeared to be translucent and whitish in colour.

pH Determination

The pH of all liposomes formulations was found to be in the range of 3.83 to 3.90. These pH values showed that formulated gel probably would not produce skin irritation.The conductivities values gel remained stable. Hence, prepared gel is suitable for topical applications.

Drug Content

Drug content estimation was determined by using UV Spectrophotometer at 294 nm. Drug content of all the formulation was found between 68.7 to 84.9%, which represent uniformity in drug content.

In vitro drug release

The percentage in vitro release of liposomes was carried out after 1 hour of interval upto 8 hours.

Determination of Particle Size and Poly Dispersity Index by the use of ZetaSizer

The liposomes formulation was characterized for their size Polydispersity index by using Zeta Sizer [Beckman Coulter]. Results of average particle size and Polydispersity index were obtained from instrumental based calculation system.

Stability studies:

The stability studies are very important evaluation parameter and they were performed in order to study whether the formulation can bear changes in temperature, humidity etc.So., stability studies were done for 3 months.The formulation was stable upto 1 months in terms of appearance and pH. But after 2 months, the formulation kept under room temperature and accelerated started to change in physical apperance and pH.

SUMMARY AND CONCLUSION

The prepared gel formulations were found to be effective formulation . The results obtained from various studies like in-vitro study, antimicrobial study and Transmission electron microscopy study. It was concluded that F2 gel formulation was having best having best anti-microbial activity when compared to other prepared

gel formulations. (F1, F3. F4). For future prospective these gel formulations can be good for the treatment of mouth ulcer and the effect of dosage form can be further studied by carried out *in-vivo* studies.

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