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FORMULATION AND EVALUATION OF 5-FLUROURACIL NIOSOMES

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ABSTRACT

5-flurouracil is a nucleotide analogue that is uracil in which the hydrogen at position 5 is replaced by fluorine.It is an antineoplastic agent which acts as an antimetabolite following conversion to the active deoxynucleotide it inhibits DNA synthesis And so slows tumor growth. It has a role a xenobiotic, a radiosentizing agent as an antineoplastic agent as an immunosuppressive agent and an antimetabolite.It is a nucleobase analogue and an organofluorine compound.It derives from uracil The concentration of drug in the formula was increased to study its impact on drug entrapment efficiency. In future invivo studies should be done for ensuring the effectiveness of the formulation.

KEYWORDS: 5-flurouracil, maldodextrin, cholesterol, uracil, span80, sodium lauryl sulphate, Diethyl ether, sodium hydroxide.

INTRODUCTION

Niosomes are multilamellar or unilamellar vehicles where in an aqueous solution of solute is enclosed in highly ordered bilateral made up of non-ionic surfactant with or without cholesterol and diethyl phosphate and exhibit behavior similar to liposomes. Many synthetic amphiphiles from vehicles, but most of them are ionic and relatively toxic, they generally unsuitable for use as drug carriers. The vehicles are formed on hydration of mixture of cholesterol and a single alkyl- chain non-ionic and non- toxic surfactant. Since then a number of non-ionic surfactant have been used to prepare vehicles polyglycerol alkyd ethers Glycosyl dialysis ethers, crown ethers, Ester- linked surfactant polyoxyethyiene alkyl ethers, Brij and series of spans and Tweens. Resultant vehicles have been termed niosomes.Non-ionic surfactant used in Niosomes are non- toxic and no toxic effects have been reported so far in animal studies due to the use of Niosomes as drug carrier. Niosomes have unique advantages over liposomes. Niosomes are quite stable structures even in the emulsified form.They require no special conditions such as low temperature or inert atmosphere for protection or storage and are chemically stable. Relatively low cost of materials makes it suitable for industrial manufacture. Niosomes entrap solute in a manner another to liposomes. They are osmotically active and are stable as their own as well as increase the stability of the entrapped drugs. Handling and storage of surfactants require no special conditions. Niosomes posses an infrastructure consisting of hydrophilic and hydrophobic moieties together and as a result can accommodate drug molecules with a wide range of solubility. They exhibit flexibility in structural characteristics and are designed according to the desired situation. Niosomes improve the oral bioavailability of poorly absorbed drugs and enhance skin penetration of drug.

MATERIALS

5-Fluorouracil was gifted sample from Biochem Pharmaceuticals, Mumbai, Cholesterol - Loba chemicals, Span 80 - SD Fine chemicals, Diethyl ether - CP laboratories, Sodium Lauryl Sulphate - Nice chemicals, Potassium Dihydrogen Phosphate - Nice chemicals, Glycerin IP - Nice chemicals, Sodium hydroxide - Fine chemicals Limited, Maltodextrin – Himedia.

Standard Calibration Curve for 5- Flurouracil Niosomes

5-Fluorouracil can be estimated spectrometrically at 304 nm using phosphate buffer pH 7.4 as dilution medium. The solution found to obey beer-lambert's law in the range of 2-14 µg/ml.

Preparation of pH 7.4 phosphate buffer

Place 50.0ml of 0.2M potassium dihydrogen phosphate in a 200ml volumetric flask, add the specified volume of 39.1ml of 0.2M sodium hydroxide and then add distilled water to volume.

Preparation of standard drug solution

Stock solution: 100mg 5- Fluorouracil was dissolved in 100ml of pH 7.4 phosphate buffer so as to get a solution 1000 µg/ml concentration.

Standard solution: 2 ml of stock solution was made to 100ml with pH 7.4 phosphate buffer thus giving a concentration of 20µg/ml. Aliquot of standard drug solution ranging from 1ml to 7ml were transferred in to 10ml volumetric flask and were diluted up to the mark with pH 7.4 phosphate buffer. Thus the final concentration ranges from 2-14 µg/ml. Absorbance of each solution was measured at 304 nm[36] against distilled water as a blank. A plot of concentration of drug versus absorbance was made.

Preparation of Niosomes

The slurry method is selected for the preparation of niosomes using maltodextrin as a carrier. The drug solution is added to the surfactant mixture, which contains ether, surfactant and cholesterol. The resultant solution is added to the flask containing maltodextrin. It is rotated in the rotary shaker to get the maltodextrin coated with surfactant mixture. Meanwhile if necessary it is kept in the water bath to evaporate the ether from the flask.

***In-vitro* drug release**

The formulation were subjected to centrifugation prior to *in vitro* drug release study. The centrifugation was done to remove unentrapped drug in the formulation, which will affect the release study of 5- Flurouracil drug from vesicles. Two –step centrifugation was carried out as done in drug entrapment. To the supernatant in the second step of centrifugation process, about 2.5 ml of pH 7.4 phosphate buffer was added. This was placed in the open – end glass cylinder in which one side was covered with dialysis bag. The glass cylinder was immersed in the beaker containing 100 ml of pH 7.4 phosphate buffer. Magnetic stirrer was used. Sample were collected for every hour upto 24 hrs. At each time when an aliquot of sample were withdrawn, the same quantity of buffer was replaced to the beaker.

RESULTS AND DISCUSSION

Preformulation Studies

Preformulation studies were performed using FT –IR spectrometer. The IR spectrum of pure drug, polymer (cholesterol) and formulation (PRN 1) were compared. The characteristic absorption peaks of 5- Flurouracil were observed in pure drug as well in the formulation PRN1, which indicate that the drug was incorporated in the formulation. Absorption peaks obtained at 1604.17, 1209.1, 2365, 1646 wave number were taken for study.

Effect of drug concentration

5-FU5,5-FU10, 5-FU20 formulation were prepared and they were tested for percentage drug entrapment, vesicles size using optical microscopywere obtained in all the formulations and the percentage drug entrapment has no significant change and found to be around 87%. Drug concentration has not altered the behavior of niosome preparation.Although the entrapment efficiency did not depend on the 5- Flurouracil concentrations, observations of niosome dispersions under optical microscope showed more crystalline drug existed in the preparation containing 20 mg of 5- Flurouracil (5-FU20)**.**In the preparation containing 10 mg of 5- Flurouracil some crystalline drug existed which can be seen in SEM photographs. 5-FU5 containing 5 mg of 5- Flurouracil shows no crystalline drug. Therefore, lower 5-Flurouracil concentration of 5 -10 mg in the formulation may be optimum.

Percent Drug Entrapment

	Percent drug entrapment	
Formulation Code	**Vesicle formation**	**Percent drug entrapment**
5-FU5	Spherical vesicles with no crystalline drug	86.3
5-FU10	Spherical vesicles with few crystalline drug	87.4
5-FU20	Spherical vesicles with numerous crystalline drug	84.9

Percent Drug Entrapment

Formulation	**Absorbance at**	**Concentration**	**Concentration**	**Dilution**
Code	304 nm	(µg/ml)	(mg/20 ml)	factor
5-FU5	0.253	4.32	1.73	400
5-FU10	0.197	3.22	1.29	400
5-FU20	0.216	3.77	1.51	400

Effect of Maltodextrin

The formulations containing various proportions of maltodextrin were prepared and tested for percent drug entrapment, solubility chacteristics in distilled water. Formulation MDN 0.5, MDN 1.0, MDN 2 have 500 mg, 1000 mg and 2000mg of maltodextrin respectively.All the formulations were soluble in 10 ml of the aqueous solution; vesicles were formed. The content of maltodextrin in niosomes powder was small in MDN 0.5. The concentration of maltodextrin has no measurable effect on entrapment efficiency of 5- Flurouracil on comparison of surfactant: maltodextrin ratios of 1:20, 1:40 (50 mg: 1000mg, 50 mg: 2000 mg).

The preparation of niosomes yields small amount when concentration of maltodextrin is 10: 1 to the surfactant (500 mg of maltodextrin) in MDN 0.5 formulation.The insufficient surface for the coating of surfactant mixture in the formulation MDN 0.5 may be due to the small

quantity of carrier used. This leads to decrease in the drug entrapment.

Percent Drug Entrapment

Formulation Code	Absorbance at 304 nm	Conc. (µg/ml)	Conc. mg / 10 ml)	Dilution factor	% Drug entrapment
MDN 0.5	0.334	6.0	2.4	400	76.1
MDN 1	0.189	3.17	1.27	400	87.3
MDN 2	0.209	3.57	1.43	400	85.7

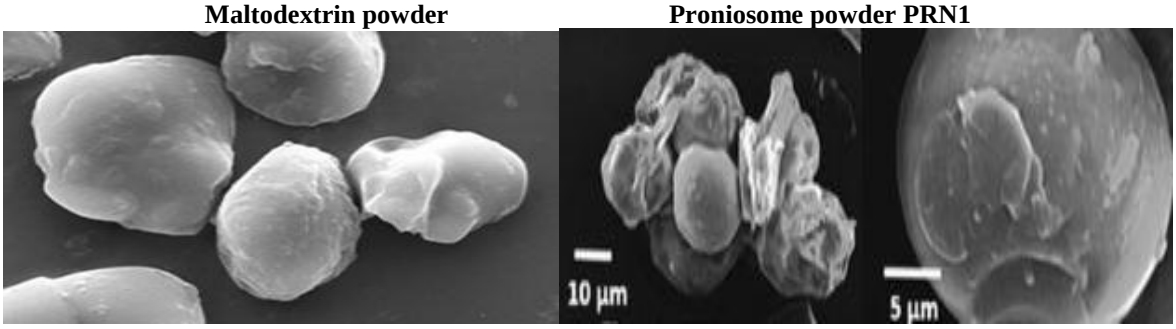
Sem (Powder Morphology)

SEM of uncoated maltodextrin and dry niosome powder reveals that there was slight difference in their appearance.Maltodextrin powder particle was magnified at 600 X and proniosome powder at 300 X. From these photographs proniosome powder was found to be large.The shapes of uncoated maltodextrin were mostly spherical and smooth surface whereas dry niosome was irregular and nearly spherical. Many adhered particles were on the surface.This shows that there was deformation after adding surfactant mixture to the carrier. From the photographs of uncoated carrier and surfactant coated preparation the drug loaded particle was clearly seen.The size of uncoated maltodextrin ranges from 200µ-1200µ whereas niosome powder ranges from 250µ - 1500µ. The size measurements add an additional confirmation to the drug loaded powder since fig 2 showed larger niosome powder particle.

Measurement of size of the particle

Magnification of size was indicated on the SEM negatives and size of particles can be measured.

Maltodextrin powder **Proniosome powder PRN1**



Rehydration Characteristics

5,10,15 ml of distilled water were added to PRN 1 formulation. They are rehydrated by agitation or shaking, Niosomal dispersions were obtained when different volumes were added to niosome powder. All the dispersions give niosome vesicle when viewed under optical microscope. This indicates that niosome powder can be rehydrated with any desired volumes of 5, 10,15ml of distilled water depending upon the requirement. It is convenient to administer the encapsulated drug through I.V (when rehydrated with 5 ml), through I.M. (when rehydrated with 10 ml), through Oral (when rehydrated with 15 ml of the distilled water).

Osmotic Shock

All the formulation were subjected to osmotic shock study by incubating in 45 ml each of three tonicity media for 3 hrs. After 3 hr, the vesicle size was observed under optical microscope. Vesicles of all the formulations that are incubated in 0.5% Nacl and 1.5 % Nacl were swelled and shrinked respectively. Whereas the size of vesicles of the formulation incubated in 0.9% Nacl was unchanged. The amount of drug leached if any was measured by talking sample at every 1 hr, for 3 hrs. The absorbance was measured using UV VIS spectrophotometer for the presence of 5-Flurouracil. Among PRN formulations, PRN 1 and among 5-FU formulations 5-FU20 was selected. The conventional niosomes was also taken for study.

Osmotic shock studies after 1 hour (in 0.5% Nacl).

Formulation Code	Absorbance at 304nm	Concentration (µg/ml)	Concentration (mg / 50 ml)	Dilution factor
PRN 1	0.531	10	0.5	------
5-FU20	0.316	6	1.2	200

Formulation Code	Absorbance at 304nm	Concentration (µg/ml)	Concentration (mg / 50 ml)	Dilution factor
PRN 1	0.309	5	1	200
5-FU20	0.701	13.5	2.7	200
CON NIO	0.356	1.3	1.3	200

Osmotic shock studies after 2 hour (in 0.5% Nacl).

Formulation Code	Absorbance at 304nm	Concentration (µg/ml)	Concentration (mg / 50 ml)	Dilution factor
PRN 1	0.587	11	2.2	200
5-FU20	0.726	14	5.6	400
CON NIO	0.644	12.5	2.5	200

Osmotic shock studies after 3 hour (in 0.5% Nacl).

Formulation Code	Absorbance at 304nm	Concentration (µg/ml)	Concentration (mg / 50 ml)	Dilution factor
PRN 1	0.627	12.5	7.7	625
5-FU20	0.589	6.47	16.1	2500
CON NIO	0.336	11.51	7.5	625

After 3 hours, all the formulations in isotonic media showed no change in the vesicle size when viewed under optical microscope. The vesicles swell in hypotonic media; whereas they skrinked incubated in hypotonic media.This swelling and skrinking were due to osmosis. The vesicle size in isotonic media (0.9% Nacl) remains unchanged. This reveals that these formulations should be dispersed in isotonic media no matter in which route it is interested to administer.

***In-Vitro* Drug Release**

Overall release profile of 5- Fluorouracil from noisome dispersions prepared from niosomes or by conventional methods show little difference.This implies that noisome formed from niosomes has satisfactory drug release in the diffusion medium.

***In vitro* Drug Release Profile of Formulation PRN 1**

Time in Hours	Absorbance at 304nm	Concentration (µg/ml)	Amount of released in mg/ml	Cumulative Amount Released in mg	Cumulative % drug release
0	0.000	0.000	0.0000	0.00	0.00
0.5	0.091	1.833	0.0183	1.83	18.33±2.34
1	0.101	2.037	0.0204	2.05	20.55±1.73
2	0.146	2.941	0.0294	2.98	29.80±1.94
4	0.219	4.405	0.0441	4.47	44.74±2.89
6	0.257	5.177	0.0518	5.29	52.89±3.67
8	0.297	5.983	0.0598	6.15	61.47±3.35
10	0.335	6.748	0.0675	6.97	69.72±4.25
12	0.368	7.413	0.0741	7.70	77.04±2.34
16	0.387	7.796	0.0780	8.16	81.61±2.50
20	0.411	8.279	0.0828	8.72	87.22±2.72
24	0.437	8.803	0.0880	9.33	93.29±1.49

SUMMARY AND CONCLUSION

Among Vesicular drug delivery systems, Niosomes are economic, easy to economic, easy to prepare. The shelf life of the preparation threatens the formulator to find an alternative form, niosomes. The dry product (niosomes) improve shelf life, entrapment efficiency of drug and possess good rehydration characteristics, appreciable flow properties. The formulations were made with various proportions of cholesterol, Span 80 and the carrier maltodextrin.The concentration of drug in the formulations was increased to study its impact on drug entrapment efficiency. The optimum concentration for the drug to be entrapped was 5 -10 mg. The optimum concentration for the carrier was found to be 1000 mg. Conventional niosomes in the ratio of 1:1 (Chol:span 80) was taken as a control for comparing Invitro drug release with prepared niosomal formulations (PRN 1, PRN 2, PRN 3). The release study of proniosomes shows no much difference with conventional niosomes.This formulation can be scalable for producing niosomes for delivery of hydrophilic and lipophilic drugs. Apart from these, niosomes are easy to handle, transportation, long stability than conventional niosomes.Maltodextrin carrier overcomes several difficulties faced by preparation using Sorbitol. Thus it is promising carrier for targeting potent drugs. In this work, invitro studies for niosomes were

conducted and found to have better release of drug. In future, invivo studies should be done for ensuring the effectiveness of the formulation.Since it contains Maltodextrin, Cholesterol, this formulation may come under Nutraceutical. Maltodextrin found to give caloric value as other polysaccharides. One of the primary goals of subsequent research was to study the effect of formulation on diabetes patients.niosomal formulation is not contraindicated to the Hypercholestremia patients. The cholesterol content in the formulation was reduced since it takes up the required cholesterol from erythrocytes and make them less vulnerable to destabilization of lipid layer.

REFERENCES

1. Agarwal, R, Katare, O.P, Vyas, S.P. Preparation and *in vitro* evaluation of niosomal delivery systems for antipsoriatic drug dithranol. Int J Pharm, 2001; 228(1-2): 43-52.
2. Agarwal, R, Katare, O.P. Preparation and *in vitro* evaluation of miconazole nitrate loaded topical liposomes. Pharm Tech, 2002; 26(11): 48-60.
3. Arunothayanun, P, Sooksawate, T, Florence, A.T, Extrusion of niosomes from capillaries: approaches to a pulsed delivery device. Journal of Controlled release, 1999; 60(2-3): 391-397.
4. Arunothayanun, P, Bernard, M.S, Craig, D.Q, Uchegbu, I.F, Florence, A.T. The effect of processing variables on the Physical characteristics of non-ionic surfactant vesicles (niosome) formed from a hexadecyl diglycerol ether. Int J Pharm, 2000; 201(1): 7-14.
5. Azeem, A, Anwer, M.K, Talegaonkar, S. Niosomes in sustained and targeted drug delivery: some recent advances. J Drug Target, 2009; 17(9): 671-89.
6. Baillie, A.J, Florence, A.T, Hume, L.R, Muirhead, G.T, Rogerson, A. Preparation and properties of niosomes – non ionic surfactant vesicles. J Pharm Pharmacol, 1985; 37(12): 863-8.
7. Balasubramanium, A, Dasaratha, D.M, Vangala, A.K, Formulation and in-vitro evaluation of niosomes encapsulated Daunorubicin hydrochloride. Indian Drugs, 2002; 39(1): 27-31.
8. Bangham, A.D, Standish, M.M, Watkins, J.C.Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol., 2000; 13(1): 238-52.
9. Barasch, A, Griffin, A.V. Miconazole revisited: New evidence of antifungal efficacy from laboratory and clinical trials. Future Microbiol, 2008; 3(3): 265-69.
10. Baroni , A, Buommino, E, Gregorio, V.D, Ruocco, E, Ruocco, V, Wolf, R.Structure and function of the epidermis related to barrier properties. Clin Dermatol, 2012; 30(3): 257-62.
11. Bayindir, Z.S, Yuksel, N. Provesicles as novel drug delivery systems. Curr Pharm Biotechnol, 2015; 16(4): 344-64.
12. Bennett, J.E. Antimircrobial agents-Antifungal agents. In: Gillman, G.A (11th ed.) The Pharmacological basis of therapeutics. New York: Mcgraw-Hill, 2006; 1225-41.
13. Bouwstra, J.A, Honeywell-nguyen, P.L. Skin structure and mode of action of vesicles. Adv Drug Deliv Rev., 2002; 54(1): S41-S55.