International Journal of Pharma and Bio Sciences V1(2)2010

DEVELOPMENT AND *IN-VITRO* EVALUATION OF LIPOSOMAL GEL OF CICLOPIROX OLAMINE

ABHAY M. L. VERMA*AND S.PALANI

Institute of Pharmacy, BundelKhand University; Jhansi, (U.P.)

*Corresponding Author meet2abhay@gmail.com, meet2abhay@indiatimes.com

ABSTRACT

The objective of the present study was to prepare liposomal gel bearing anti-fungal drug (Ciclopirox olamine) to provide controlled release of drug for topical treatment of patients with dermal infections and enhancing local effect. Different formulations of Liposomes were prepared by using thin film hydration method by varying the lipid phase composition (Phosphatidylcholine / cholesterol mass ratio) with drug. These prepared liposomes were characterized for their drug loading, encapsulation efficiency, morphological character, surface charge and size analysis as well as for transmission electron microscopy. Topical liposomal gels were prepared by incorporation of liposomes into different polymers (0.5% w/V Agar base, 0.5% w/V Carrageenan and 0.5% w/V Carrageenan/Agar in 1:1 Ratio) bases and their release characteristics were investigated.

KEYWORDS

Liposome; Ciclopirox Olamine; Agar-Carrageenan Gel; Release studies

INTRODUCTION

Liposomes are single or multilayered vesicles that completely enclose an aqueous phase within one or several phospholipid bilayer membrane(s). An important aspect of liposomes is the protection that they afford as an encapsulating agent against potentially damaging conditions in external environments. Liposomes are also an important system in their own right in medical, cosmetic, and industrial applications [1, 2].

Liposomes can substantially improve drug loading, drug delivery and sustained release, thereby offering clear-cut advantages over traditional dosage forms[3]. Liposomes were first produced in England in 1961 by Alec D. Bangham, who was studying phospholipids and blood clotting[4]. It was found that phospholipids combined with water immediately formed a sphere because one end of each molecule was water soluble, while the opposite end is water insoluble. Water-soluble medications added to the water were trapped inside the aggregation of the hydrophobic ends; fat-soluble medications were incorporated into the phospholipid layer[5, 6]. There was so much research was also investigated on liposomes with various hydrophilic/hydrophobic drugs for localized and targeted drug delivery[7, 1, 8, 9].Liposomes have been proposed to be effective vesicles for delivery of antifungal drugsat the site of the infection, as their topical application results in high local concentrations of drug[10, 11, 12]. Moreover, antifungal drug released from liposome vesicles may interfere with the process of fungal growth[13 14, 15].

The aim of this paper is to evaluate liberation of Ciclopirox olamine from the gel preparations with respect to liberate antifungal drug in controlled manner to provide better

DEVELOPMENT AND *IN-VITRO* EVALUATION OF LIPOSOMAL GEL OF CICLOPIROX OLAMINE

therapeutics[16]. The polymers used for preparation of gels are hydrogel category[17, 18, 19], namely agar, Carragenan[20]. The blending effect of polymer is also performed Ciclopirox olamine is an effective agent in the protection of various fungal infection[21].

MATERIALS AND METHODS

Ciclopirox olamine (CPO) was used as model drug obtained as gift sample (Kumar organic pvt. Ltd., Bangalore). Phosphatidylcholine (PC) from Lipoid, GmbH, Germany; Cholesterol (CHL) from CDH, New Delhi were used as polymers for liposome preparation. Agar (CDH, New Delhi) and Carrageenan (Himedia laboratories, Mumbai) were used as polymers for gel preparation. Methanol and Chloroform were used as a solvent (Merck India Ltd, Mumbai). Sodium lauryl sulfate was used as surfactant (CDH, New Delhi), di-Sodium hydrogen phosphate and potassium dihydrogen phosphate (CDH, New Delhi). All chemicals used in the experiment were analytical grade and purchased from their respective commercial sources.

1. Preparation of Liposomes

Phosphatidylcholine, cholesterol and Ciclopirox olamine (CPO) were dissolved in chloroform/methanol (2:1, v/v) mixture and subsequently transferred into a pear-shaped flask connected to a Rotavapor (Büchi- type). Speed was maintained at180 r/min, vacuum applied and the thin film were formed by slow removal of the solvents at 40^0C. The lipid film was maintained under vacuum for 12hr in a desiccator to remove solvent traces and subsequently it was hydrated with a 0.9% NaCl of pH 7.4 solution at 40^0C under continuous rotation of the flask until a dispersion was formed (about 1h). The final suspension consisted of multilamellar vesicles was subjected to vertexing for two 5-min periods and kept for 30 minutes.

2. Preparation of Liposomal gels

The gels used in this study were prepared from agar, carrageen and blend of both. These gels served as drug release material after incorporating an appropriate amount of liposomes. It was done by taking 0.5 g of polymer and than slowly heated to around 80^0C and 90^0C for k-Carrageenan and agar gels respectively, for blend of polymers gel, the higher melting points temperature. Gentle stirring was needed to avoid bubble in final gel solution. All the located solution/solution mixture was cooled up to room temperature and specific amount of liposome pellet was mixed to the blend of polymers (Agar and k-Carrageenan) consisting of 50 wt.% respectively.

RESULTS

Liposomes of Ciclopirox-olamine were prepared by thin film hydration technique. These liposomes were incorporate in various gel matrices and research investigated for various characteristics and processed.

1. Characterization of Ciclopirox-olamine liposomes

The effect of the formulation variable, the lipid phase composition (PC/CHL mass ratio) on the drug entrapment efficiency and mean vesicle size of liposome vesicle is compiling in table no.1.

DEVELOPMENT AND *IN-VITRO* EVALUATION OF LIPOSOMAL GEL OF CICLOPIROX OLAMINE

Table1.
Formulation Composition and Physicochemical Properties of prepared liposomes.

Formulation Code	Composition ratio by weight (PC:CH:DRUG)	Drug Entrapped* (Mean:SD)	Percent Encapsulation Efficiency	Ratio of drug to lipid (µg of drug, mg of lipid)	Total Lipid (mg)
CPO-1	100: 10:10	7.924±0.12	79.24	72.03:1	120
CPO-2	100: 20:10	7.858±0.16	78.58	65.48:1	130
CPO-3	100: 30:10	8.131±0.13	81.31	62.55:1	140
CPO-4	100: 40:10	8.645±0.24	86.45	61.75:1	150
CPO-5	100: 50:10	8.280±0.28	82.80	55.20:1	160

Value indicates mean ± S.D. (n=3)

On increasing the amount of cholesterol in formation the encapsulation efficiency decreases that shows drug substance how high affinity for the lipid phase. Liposomes are round shape and have size in range of 307 to 843 nm (Fig 1).

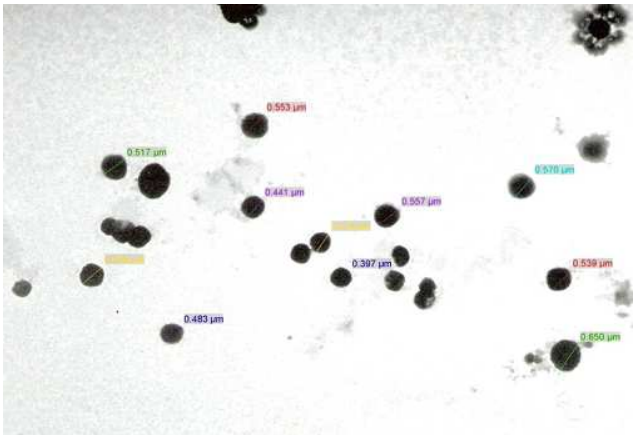

Fig.1. ***TEM photographs of drug loaded liposomes.***

Vesicle number were counted by using hemocytometer in optical Microscope (Magnus MLX-DX) and found in following ranges (table no.2).

DEVELOPMENT AND *IN-VITRO* EVALUATION OF LIPOSOMAL GEL OF CICLOPIROX OLAMINE

Table 2.

Zeta Analysis

S. No.	Formulation Code	Composition PC: Chol: Drug (mg)	Zeta Size (nm)	Zeta Analysis		Vesicle Count mm^3×1000 (n=3)
				Potential (mV)	Deviation	
1	CPO-1	400:40:40	307.4656	-23. 3	16.6	16.25±2.43
2	CPO-2	400:80:40	789.1899	-21. 5	13.2	14.38±1.95
3	CPO-3	400:120:40	656.7279	-10. 2	62.7	26.73±1.58
4	CPO-4	400:160:40	715.8384	-19. 3	15.9	21.49±2.46
5	CPO-5	400:200:40	843.5162	-20. 3	20.2	15.45±1.99

2. Micromeritic properties of the liposomes

Particle size, Size distribution and surface charge analysis is done, that shows increase in the lipid mass ratio causes increase in vesicle & vesicle size found was greater than 500 nm. This indicates that all vesicles are multilamellar in nature. Surface charges on all liposomes are negative (-) in nature**.**

3. In-vitro release studies

Physiological saline was potentially most appropriate receptor phase for drug release studies, But CPO is sparingly soluble in water, thus an appropriate medium was required that could provide sufficient solubility for the drug to maintain the required sink condition during permeation studies. To solve this problem 0.1% SLS by wt. is dissolve in the dissolution medium pH of Phosphate Saline Buffer.

The release of drug through liposomal suspension is found more than about 90% of entrapped drug, but in case of liposomal gel the release of drug is lower than 75% after 24 hours.

4. Drugs release from single polymer

The lowest Drug release through agar gel in CPO4A i.e.68.443 ± 0.56 % and in Carrageen gel was found 70.456 ± 1.03from CPO-4CA. But release from both gels is lower in comparison with liposomal suspension.

5. Drug Release through blend of polymer

Drug release from the blend of polymer gel is lower in comparison with single polymer gel it show highest release 75.162 ± 0.93 % & in case of CPO-2CA and lowest in case of CPO-4CA i.e. 68.395 ± 0.88 % was found.

CONCLUSIONS

Ciclopirox olamine (CPO) was selected for encapsulate within the phospholipid bound closed lamellar systems to explore its potential for topical application.

The increase in entrapment efficiency was attributed to the ability of cholesterol to cementing the bi-layered membrane. The entrapment efficiency data clearly suggest that the PC to CHL ratio is crucial because an enhanced CHOL level disturbs entrapment the amount of drug loaded in to liposomal vesicles. These systems were found to have good size and stability characteristics.

DEVELOPMENT AND *IN-VITRO* EVALUATION OF LIPOSOMAL GEL OF CICLOPIROX OLAMINE

In vitro drug release characteristic was performed for 24 hrs, (on liposomal suspension, single polymer gels and blend of polymers) and drug release from the liposome single polymer gels and blend of polymers was found much lower in comparison of liposomal suspensions.

Kinetic Analysis

Mathematical processing of the in vitro release data Showed that the release of Ciclopirox olamine from suspension follows concentration dependent release model (First order release of Kinetics). Single polymer gels follow Higuchi release of Kinetics (diffusion controlled release model) and the blend of polymeric liposomal gels follows Peppas & Korsmeyer model.

ACKNOWLEDGEMENTS

The authors are very thankful to Kumar Organic Pvt. Ltd. Bangalore, India, for generously providing the gift sample of Ciclopirox olamine.

REFERENCES

1. D.D. Lasic, Novel application of liposomes. Tibtech, 1998, 16: 8-17
2. D.W. Osborne, A.H. Anann, Topical Drug Delivery formulations, Marcel Dekker Inc. 42, 468-477
3. G. Storm, D. J. A. crommelin; Liposomes: quo vadis? Research focus. 1998; PSTT, 1, 1: 19-31.
4. Encyclopedia of controlled release, 1: 461-485.
5. A.D. Sezer, A.L. Bas, J. Akbuga *et al*. Encapsulation of Enrofloxacin in Liposomes I: Preparation and in vitro characterization of LUV, J. Lipo. Res., 2004, 14, 1 & 2: 77-86.
6. S.L. Law, K.J. Huang, V.H.Y. Chou, *et al*. Stability of Desmopressin loaded in Liposomes; J. Lipo. Res., 2003. 13, 3 & 4: 269 –277.
7. M.G. Dodov, E.F. Kumbaradzi, K. Goraciuova, S. Calis, M. Simonoska, A.A. Hincal, *et al*., 5-Flurouracil in topical liposome gels for automates treatment – formulation and evaluation, Acta Pharm, 2003, 53, pp. 241-250.
8. S.P. Vyas, R.K. Khar, Targeted and Controlled drug delivery; Novel Carrier system. First reprint ed., C.B.S. publications. 2004: 835-839.
9. M. Anderson, A. Onria., *et al*. The effect of different lipid components on the in-vitro stability and release kinetics of liposome formulation. Drug delivery, 2004. 11(1): 33-39.
10. A. Bhatia, O.P. Katare, R. kumar, *et al*. Tamoxifen in topical liposomes; Development, characterization and in-vitro evaluation. J. Pharm. Pharmaceut. Sci. 2004, 7(2): 252-259.
11. R. Ted, S. Barbara, O. Ida *et al*. Anti-inflammatory activity of antifungal preparations.
12. In. J. Dermatology, 1997, 36 (10): 788.
13. R. Barbara, T. Giovanni, F. Flavio, V.M. Angela, *et al*. Ketorolac Tromethamine liposomes; Encapsulation and release studies, J. Lipo. Res., 2005, 15: 175-185.
14. R. Agarwal, O. P. katare, *et al*. Preparation and in-vitro evaluation of miconazole nitrate loaded topical liposomes. Pharmaceutical technology, 2002: 48-59.
15. A. Bhatia, R. kumar, O.P. kalare *et al*. Tamoxigen in topical liposomes; development, characterization and in-vitro evaluation. J. Pharm. Pharma. Sci, 2004, (2): 252-259.
16. Z. Pavelic, N. S. Basnet, I. Jalsenjak *et al*. liposomal get with chloramphenicol; characterization and in-vitro release. Acta Pharm., 2004, 54: 319-330.
17. N. Markus, K. Donica, S. Michael, S. Martin, K.H. Christian, H. Bernhard. *et al*. Ciclopirox olamine treatment affects the expression pattern of *Candida albicans* Genes encoding virulence factors, iron metabolism proteins,

DEVELOPMENT AND *IN-VITRO* EVALUATION OF LIPOSOMAL GEL OF CICLOPIROX OLAMINE

and drug resistance factors. 2003, 47, 6: 1805-1817.

18. E. Amici, A.H. Clark, V. Norm, N.B. Johnson, *et al*. Interpenetrating network formation in gellan-agarose composites. Biomacromolecules, 2000, 1: 721-729.
19. A.S. Hoffman, Hydrogels for biomedical applications. Adv. Drug Delivery Rev., 2002, 43: 3-12.
20. Z. Vitkova, M. Subova, E. Cirbusova, M. Kmet'ova, J. Cizmarik, *et al*. Pharmaceutical availability of potential drugs from hydrogels. Acta Facultatis pharmaceuticae Universitatis Comenianae, Tomus LI. 2004: 245-250.
21. E. Amici, A.H. Clark, V. Norm, N.B. Johnson, *et al*. Interpenetrating network formation in agarose- k-Carrageenan composites. Biomacromolecules, 2000, 1: 721-729.
22. J. Liu, S. Lin, L. Li, E. Liu *et al*. Release of Theophylline from polymer blend hydrogels. Int. J. Pharm., 2005, 298: 117-125.