ASIAN JOURNAL OF PHARMACY & LIFE SCIENCE

In-vitro drug release profile of Acyclovir from Niosomes formed with different Sorbitan esters

Anupriya kapoor*, R.Gahoi, D.Kumar

Advance institute of pharmaceutical engineering and research

Faculty of pharmacy, integral university Kanpur India

Abstract

Niosomes have been reported as a possible approach to deliver the drug to ophthalmic cavity. Niosomes were formed using sorbitan esters (Span 20, 40, 60, and 80) and cholesterol in different molar ratio using Acyclovir as the model drug; Niosomes were formed using Reverse phase evaporation method. The so formed Niosomes were characterized for their in-vitro drug release efficiency, the results indicated that more sustained release pattern can be obtained by incorporating the drug in Niosomes formed with Span60.

Key words: *Sorbitan esters, Niosome, Reverse phase evaporation*

Corresponding Author's E-mail: - anupriya321@gmail.com

Received: 01/02/2011 Accepted: 03/03/2011

Introduction

Non-ionic surfactant vesicles (Niosomes or NSVS) are widely studied as an alternative to hydrated surfactant monomers. Non-ionic surfactants of wide structural types have been found to be useful alternatives to phospholipids in fabrication of vesicular system.

They are the microlamellar structures formed on admixing of Non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media.

The aim of the present study was to prepare stable Niosomes of Acyclovir for ocular use, which has got advantages over conventional dosage forms. Vesicles were prepared with the help of chemically stable surfactants, ie. Sorbitan esters (span) using cholesterol as a stabilizing agent. The formed Niosomes were characterized for their in-vitro release profile in phosphate buffer, Acyclovir is an antiviral drug used against Herpes and Varicella Zoster virus. It is a deoxyguanosine analogue which is used in the treatment of Herpes simplex keratitis, frequent application of eye ointment, 5 times a day is required since there are chances of drug drainage due to lacrimation, tear dilution of drug etc. to overcome this loss of drug Niosomes of Acyclovir were prepared.

Materials and method

Materials

Cholesterol, dicetyl phosphate (DCP), Sorbitan esters, Chloroform were purchased from CDH (India), Acyclovir was obtained as gift sample. Dialysis membrane was purchased form Himedia Laboratories Ltd, Mumbai, India. Methanol was purchased from E. Merck India Ltd. Mumbai, India.

Method

Niosomes of Acyclovir were prepared by reverse phase evaporation method, accurately weighed amount of surfactant and cholesterol along with dicetyl phosphate were dissolved in Chloroform and Methanol mixture (2:1). The solvent system containing surfactant and cholesterol was placed in a round bottom flask, chloroform methanol mixture was evaporated at 55ºC under reduced pressure at 150 rpm using Rotary evaporator (Buchi 461, Switzerland), after the solvent was evaporated the thin film formed at the walls of the flask was re-dissolved by using ether and drug in 4 ml of acetone, 6 ml of phosphate buffer of pH 7.4, the mixture was vortexed for 5 minutes and then swirled by hand, again it was vortexed for 10 minutes. The dispersion was allowed to evaporate; hydration was done by using phosphate buffer of pH 7.4 which was followed by Rotary evaporation for 15 minutes. Left for overnight, all the steps were carried under laminar air flow bench.

Formulation code

Formulation code for Span20

Formulation code	Surfactant (mmol)	Cholesterol (mmol)	Chloroform : methanol (ml)	Drug (mmol)	DCP (mg)
NMA1	10	0	2:1	0.886	5
NMA2	9	1	2:1	0.886	5
NMA3	8	2	2:1	0.886	5
NMA4	7	3	2:1	0.886	5
NMA5	6	4	2:1	0.886	5
NMA6	5	5	2:1	0.886	5
NMA7	4	6	2:1	0.886	5

Similarly NMB, NMC, NMD for Span 40, 60, 80 respectively

In-vitro release study through dialysis bag

The niosomal preparation of Acyclovir was placed in a dialysis bag of effective length 8 cm, which acts as a donor compartment. Dialysis bag was placed in a beaker containing 250 ml of phosphate buffer saline of pH 7.4, which acts as a receptor compartment. The temperature of receptor compartment was maintained at 37±1ºC and the media was agitated at a moderate speed using a magnetic stirrer. Aliquots of sample (5 ml) were withdrawn periodically at regular interval of time for 9 hours, and replaced with same volume of fresh phosphate buffer after each withdrawal. The collected samples were analyzed at 252 nm by using phosphate buffer saline as blank.

Data treatment

In-vitro dissolution has been recognized as an important element in drug development. Under certain condition it has been used as a substitute for the assessment of bioequivalence. Several theories/kinetics models describe drug dissolution from immediate and modified release dosage form. The quantitative interpretation of the values obtained in the dissolution assay is facilitated by the use of a generic equation that mathematically translates the dissolution curve in function of some parameters related with the pharmaceutical dosage forms. The release of drug from a polymeric matrix is complicated. It often involves drug diffusion, interface movement and various interactions.

Many authors describe the release rate process by simply comparing the correlation coefficient values of lines collected from graphical presentation of different mathematical models. In order to determine the mechanism of drug release from sustained release floating matrix tablets, the data were treated using following mathematical models-

1. Zero order (cumulative percentage of drug released versus time)
2. First order (log percent of drug unreleased versus time)
3. Higuchi square root law (cumulative percentage of drug released versus square root of time)

The released data were plotted according to following equations,

1. Zero order :

$$M = M_0 - K_0 t$$

2. First order :

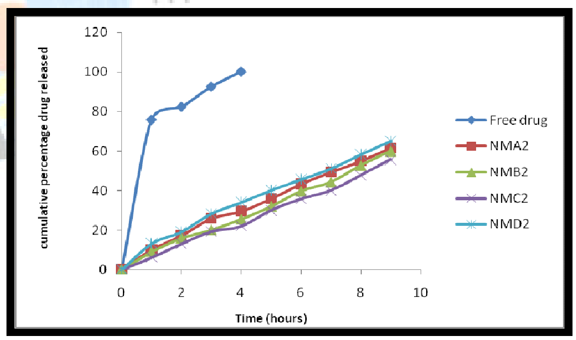
$$\text{Log } C = \text{Log } C_0 - K_t/2.303$$

3. Higuchi square root law :

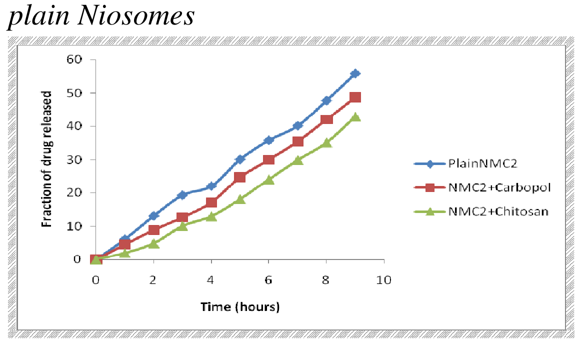
$$Q = kt^{1/2}$$

Where, M, C and Q is the amount of drug released at time t, M_0 and C_0 is total amount of drug and K_0, K_t and k are corresponding rate constants.

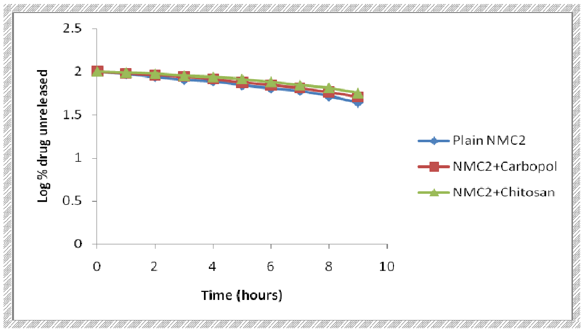
From the above equations the correlation coefficient values for different formulations have been calculated to identify the drug release mechanism and are shown in table 1.



Graph 1: *graph for in-vitro drug release from plain Niosomes*



Graph 2: *graph for Zero order data treatment*



Graph 3: *graph for first order data treatment*

a) First order treatment

Table1: Drug release profile for Plain Niosomal Formulations

Time (hours)	Free drug	NMA2	NMB2	NMC2	NMD2
0	0.00	0.00	0.00	0.00	0.00
1	75.68 ± 0.654	10.16 ± 0.087	9.00 ± 0.132	6.17 ± 0.182	13.19 ± 0.192
2	82.23 ± 0.067	17.27 ± 0.192	15.91 ± 0.078	13.10 ± 0.192	19.19 ± 0.011
3	92.45 ± 0.213	25.92 ± 0.342	20.11 ± 0.056	19.28 ± 0.083	28.32 ± 0.365
4	100.00	29.56 ± 0.341	25.48 ± 0.165	22.02 ± 0.651	34.00 ± 0.143
5		35.98 ± 0.123	32.01 ± 0.127	30.10 ± 0.610	40.12 ± 0.574
6		43.34 ± 0.441	39.67 ± 0.178	35.77 ± 0.012	45.56 ± 0.065
7		49.39 ± 0.875	44.31 ± 0.091	40.10 ± 0.089	51.01 ± 0.187
8		55.01 ± 0.054	52.71 ± 0.165	47.76 ± 0.154	58.19 ± 0.234
9		61.67 ± 0.165	59.97 ± 0.453	55.81 ± 0.098	65.00 ± 0.121

Values represented as mean ± SD (n=3), ($p < 0.05$)

Data treatment

b) Zero order kinetic treatment for mucoadhesive Niosomes of Acyclovir

Table 2:- Zero order kinetic treatment of data

Formulation	**Equation of line**	**Correlation coefficient**
Plain NMC2	Y= 5.975x+0.120	0.995
NMC2+Carbopol	Y= 4.785x-3.521	0.979
NMC2+Chitosan	Y= 5.414x-1.948	0.992

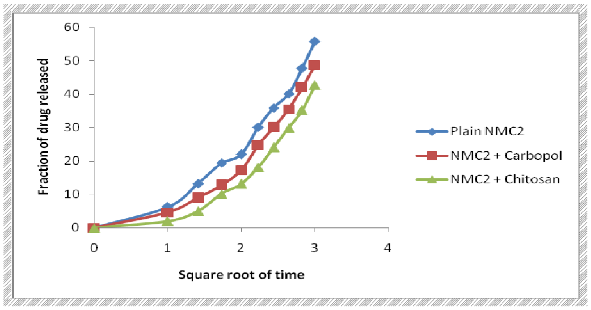
Table 3: first order kinetic treatment of data

Formulation	**Equation of line**	**Correlation coefficient**
Plain NMC2	y= -0.037x+2.016	0.975
NMC2+Carbopol	Y= -0.031x+2.021	0.972
NMC2+Chitosan	Y= -0.026x+2.025	0.954

c) Higuchi square root treatment

Table4: Higuchi square root treatment of data

Formulation	Equation of line	Correlation coefficient
Plain NMC2	Y=18.68x-9.020	0.909
NMC2+Carbopol	Y=16.60x-9.610	0.872
NMC2+Chitosan	Y=14.41x-9.800	0.831



Graph 4: *graph for Higuchi treatment*

Result and discussion

The rate of release of drug from a delivery system is a critical factor and has to be investigated to achieve an optimal system with desired drug release profile. The *in-vitro* release study is performed to predict how a delivery system may work under ideal conditions, which might give some indication of its *in-vivo* performance. In current study, release of Acyclovir entrapped in large unilamellar vesicles composed of surfactant/cholesterol (90% surfactant/10%cholesterol), and Acyclovir entrapped in large unilamellar vesicles composed of surfactant/cholesterol (90% surfactant/10%cholesterol), it appears that Acyclovir efflux form niosomes is a process containing slower release phase achieved within 2-4 hours in solution. The initial phase of slight rapid release may be due to the desorption of drug adsorbed at the surface of niosomes and the slower phase may be due to the diffusion of Acyclovir through the bilayers.

The release rate was calculated from the corresponding release profiles beyond 1 hour from the beginning of release tests for free and niosomal drug dispersion respectively. Free drug solution gave a high initial percentage durg release of 75.68% after 1 hour, where as plain niosomal dispersion of Span 60 gave only 6.17% release after 1 hour. The niosomal formulations NMC2 gave significantly slow release (55.8 ± 0.098), in comparison to free drug. Form the results it can be inferred that Acyclovir loaded niosomes retard the transfer of drug molecule across the bilayered compartment when compared to plain drug solution.

Data treatment

To find out the kinetics and mechanism of drug released from all the formulations of Acyclovir encapsulated niosome, the data were treated according to zero order, first order and Higuchi's equation pattern. As clearly indicated in table, the correlation coefficient of the formulation NMC2 was found to be 0.995, 0.979 for NMC2 dispersed in carbopol and 0.992 for NMC2 dispersed in chitosan. When the data was plotted for first order the values were found to be 0.975, 0.972, and 0.954 respectively. Hence the formulations followed mixed order kinetics. The data were best fitted to Higuchi's equation with average correlation coefficient value of 0.909, 0.872, and 0.831 respectively. The results pointed out the sustained release character with a Higuchi pattern of drug release, where niosomes dispersed in polymeric solutions act as reservoir system for continuous drug release.

Conclusion

From the result of in-vitro study performed by using dyalisis membrane it can be concluded that Span60 retards the drug release to a greater extent, as the vesicles formed are large in size and chances of drug lekage as observed in case of Span80 Niosomes, is not observed with Span 60 Niosomes.

Refrences

1. Vyas SP, Khar RK: Targetted & Controlled Drug Deliery Novel Carrier System, First edition, CBS publication, 2002, 249.
2. Uchegbu IF, Vyas SP: Non-ionic Surfactant based Vesicles (Niosomes) in Drug Delivery. Int. J. Pharmaceutics 1998; 172: 33-70.
3. Ludwig A, van Ooteghem M. Influence of viscolysers on the residence of ophthalmic solutions evaluated by slit lamp fluorophotometry, STP Pharm. Sci. 1992, 2, 81-87.
4. Aggarwal Deepika, Kaur Indu P. Improved pharmacokinetic dynamics of timolol maleated from a mucoadhesive niosomal ophthalmic drug delivery system, Int. J. Pharm. 2005, 290, 155 – 159.
5. Yongmei Hao, Fenglin Zhao, Na Li, Yanhong Yang, Ke'an Li. Studies on a high encapsulation of colchicines by a noisome system, Int. J. Pharm. 2002, 244, 73-80.
6. Ijeoma F. Uchegbu, Suresh P. Vyas. Non-ionic surfactant based vesicles (niosomes) in drug delivery, Int. J. Pharm. 1998,172, 33-70.
7. Rogerson A., Cummings J., Willmott N. and Florence A.T. The distribution of doxorubicin in mice following administration in niosomes. J Pharm Pharmacol. 1988; 40(5): 337–342.
8. Baillie A.J., Coombs G.H. and Dolan T.F. Non-ionic surfactant vesicles, niosomes, as delivery system for the anti-leishmanial drug, sodium stribogluconate J.Pharm.Pharmacol. 1986; 38: 502-505.
9. Raja Naresh R.A., Chandrashekhar G., Pillai G.K. and Udupa N. Antiinflammatory activity of Niosome encapsulated diclofenac sodium with Tween -85 in Arthitic rats. Ind.J.Pharmacol. 1994; 26:46-48.
10. Maver L.D. Bally M.B. Hope. M.J. Cullis P.R. Biochem Biophys. Acta (1985), 816:294-302. .
11. Chauhan S. and Luorence M.J. The preparation of polyoxyethylene containing non-ionic surfactant. vesicles. J. Pharm. Pharmacol. 1989; 41: 6p.
12. Blazek-Walsh A.I. and Rhodes D.G. Pharm. Res. SEM imaging predicts quality of niosomes from maltodextrin-based proniosomes. 2001; 18: 656-661.
13. Chauhan S. and Luorence M.J. The preparation of polyoxyethylene containing non-ionic surfactant. vesicles. J. Pharm. Pharmacol. 1989; 41: 6p.
14. Yoshioka T., Stermberg B. and Florence A.T. Preparation and properties of vesicles (niosomes) of sobitan monoesters (Span 20, 40, 60, and 80) and a sorbitan triester (Span 85). Int J Pharm. 1994; 105:1-6.

15. Yoshioka T., Stermberg B. and Florence A.T. Preparation and properties of vesicles (niosomes) of sobitan monoesters (Span 20, 40, 60, and 80) and a sorbitan triester (Span 85). Int J Pharm. 1994; 105:1-6.
16. Ahmed S. Guinedi, Nahed D. Mortada, Samar Mansour, Rania M. Hathout. Preparation and evaluation of reverse-phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide. Int. J. Pharm. 2005, 306, 71-82.
17. Ame´lie Bochot, Elias Fattal , Jean Louis Grossiord, Francis Puisieux, Patrick Couvreur. Characterization of a new ocular delivery system based on a dispersion of liposomes in a thermosensitive gel, Int. J. Pharm. 1998, 162, 119–127.
18. Aranya manosroi, Paveena wongtrakul, Jiradej manosroi, Hideki sakai, Fumio sugawara, Makoto yuasa, Masahiko abe. Characterization of vesicles prepared with various non-ionic surfactants mixed with cholesterol, Colloids and Surfaces B: Biointerfaces 2003, 30, 129-138.
19. Arunothayanun P, Uchegbu IF, Craig DQM, Turton JA, Florence AT. In vitro/Invivo characterization of polyhedral niosomes, Int. J. Pharm. 1999,183, 57-61.
20. Ahmed S. Guinedi, Nahed D. Mortada, Samar Mansour, Rania M. Hathout. Preparation and evaluation of reverse-phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide. Int. J. Pharm. 2005, 306, 71-82.
21. Aggarwal Deepika, Kaur Indu P. Improved pharmacokinetic dynamics of timolol maleated from a mucoadhesive niosomal ophthalmic drug delivery system, Int. J. Pharm. 2005, 290, 155 – 159.
22. Ahmed S. Guinedi, Nahed D. Mortada, Samar Mansour, Rania M. Hathout. Preparation and evaluation of reverse-phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide. Int. J. Pharm. 2005, 306, 71-82.
23. Indu Pal Kaur, Manjit Singh, Meenakshi Kanwar. Formulation and evaluation of ophthalmic preparations of acetazolamide, Int. J. Pharm. 2000,199, 119-127.
24. Deepika Aggarwal, Dhananjay Pal, Ashim K. Mitra, Indu P. Kaur. Study of the extent of ocular absorption of acetazolamide from a developed niosomal formulation, by microdialysis sampling of aqueous humor, Int. J. Phar. 2007, 338, 21-26.
25. Faruk Öztürk, Emin Kurt, Ümit Übeyt İnan, Selim Kortunay, Süleyman sami İlker, Nursabah E. Başçı, Atila Bozkurt, Penetration of topical and oral ofloxacin into the aquous and vitreous humor of inflamed rabbit eyes, Int. J. Pharm. 2000, 204, 91-95.