Research Article

FORMULATION AND IN VITRO EVALUATION OF NIOSOME ENCAPSULATED ACYCLOVIR

Manivannan Rangasamy*, Balasubramaniam Ayyasamy, Senthilkumar Raju[1],Sandeep Gummadevelly[2] and Sanaullah Shaik[2]

[1] Department of Pharm.Chemistry, Swamy Vivekanandha College of pharmacy, Elayampalayam, Namakkal, Tamilnadu, India.

[2]Department of Pharmaceutics, J.K.K.M.M.R.F, College of Pharmacy, B.Komarapalayam, Pin Code: 638 183, Namakkal, Tamilnadu, India.

*For correspondence:*Manivannan Rangasamy, Dept.of Pharmaceutics, JKKMMRF's college of Pharmacy, B. Komarapalayam, Namakkal, Tamilnadu, India.
E-mail: manivannan_biotech @ yahoo.co.in
Received on: 10-07-2008; Accepted on :02-10-2008

ABSTRACT

Acyclovir entrapped niosomes were prepared by hand shaking and ether injection process with different ratios of (1:1, 1;2 and 1:3) cholesterol (CHOL) and Span-80 (Non-ionic surfactant).The niosomes prepared were in the size range of 0.5-5 microns in the case of hand shaking process and 0.5-2.5 microns in the case of Ether injection process. The order of encapsulation efficiency increases when span-80 concentration was increased. In-vitro release study on acyclovir niosomes indicates 76.64% release for formulation prepared with CHOL: Span-80 (1:1) and it takes an extended period of 1 day and 16 h for release.

Key words: Acyclovir, Niosomes, Cholesterol, Span-80, Encapsulation.

INTRODUCTION

Non-ionic surfactant vesicles (Niosomes) are similar to liposome can be prepared with cholesterol, surfactant and water[1]. In recent years, niosomes have been extensively studied for their potential to serve as carrier for delivery of drugs, antigens, hormones and other bioactive agents. Niosomes are biodegradable, biocompatible, non-toxic and capable of encapsulating large quantities of material in relatively smaller volume of vesicles[2]. Encapsulation of a drug in vesicular structure can be predicted to prolong the existence of the drug in the systemic circulation and thus enhance penetration into target tissue and reduce toxicity[3].

Acyclovir is 2-amino-1, 9- [(2-hydroxy ethoxy) methyl]-6h-purine-6-one. It is currently used for the prevention and treatment of Herps simplex virus (HSV) and Varicelle-Zoster virus (VZV) infections[4]. Acyclovir has a short biological half life of 2.5h and oral bioavailability is (15%-30%)[5], which necessitates multiply daily dosing and hence a novel delivery system such as niosomes, can be used to encapsulate the drug so that it maintain a therapeutic plasma concentration for a longer period of time, thereby increasing the bioavailability of the drug.

In the present study, acyclovir encapsulated niosomes were formulated and evaluated for their in-vitro characteristic and an attempt to improve the oral bioavailability of the drug.

MATERIALS AND METHODS

Acyclovir was a gift sample from Micro labs, Bangalore, India. Cholesterol, Span-80 was procured from Loba chemicals and S.D. Fine chemicals, Mumbai, India. All other chemicals used were of analytical grade.

Hand-Shaking process

Niosomes containing acyclovir was prepared with cholesterol, Span-80 in the proportion of 1:1 ratio by taking about 50mg of CHOL and Span-80 (50mg equivalent), accurately weighed and transferred into a clean round bottom flask. Then 10ml of diethyl ether was added and flask was continuously vortexed to form a thin film along the sides of the flask. An appropriate amount of acyclovir was dissolved in phosphate buffer saline (pH 7.4), and this was added slowly to the round bottom flask having thin film of surfactant and cholesterol and vortexed continuously for a period of 30min at room temperature until a good dispersion of the mixture was obtained. Similarly other two ratios 1:2 and 1:3 were prepared. Dispersions were immediately stored in the refrigerator [6&7].

Ether Injection process

Niosomes containing acyclovir of 1:1 ratio was prepared by taking CHOL (50mg), Span-80 (50mg equivalent) in a 50ml beaker. The mixture was dissolved in diethyl ether and the solution was slowly injected into a beaker containing acyclovir in phosphate buffer saline (pH 7.4). The temperature maintained during the injection was 40-60^{0}C. The differences in temperature between phases cause rapid vaporization of ether resulting in spontaneous vesiculation. Similarly other two ratios 1:2 and 1:3 were prepared. In all the six ratios, constant amount of cholesterol and drug were used [8&9].

VESICLE SIZE DETERMINATION

It was carried out using an optical microscopy with a calibrated eyepiece micrometer. About 200 niosomes were measured individually, average was taken, and their size range, mean diameter were calculated.

ENCAPSULATION EFFICIENCY

Acyclovir encapsulated niosomes were separated from unentrapped drug by dialysis method for 24 hrs. The formulation were transferred into a standard flask, lysed with 2.5% Sodium Lauryl Sulphate solution, and then incubated at 37 ± 1^{0}C for 2hrs. Then it is filtered through whatman filter paper and the absorbance of the resulting solution was measured spectrometrically at 253 nm[10].

IN-VITRO RELEASE STUDIES

The niosomal preparation of acyclovir was placed in a dialysis bag with an effective length of 8cm, which acts as a donar compartment. Dialysis bag was placed in a beaker containing 250ml of phosphate buffer saline of pH 7.4, which acts as receptor compartment. The temperature of receptor medium maintained at 37 ± 1^{0}C and the medium was agitated at a moderate speed using magnetic stirrer. Aliquots of 5ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analyzed at 253nm. Phosphate buffer saline was used as blank[11&12].

RESULTS AND DISCUSSION

Prepared niosomes reveal that they are discrete and spherical shape, and some vesicles are slightly elongated. The size range for the niosomes prepared by hand-shaking process was from (0.5-5µ) with an average size of 2.7µ. Acyclovir niosomes prepared by ether injection method has a size range of 0.5-2.5µ with an average size of 1.5µ. The niosome vesicles prepared by hand shaking process was larger in size than vesicles prepared by ether injection process. This may be due to the passage of cholesterol and span-80 solution through an orifice into the drug solution in a beaker.

The entrapment efficiency was 56% for formulation F1whereas it was 65%, 84%, 45%, 54%, and 70% for formulations F2, F3, F4, F5 and F6, respectively. This explains that the encapsulation efficiency was increased when Span-80 concentration was increased, while cholesterol content was maintained at a constant value. The amount of drug entrapped by hand shaking process was little more than ether injection process. This may be due to vortexing was only carried out during hand shaking process, whereas in the case of ether injection process vortexing and injection takes place simultaneously. Moreover, the vesicles obtained by hand shaking process were larger in size, due to this chance for increased entrapment of drug in the aqueous compartment or in the lipid bilayers. The vesicles obtained by ether injection were more or less small and thereby it may lead to decreased entrapment.The Fig.1 and Fig.2 depicts the in-vitro cumulative drug release of acyclovir niosomes prepared by hand shaking and ether injection process.

From the graph of in-vitro drug release studies, it observed that in the formulation F1 & F4, the entrapment was less, but entrapped drug takes longer time for release,1day and 16h.But in case of F3 & F6, the amount of drug entrapped was more and it takes shorter time period for release, 24h

Table 1: Encapsulation efficiency of acyclovir niosomes

Formulation code	Cholesterol:Span-80	Method of Preparation	Amount of Drug Incorporated (in mg)	Amount of Drug Entrapped (in mg)	Percentage of acyclovir Entrapped in niosome
F1	1:1	Hand Shaking Process	50	27.84	56.12
F2	1:2			32.68	65.26
F3	1:3			41.92	84.22
F4	1:1	Ether Injection process	50	22.84	45.16
F5	1:2			27.02	53.44
F6	1:3			35.14	69.72

Fig.1: In-vitro cumulative release of acyclovir Niosomes prepared by hand shaking method

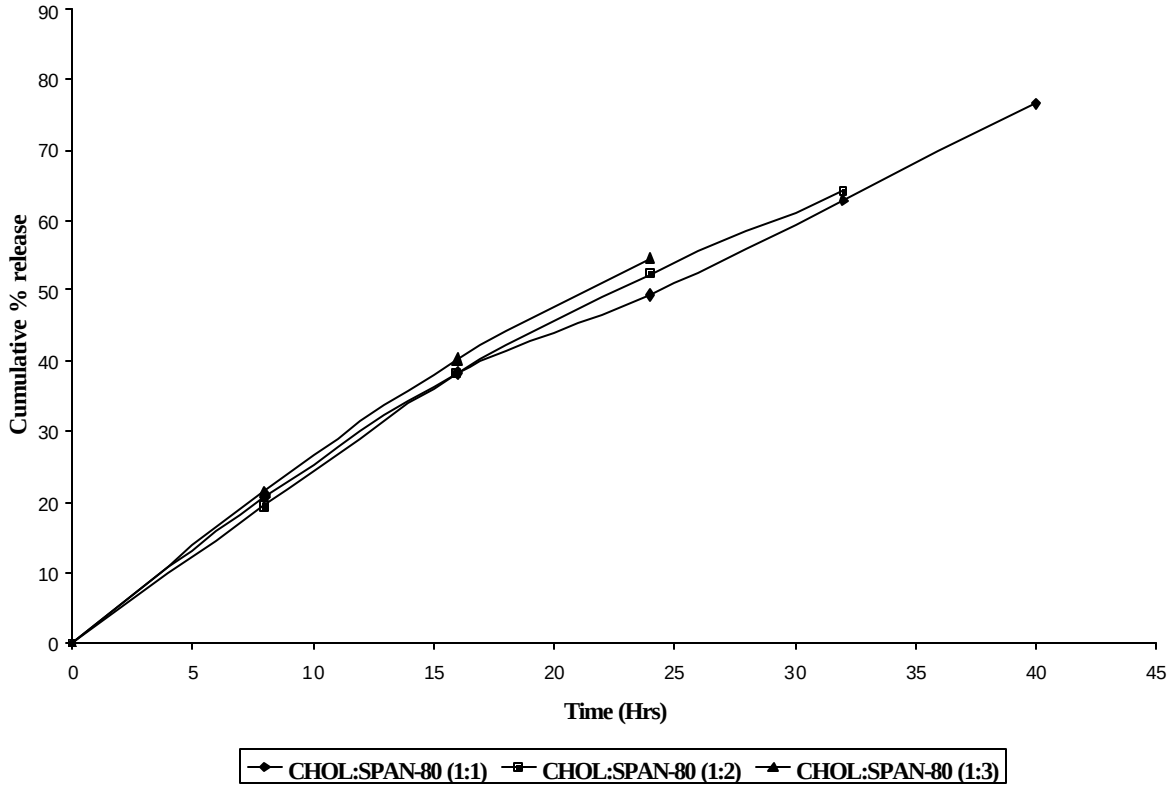
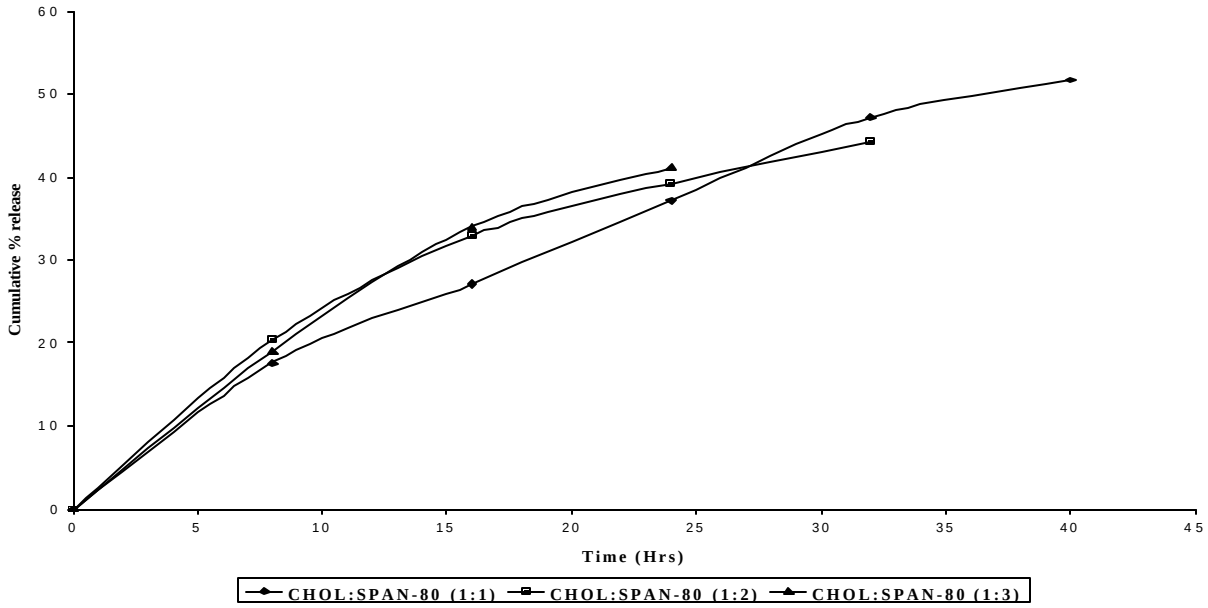


Fig 2: In vitro cumulative release of acyclovir niosomes prepared by ether injection method



only. This shows that cholesterol forms a film around the vesicles and increases the micro viscosity of the bi-layer. The thickness of the film depends upon the concentration of Cholesterol. This shows inclusion of cholesterol improves drug retention time and thus reduces permeability.

To conclude, acyclovir was successfully encapsulated into niosomes by hand shaking and ether injection process. The vesicles were quite stable and the drug release was extended upto 1 day, 16h. Niosomes could be used as a drug carrier for Acyclovir, for producing prolonged activity and simultaneously reducing side effects.

REFERENCES

1. Chandraprakash, K.S, Udupa, N, Umadevi, P and Pillai G.K, Formulation and Evaluation of Methotrexate niosomes, Ind. J. Pharm. Sci, 1992, 28, 197-200.
2. Malhotra, M and Jain N.K, Niosomes as drug carriers, Indian Drugs, 1994, 31(3), 81-86.
3. Toadd, J.S, Modest, L.T, Rassow, P.W and Tokes, A.A, Biochem. Pharmcol, 1982, 31, 542-546.
4. Erlich K.S, Management of Herps simplex and Varicella-Zoster virus infections, West. J. Med, 1987, 166, 211-215.
5. Miranda P, Krasny H.C, Page D.A, Elion C.B, Species differences in the disposition of acyclovir, Ame. J. med, 1982, 73, 31-35.
6. Paola Palozza, Rita Muzzalupo, Sonia Trombino, Ambra Valdannini and Nevio Picci, Solubilization and Stabilization of ß-carotene in niosomes: Delivery to cultured cells, Chemistry and Physics of lipids, 139 (1), 2006, 32-42.
7. Arunya Manosroi, Paveena Wongtrakul and Hidei Sakai, Characterization of Vesicles prepared with various non-ionic surfactants mixed with cholesterol, Colloids and surfaces B, Biointerfaces, 30, 2003, 129-138.
8. Alok Namdeo, P.R, Mishra, A.J, Khopade and Jain, N.K, Formulation and Evaluation of niosomes encapsulated Indomethacin, Indian Drugs, 36 (6), 1999, 378-380.
9. Liberman, H.A, Reiger, M and Banker, G.S, In Pharmaceutical dosage forms disperse systems, 2nd edition, Vol.2, Marcel Dekker, Newyork, 1989, 567-602.
10. Parthasarathi, G, Udupa, N, Pillai G.K, Formulation and In-vitro Evaluation of Vincristine Encapsulated niosomes, Ind. J. Pharm. Sci, 1994, 56(3), 90-95.
11. Balasubramaniam, A, Dasaratha, D.M and Vangala A.K, Formulation and In-vitro Evaluation of niosomes encapsulated Daunorubicin hydrochloride, Indian Drugs, 39 (1), 2002, 27-31.
12. Stafford's, Baillie, A.J and Florence, A.T, J. Pharm. Pharmacol. Suppl, 1988, 40, 26.

Source of support: Nil, Conflict of interest: None Declared