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RESEARCH ARTICLE

Design and Development of Liposome by Microencapsulation Vesicle Method and Vaginal Delivery System for Fluconazole Gel: *In-Vitro* Diffusion Study

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ABSTRACT

The present investigation constitutes the design and development of liposome by microencapsulation vesicle method and vaginal delivery system for Fluconazole and its *in-vitro* diffusion study. Amount of polymer and the number of sonication cycle was varied in different batches of formulations. Size, size distribution, surface charge, entrapment efficiency and drug content were studied for each formulation batch. Optimization of formulation and process parameter resulted in the production of Fluconazole loaded liposomal suspension with particle size distribution ranging between 130 to 240 nm and entrapment efficiency around 55%. *In-vitro* diffusion characterization using dialysis membrane was carried out to evaluate the release characteristics of the drug from optimized liposomal gel formulations with respect to the pure drug in gel.

Key words: Liposome, microencapsulation, Fluconazole, *in-vitro* diffusion, polymer.

1. INTRODUCTION

Drugs in the form of vegetation and minerals. Have existed longer than man himself. Human disease and man sinstict to survive have, through the ages led to their discovery [1]. Pharmaceuticals knowledge has grown exponentially over the years. We now have a much clearer understanding of how drugs are absorbed into, distributed within, and cleared from the body.

Development of new drug requires much of research a long development time and also coordinated team effort of a large group of researcher in various fields. Instead of searching for new drugs using random hits or miss approach the development of superior drug delivery system which enhances the therapeutic efficacy of conventional drugs by controlling the release rate or targeting to the tissue site may be an effective approach to improve the efficacy of chemotherapeutic agents. Now a day, much of the research work is focused on development of controlled sustained release medications for enhancement of clinical efficacy.

A more advanced version of controlled delivery is the targeted delivery. Targeted delivery exhibit all the advantages of the controlled delivery and at the same time facilitates delivery of the drug to the target site [2].

LIPOSOME

Liposome has been widely investigated since 1970 as drug carriers for improving the delivery of therapeutic agent to specific site of body. It plays a significant role in formulation of potent drugs to improve therapeutics .Liposomes are microscopic vesicle composed of one or more lipid membranes surrounding discrete aqueous compartments. These vesicles can encapsulate water soluble drug in their aqueous spaces and lipid soluble drugs within the membrane itself.

2. OBJECTIVE

To prepare and optimize liposomal gel formulation containing anti-fungal agent, Fluconazole for the treatment of vaginitis (*Candida albican*) and to carry out different characterization for liposomal formulation that includes comparison between *in vitro* and *ex vivo* analysis

3. MATERIAL

MATERIAL SPECIFICATIONS

Table 1: List of materials used

Material	Manufacturer
Fluconazole	Gifted sample
Soya lecithin	Himedia laboratories pvt.ltd. , Vadhani ,

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Chloroform	Qualigens fine chemicals , GSK Pharmaceutical , Mumbai
Double Distilled Water	Double Distillation Unit, Borosil
Cholesterol	Burgoyne urbidges & co. Mumbai
Carbopol 934	Himedia laboratories pvt.ltd. , Vadhani , Mumbai
Potassium dihydrogen phosphate	Merck Specialties pvt ltd. Worli, Mumbai.
Propylene glycol	Qualigens fine chemicals , GSK Pharmaceutical , Mumbai
Sodium Hydroxide	Central Drug House (P) LTD , New Delhi-110002

INSTRUMENT SPECIFICATIONS

Table 2: List of equipments used

Equipment	Specification
Four Decimal Digital Balance	Acculab Sartorious Group Pvt. Ltd Sr. No. ALC-2104
Two Decimal Digital Balance	Denver Instruments Pvt.Ltd MXX-212
pH meter	Hanna Instruments pHep®, Model PHEP
Digital Mechanical Stirrer	REMI Instrument Division, Vasai-08 CAT No. RQ-121
Probe Ultra-Sonicator	Lark Innovative Fine Teknowledge Chennai-80
Ultracentrifuge	REMI Instrument Division, Mumbai-53 CAT No. R4C
Dissolution Apparatus	Electrolab Tablet Dissolution Tester, Mumbai Model NO. TDT-06P, Sr. No. 0807019
UV-Visible spectrophotometer	Jasco V-630 Spectrophotometer, Japan, Sr. No. AI25961148
Zeta potential	Malvern Instrument
Scanning Electron Microscope	Joel JSM-1600, Tokyo, Japan
Double Distillation Unit	Mono Quartz Distillation Unit, Borosil CAT No. 3363

4. Method Development for Preparation of Fluconazole loaded Liposomal suspension

The micro encapsulation vesicle (MCV) method is a liposome preparation technique that reproducibly produces liposomes with homogeneous particle sizes with high encapsulation efficiency. Liposomes encapsulating water-soluble drugs, lipophilic drugs and an amphiphilic drug were prepared by the MCV method and the encapsulation efficiency of the drugs was examined.

METHOD OF PREPARATION OF LIPOSOMAL SUSPENSION

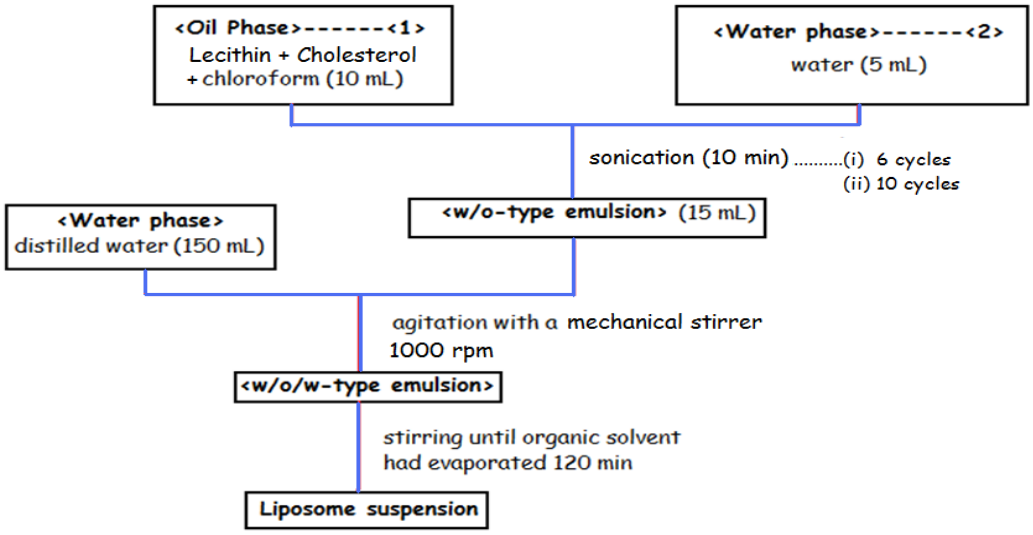

Fig 1: Schematic Representation of Preparation of Fluconazole loaded Liposomal suspension by MCV method

Table 3: Batch Specification of FZ Liposomal Suspension

Formulation code	PC:CH ratio	Amount of PC (mg)	Amount of PC (mg)	Amount of Drug (mg)	Sonication cycles
LP 1	9:1	180	20	20	6
LP 2	10:1	200	20	20	6
LP 3	12:1	220	20	20	6
LP 4	9:1	180	20	20	10
LP 5	10:1	200	20	20	10
LP 6	12:1	220	20	20	10

Sonication parameters:

Probe size - 06
Pulse on time - 2 seconds
Pulse off time - 2 seconds
Pulse ratio - 30%
Temperature - 38^{o}C

LP1, LP2, LP3:

Sonication Period - Total Time: 600 seconds (10 minutes)
Cycles: 6 cycles (each cycle of 100 sec.)

LP4, LP5, LP6:

Sonication Period - Total time: 600 seconds (10 minutes)
Cycles: 10 cycles (each cycle of 60 sec.)
Time gap between cycles - 2 seconds

PREPARATION OF LIPOSOMAL GEL

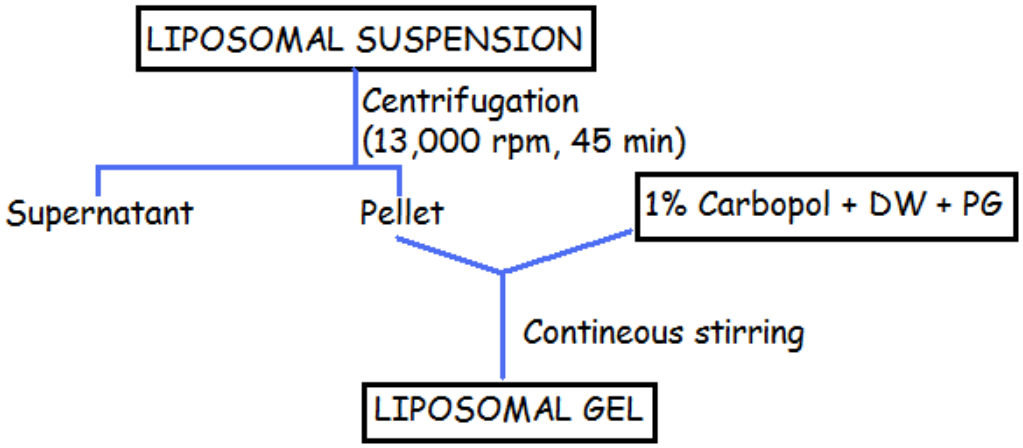

Fig 2: Schematic Representation of Preparation of Liposomal Gel

PREPARATION OF 1% OF CARBOPOL GEL

As a vehicle for incorporation of liposomes for vaginal delivery, bioadhesive gel was made .Carbopol 934 (100mg) was dispersed in demineralised water (4gm) by continuous stirring with glass rod. Then propylene glycol (6gm) was added and the mixture was neutralized by drop-wise addition of 10% NaOH. Mixing was continued until a transparent gel appeared, whereas the amount of base was adjusted to achieve a gel with pH 4.5.

INCORPORATION OF LIPOSOMES INTO THE GEL

After preparation of liposomal suspension, go for the centrifugation at 13,000rpm for 45min.to get the liposomal pellet containing Fluconazole drug. Take known amount of pellet (containing 1.5mg drug) and mixed with the previously prepared 1% carbopol gel.

5. Characterization Process of Fluconazole Loaded Liposomal suspension

Table 4: Characterization methods of Fluconazole Loaded Liposomal suspension

Parameter	Instrument used
Vesicle size determination	Malvern Zetasizer
Surface and Internal Morphology	SEM ,TEM
Charge determination	Zeta Potentiometer
Polydispersity index	Malvern Zetasizer

5.1. *Vesicle size and surface charge determination:-*

The average diameter of the vesicles and their Zeta potential were determined using a Zetamaster apparatus (Malvern Instruments, Malvern, UK) at a temperature of 25 ± 0.1^{o} C. Samples were analyzed 24 h after their preparation. For the particle size measurements, the liposome suspensions were suitably diluted with distilled water in order to avoid multi-scattering phenomena.

5.2. *Scanning electron microscope analysis:*

The samples for SEM were prepared by lightly sprinkling the liposomal suspension and liposomal gel on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (Jeol JSM-1600, Tokyo, Japan). Electron micrographs were taken at magnifications of 7500 using 10KV accelerating voltage.

5.3. *Encapsulation efficiency:*

The encapsulation efficiency was calculated according to a method as we reported previously (Ishii and Nagasaka, 2001).

Encapsulation efficiency (%) = $C_{total} - C_{out} / C_{total}$ *100

where C_{out} : is the liposome suspension diluted to 4fold with chloroform water and centrifugation at 25^{0}C , 13,000rpm for 45min in a R-24 (Remi, India) research centrifuge.

5.4. *Drug content:*

Prepare liposomal suspension of different formulation and centrifuged at 13,000rpm for 45 min., collect the pellet and redispersed in 50ml of 95% ethanol. From this solution, take 2ml and the absorbance was measured after suitable dilution at 256nm against the corresponding blank solution.

5.5. Release Kinetics:

Data obtained from In-vitro release studies were fitted to various kinetics models to find out the mechanism of drug release from microspheres.

Kinetics models used for In-vitro drug release from microspheres are

- Zero order release kinetic model.

- Higuchi Model [T.Higuchi 1963 & W.I. Higuchi 1967]

6. EVALUATION OF LIPOSOMAL GEL

In-vitro diffusion study:

The *in-vitro* diffusion study of the gels was performed using artificial dialysis membrane (Sigma Inc, MO, USA;Cat.No.:250-7U;dry , unwashed ,pre-cut and open ended; flat width:35mm; inflated diameter:21mm; lenth:30mm).The membrane soaked in phosphate buffer, pH 4.5 which is equivalent to human vaginal pH, for 8hrs was clamped carefully to one end of the hollow glass tube of dialysis cell (3.14cm^2 area) .200ml of PBS was taken in 900-mL vessel of the USP 8 basket dissolution test apparatus, which was used as receptor compartment. Then 10gm of liposomal carbopol gel containing1.5mg equivalent of the Fluconazole was spread uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37^{0}C.

Table 5: Correlation coefficients of optimized formulations

Formulation code	Zero order	Higuchi
Pure drug, Fluconazole	0.9697	0.9737
LP 1	0.9425	0.8712
LP 2	0.9498	0.9553
LP 4	0.9722	0.9612
LP 5	0.9818	0.9685

7. RESULTS AND DISCUSSION

7.1. Zeta potential report

Table 6: Zeta potential, Average size, PDI and % Entrapment of all liposomal suspensions.

Formulation code	Zeta potential (mV)	Z – avg. size (nm)	PDI	%Entrapment efficiency (mean ± SD, n = 3)
LP 1	-48.0	183.6	0.551	53.303 ± 1.709
LP 2	-53.2	206.3	0.539	47.666 ± 1.900
LP 3	-44.3	134.5	0.469	36.233 ± 0.802
LP 4	-48	228	0.645	53.433 ± 3.023
LP 5	-44.7	238.3	0.655	52.533 ± 1.096
LP 6	-38	175.8	0.505	31.533 ± 1.331

a. Average Particle Size and Polydispersity Index

It is evident from Table 6 that with the increase in the number of sonication cycles the average particle size and polydispersity index increases. With 2 seconds gap time between the cycles the 6 cycled sonication has 5 gaps counting to 10 seconds gap in total while, 10 cycled sonication has 9 gaps counting to 18 seconds gap in total. Thus, in 6 cycled sonication the shear stress on the particles is quite continuous (100 seconds in each cycle for 10 minutes) with respect to the 10 cycled sonication (60 seconds for 10 minutes). Because of the increase in the shear stress there occurs decrease in the average particle size on the particles formed by 6 cycled sonication than the particles formed by the 10 cycled sonication.

b. Zeta Potential

As the sonication cycle increases from 6 to 10, the zeta potential value decreases. This may be due to the increase in the particle sizes from 6 cycles to 10 cycles. And as particle size increases the surface charges on the particle decreases. The zeta potential of a system is governed by both the stabilizer and the drug itself. In order to obtain a system exhibiting good stability, zeta potential ranging from ±30mV to ±60mV is essentially required. All the formulations, LP 1 to LP 6 find there place in this range, thus proving to be stable formulations.

c. Entrapment Efficiency

The encapsulation efficiencies of Fluconazole laoded in liposomal suspension was characterised by variation in sonication cycle with constant sonication time. The encapsulation efficiency prepared with soya lecithin was 53.303 ± 1.709, 47.666 ± 1.900, 36.233 ± 0.802 for LP1, LP2, LP3 of 6 cycled sonication and also for 10 cycled sonication the LP4, LP5, LP6 having encapsulation efficiency of 53.433 ± 3.023, 52.533 ± 1.096, 31.533 ± 1.331 respectively.

7.2. DRUG CONTENT

Table 7: Drug content of optimized formulations

Formulation	Drug Content
LP 1- 9:1 (6)	3.4 ± 0.213
LP 2- 10:1 (6)	6.2 ± 0.405
LP 4- 9:1 (10)	1.5 ± 0.435
LP 5- 10:1 (10)	4.4 ± 0.374

It is clearly seen from the above Table No. 7 that the formulations containing 10:1 polymer: drug ratio gives higher drug content than their corresponding 9:1, polymer: drug ratio formulation. This may be because of the higher average sizes showed by the formulation LP 2 and LP 5.

7.3. *IN-VITRO* DIFFUSION STUDY

Table 8: Correlation coefficients of optimized formulations

Formulation Code	Zero order	Higuchi
Pure drug, Fluconazole	0.9697	0.9737
LP 1	0.9425	0.8712
LP 2	0.9498	0.9553

LP 4	0.9722	0.9612
LP 5	0.9818	0.9685

The examination of correlation coefficients 'r^2' indicated that the drug release followed diffusion controlled mechanism from the gel, as the values of 'r^2' for the zero order (ranged from 0.9425 to 0.9818) and Higuchi square root of time (ranged from 0.8712 to 0.9737)

8. Conclusion

In conclusion, Fluconazole loaded liposomal gel for vaginitis was prepared by microencapsulation vesicle method varying the polymer amount and the number of sonication cycle. Influence of both the formulation and process parameters in formulation of Fluconazole loaded liposomal gel was characterized with respect to the size, size distribution, surface charge, entrapment efficiency and drug content. *In-vitro and ex-vivo* characterization was carried out to evaluate the release characteristics of the drug from liposomal gel with respect to the pure drug. Increase in lipid concentration in liposomal gel was able to control the release of the active for longer period of time, which shows the sustained release behavior of formulation.

Future scope

Ex-vivo characterization may be carried out to evaluate the release aracteristics of the drug from liposomal gel with respect to the pure drug. the Further, thixotropic behavior of the liposomal gel *and in-vivo* studies in animal models and stability studies are needed to prove the enhanced bioavailability of Fluconazole loaded liposomal gel.

REFERENCE

1. Howard & Ansel, Nicholas G Popovich [edn], In Pharmaceutical dosage form & drug delivery, 5th Edn, 1990, Philadelphia.
2. Chien Y W in Transdermal controlled release drug administration in Novel Drug Delivery System., fundamentals , Developmental concepts & Biomedical application , 1982 Marcel Dekker , New York , chapter 5.
3. Scheuplin.Rj , Mechanism of percutaneous absorption route of penetration & influence of solubility J. Invest dermatol 45 , 334 , 1965.
4. Li, V.H.K., Robinson, J.R and Lee, V.H.L., In; Controlled Drug Delivery: Fundamentals and Applications, 2nd Edn., Vol29, Marcel Dekker, Inc., NY, 1987 , 7
5. Goldberg, E. P. Eds., In; Targeted Drugs, 2nd Edn., Wiley , New York, 1983, 312.
6. Riaz.M review article on Liposomes Preparation Methods, Pakistan Journal of Pharmaceutical Sciences Vol.19(1), January 1996, pp.65-77.
7. Khar RK, Vyas SP. Targeted and Controlled drug delivery novel carrier systems. 1st ed. New Delhi; CBS Publishers and Distributors; 2002.p. 384.
8. Vemuri.S Rhodes.C.T.,Preparation and Characterisation of Liposomes as therapeutic delivery systems:a review , Pharmaceutica acta Helvetiae, 70 1995 ,95-111.
9. Florkin, M., and Stotz, E.H. (Eds.) Lipid Metabolism of Comprehensive Biochemistry , 1967 Vol. 18. Elsevier , New York .
10. Damen, J., Regts, J. and Scherphof, G. Transfer and exchange of phospholipid between small unilamellar liposomes and rat plasma high density lipoproteins. Dependence on cholesterol content and phospholipid composition. Biochim. Biophys. Acta 1981 , 665 , 535-545.
11. Papahadjopoulos, D. (ed.), 1978, Liposomes and their use in biology and medicine, Ann. NY Acad.Sci. **308**, 1–412.
12. Lasic, D.D., Liposomes, Am. Sci. 1992, **80**, 20–31.
13. Lipowsky, R., The conformation of membranes, Nature 1992, **349**, 475–481.
14. Sharma, A., Liposomal delivery of antineoplastic drugs: preparation, characterization and antitumor activity of liposomal drug formulations. Ph.D. Thesis, State University of New York at Buffalo, 1994.
15. Sharma, A., Mayhew, E., Bolcsak, L., Cavanaugh, C., Harmon, P., Janoff, A., Bernacki, R.J., Activity of paclitaxel liposome formulations against human ovarian tumor xenografts. Int. J. Cancer. 1997. 71, 103- 107.
16. Sharma, A., Mayhew, E., Straubinger, R.M.,. Antitumor effect of taxol-containing liposomes in a taxol-resistant murine tumor model. Cancer Res. 1993 53, 5877 5881.
17. Sharma, A., Sharma, U.S., Straubinger, R.M., Paclitaxel-liposomes for local treatment of intraperitoneal P388 leukemia. Cancer lett. 1996. 107, 265 272.

18. Heath, T.D., Brown, C.S., Liposome dependent delivery of N-(phosphonacetyl)-L-aspartic acid to cells in vitro. J.Liposome Res. 1989.1,301-303.
19. White, J.C., Hines , L.H., Role of endocytosis and lysosomal pH in uptake of N-(phosphonacetyl)-L-aspartate and its inhibition of pyrimidine synthesis. Cancer Res. 44, 1984, 507-513.