Volume-3-(3)-2015,Page-s15-s21
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Design and Evaluation of Niosomes Containing Salbutamol Sulphate Anti-Asthmatic Drug

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ARTICLE INFO

Received: 17 May 2015
Accepted: 05 Jun 2015

ABSTRACT

Niosomes are promising vehicle for drug delivery and being nonionic, it is less toxic and improves the therapeutic index of drug by restricting its action to target cells. Hence, in present study salbutamol sulphate was encapsulated in niosomes using non- ionic surfactants like span 40, span 60 and span 80. The preparation of niosomes was done by Thin film Hydration Technique using Rotary flash evaporator. The formulated niosomes were characterized for optical microscopy, SEM, particle size distribution, entrapment efficiency, *in-vitro* drug release, sterility test, stability studies was compared . The formulated niosomes were viewed through optical microscope. Less spherical vesicles are formed in F-I containing span 40. Numerous spherical vesicles are formed in F-II containing span 60. F-III containing span 80 showed more spherical vesicles than F-I but less than that of F-II. Microscopic examination revealed that the vesicle diameter complies within the niosomal size range of 100-300nm. The average mean particle size range was 100nm, 270 nm and 200nm for F-I, F-II and F-III respectively, revealed that the mean particle size of all the three formulations complies within the niosomal size range of 100-300nm. The entrapment efficiency of drug in F II containing span 60 was found to be 76.61% which showed maximum percent drug entrapment where as those containing span 40 and span 80 (F-I and F-III) was found to encapsulate 58.53% and 66.42% respectively. Niosomes were subjected to *in vitro* drug release using 0.1N HCl as the medium in sigma dialysis membrane. These results showed that niosomal salbutamol sulphate has sustained release upto 20 hours whereas free salbutamol sulphate was released within 2.5 hours. This is because the drug is released slowly for a prolonged period of time in niosomal salbutamol sulphate. Also, F-II containing span 60 showed higher release when compared to F-I containing span 40 and F-III containing span 80.Therefore, F-II is selected for further studies like sterility test, stability studies and pharmacological studies.The stability studies was performed by testing drug leakage from the niosomal formulation were analysed in terms of percent drug retained at the end of every month. Storage under refrigerated condition showed greater stability with 93.12% of drug content at the end of 3 months whereas storage under room temperature and at 400C ± 20C R-H 70 % ± 5% showed drug content of 88.92% and 81.26% at the end of three months

Keywords: Niosome, Salbutamol Sulphate, Stability, Storage, Entrapment

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1. INTRODUCTION

Niosomes have been used to prolong the circulation of the drugs, to alter the distribution of drugs and they offer a host of other advantages. Niosomes favour selective delivery of drugs and improves the therapeutic efficacy and reduces the severity of side effects. The need for present study is to encapsulate the drug in the niosomes vesicles for effective Respiratory system drug delivery for a prolonged period of time. Salbutamol Sulphate is one of the most effective drug in the treatment of Anti-asthmatic. My objective of this study is to treat Asthmatic with Salbutamol Sulphate niosomes, since it is an short-acting, selective beta2-adrenergic receptor agonist used in the treatment of asthma and COPD. It is 29 times more selective for beta2 receptors than beta1 receptors giving it higher specificity for pulmonary beta receptors versus beta1-adrenergic receptors located in the heart. Salbutamol is a beta(2)-adrenergic agonist and thus it stimulates beta(2)-adrenergic receptors. Binding of albuterol to beta(2)-receptors in the lungs results in relaxation of bronchial smooth muscles. It is believed that salbutamol increases cAMP production by activating adenylate cyclase, and the actions of salbutamol are mediated by cAMP. Increased intracellular cyclic AMP increases the activity of cAMP-dependent protein kinase A, which inhibits the phosphorylation of myosin and lowers intracellular calcium concentrations. A lowered intracellular calcium concentration leads to a smooth muscle relaxation and bronchodilation. In addition to bronchodilation, salbutamol inhibits the release of bronchoconstricting agents from mast cells, inhibits microvascular leakage, and enhances mucociliary clearance. It has short biological half life of 1-2 hours and requires frequent administration for a prolonged period of time. Since, niosomes prolong the circulation of many drugs and alters the distribution of drugs, I aimed at formulating Salbutamol Sulphate in niosomal drug delivery, thereby minimizing the dose and also to achieve sustained release for a prolonged period of time and to compare the antiasthmatic activity of Salbutamol Sulphate niosomes at different concentrations of the drug with a marketed tablet formulation. The reason for selecting niosomal drug delivery is that niosomes can entrap both hydrophilic and lipophilic drugs and also have better stability than liposomes. [1, 2]

2. MATERIALS AND METHODS

Salbutamol sulphate were procured from Micro Labs, Hosur and Cholesterol Span 40,60 and 80 was purchased from Qualigens Fine Chemicals, Mumbai.

Research methodology:

Pure drug (Salbutamol sulphate) was identified using FTIR Technique. IR Spectra was taken for Cholesterol, Span 40, Span 60, Span 80 and for mixture of drug, cholesterol and Surfactants. [3, 4]

Standard Curve for Salbutamol Sulphate

100 mg of Salbutamol sulphate pure drug was dissolved in 100ml of 0.1N HCl. From this stock solution, 2.5 ml was pipetted out and made upto 25ml with 0.1N HCl. From this solution, serial dilutions were made to produce 5, 10, 15, 20, 30, 40, 50 µg/ml concentrations. These samples were analysed spectrophotometrically at 255 nm using 0.1N HCl as blank.

Preparation of Salbutamol Sulphate Niosomes

Salbutamol sulphate niosomes were prepared by Thin Film Hydration Technique using Rotary flash Evaporator. According to this method, accurately weighed quantity of cholesterol and non-ionic surfactant were dissolved in 10ml of chloroform and poured into a round bottom flask. The flask was rotated at 1.5 cm above a water bath at $60^{o}C\pm 2^{o}C$ under reduced pressure, until all the organic phase evaporated and a thin layer was formed on the wall of a round bottom flask. Then accurately weighed quantity of drug

was dissolved in 10ml of phosphate buffer saline pH 7.4. The dried non- ionic surfactant and cholesterol film was subsequently hydrated with this drug solution and the mixture was rotated by immersing in a water bath at 60°C± 2°C for 1 hour until a good dispersion of mixture was obtained. The niosomes vesicles containing Salbutamol sulphate vesicles were subsequently formed. The suspension was then sonicated to form unilamellar vesicles. [5, 6]

Table 1: Preparation of salbutamol sulphate niosomes

F.Code	Drug	Cholesterol	Span 40	Span 60	Span 80	Chloroform	PBS pH 7.4	C.S ratio
F-I	10mg	30mg	30mg	-	-	10ml	10ml	1:1
F-II	10mg	30mg	-	30mg	-	10ml	10ml	1:1
F-III	10mg	30mg	-	-	30mg	10ml	10lm	1:1

Table 2: Percentage Drug Entrapment of the formulated Niosomes

Formulation code	% Drug Entrapment
F-I (Span 40)	58.53 %
F-I (Span 60)	76.61 %
F-I (Span 80)	66.42 %

Table 3: In-vitro Release Study of Drug

Time (min)	Absorbance at 255 nm	Concentration (mcg/ml)	Amount diffused (mg)	% Amount diffused
15	0.0197	3.47	0.69	13.9
30	0.0466	4.58	0.91	18.34
45	0.1031	6.92	1.38	27.68
60	0.1679	9.59	1.91	38.39
75	0.2191	11.71	2.34	46.85
90	0.296	14.89	2.97	59.57
105	0.3746	18.14	3.62	72.56
120	0.4427	20.95	4.19	83.81
135	0.5089	23.69	4.73	94.76
150	0.5348	24.76	4.95	99.04

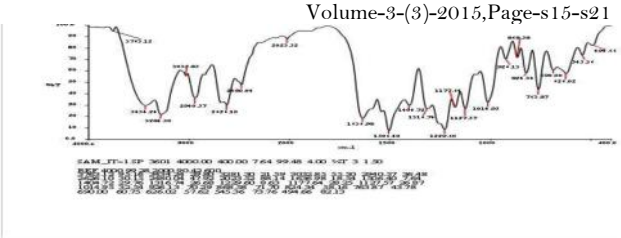

Fig 1: FTIR of drug+cholesterol+span 60

Characterization of niosomes

***Optical microscopy*:** The formulated niosomes were viewed through optical microscope. The microscopic methods include the use of Bright field, phase contrast microscope and fluorescent microscope and are useful in evaluating the vesicle size of large vesicles (>1µm) particularly the upper end of the size distribution for miltilamellar vesicles. Vesicular dispersion appropriately diluted are wet mounted on a haemocytometer and photographed with a phase contrast microscope. The negatives then can be projected on a piece of calibrated paper using a photographic enlarger at X 1250 (Vyas and Katar, 1991), diameters of approximately 500 vesicles are measured and thus this method is tedious and coupled with the limitation of resolution, hence electron microscopic methods with greater resolutions are preferred.

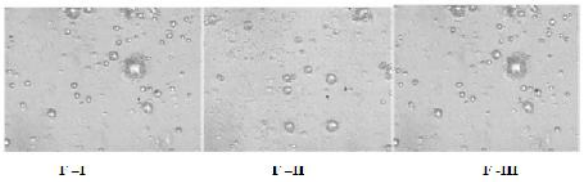

Fig 2: Optical Microscopy of Formulations

Morphological Studies

Scanning Electron Microscopy: Niosomes were characterized by SEM (JEOL) . Niosomes containing Salbutamol sulphate was taken in a cover glass and transferred on a specimen stub. Dried samples were coated with platinum alloy to a thickness of 100 A using a sputter coater. After coating, scanning was done to examine the shape and size. [7]

Determination of Drug Entrapment Efficiency

1 ml of the sample is taken and centrifuged at 13000 RPM at 40C for 90 minutes using Remi centrifuge. Supernatant was separated without disturbing the sediment layer using micropipette. Then the supernatant layer (free drug) was diluted using PBS pH 7.4 and analysed using UV spectrophotometer.

$$\text{Percentage drug entrapment} = \frac{\text{Amount of drug entrapped}}{\text{Initial amount of drug}} \times 100$$

In Vitro Release Studies

***In-vitro* release for Pure Drug Salbutamol sulphate:** 10mg of pure drug was dissolved in 10ml of 0.1N HCl. 5ml of solution was taken in a dialysis tube and placed in 200ml of 0.1 N HCl. The medium was stirred by using the magnetic stirrer and the temperature was maintained at 37±20C. Periodically 5 ml of samples were withdrawn and after each withdrawal same volume of medium was replaced. Then the samples were assayed spectrophotometrically at 255nm using 0.1 N HCl as blank. [8]

***In-vitro* release of Salbutamol sulphate niosomes:** The *in vitro* release of niosomes were studied by using simple diffusion cell apparatus. The diffusion cell apparatus consists of a glass tube with an inner diameter of 2.5cm, open at both ends, one end of the tube is tied with Sigma dialysis membrane, which serves as a donor compartment. Niosomes equivalent to 5mg of Salbutamol sulphate was taken in a dialysis tube and placed in 200ml of 0.1 M Hcl. The medium was stirred by using the magnetic stirrer and the temperature was maintained at 37±20C. Periodically 5 ml of samples were withdrawn and after each withdrawal same volume of medium was replaced. Then the samples were assayed spectrophotometrically at 255nm using 0.1 M Hcl as blank.

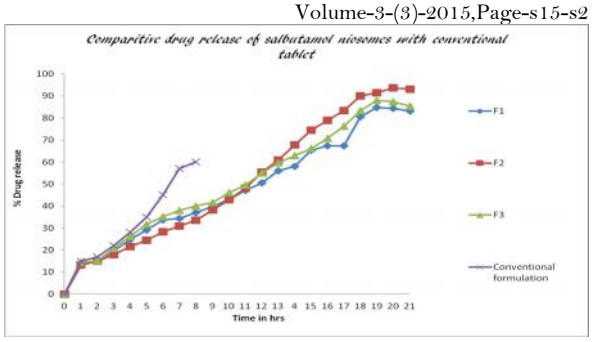

Fig 3: Comparative invitro relese study of formulationa and marketed formulation

Sterility Test For Salbutamol Sulphate Niosomes

After sterilization of Salbutamol sulphate niosomes**,** the formulations were subjected to sterility test. The test for sterility is intended for detecting the presence of viable forms of bacteria, fungi and yeast in preparations. The tests were carried out under aseptic conditions designed to avoid accidental contamination of the product during the test. [9-12]

Culture Media

a. For aerobic Bacteria and Fungi: Fluid thioglycollate medium: This medium can be used for the detection of aerobic bacteria and fungi.

Preparation of fluid thioglycollate medium

Trypticase 15g, L-cystine 0.5 g, Dextrose 5g, Yeast extract 5g, Sodium chloride 2.5 g Sodium Thioglycollate 0.5 g, Reazurin 0.001 g, Agar 0.75 g

The above ingredients were dissolved completely in 1000ml of distilled water and the medium was boiled for 10 minutes. The pH was adjusted to 7.2 ± 0.2. Media was distributed into 4 ½ size test tubes and sterilized by autoclaving at a pressure of 15 lbs/in 2 and at a temperature of 1210C for 15 minutes. Autoclaved medium was kept in room temperature.

For Anaerobic Bacteria

Chopped meat (CM) medium: This medium can be used for the detection of anaerobic bacteria.

Preparation of chopped meant medium

a. Lean ground beef 500 g, Distilled water 1000 ml, Sodium hydroxide (In Solution) 25 ml

b. Trypticase 30 g, Yeast extract 5 g, Dipotassim hydrogen phosphate 5 g

L-cystine 0.5 g, Hemin (1% solution) 0.5 g, Vitamin K (1% alcoholic solution) 0.1 ml

Procedure

1. 500g meat was mixed with 1000ml distilled water and 25ml sodium hydroxide.It was heated to boiling while stirring. After mixture has cooled,it was refrigerated overnight at 40C. Mixture was filtered through two layers of gauze. Meat particles were retained and liquid was filtered. Enough distilled water is added to filtrate to give final volume of 1000ml. All ingredients were added into above two liquids except Lcystine. It was heated until ingredients dissolved completely, cooled to less than 500C and L-cystine was added and mixed to dissolve it completely.The pH of broth was adjusted to 7.4. Meat particles were washed several times with distilled water to remove excess sodium hydroxide and spreaded thinly on clean towel to partially dry. About 0.5g meat particles was dispensed with small scoop into 15X90mm screw cap tubes. 7ml enriched broth filtrate was added to each tube. The tubes were autoclaved at 1210C for 15 minutes. Tubes were cooled and with caps loosened,they were passed into anaerobic glove box to cover the surface of the media with white petroleum jelly, so that atmosphere of approximately 85% nitrogen (N2), 10% hydrogen (H2) 5%carbon dioxide (CO2) replaces air in tubes. After caps are tightened securely, tubes were removed from glove box. Chopped meat (cm) medium tubes were stored in refrigerator at 40C at ambient temperature.

Procedure

The Salbutamol sulphate niosomal formulation was added to the fluid thioglycollate medium and incubated at 20-25^{0}C for not less than seven days. Chopped meat medium is incubated for not less than fifteen days. At intervals during the incubation period, the media were examined visually for microbial growth.In order to support the above performed test, a positive control and

negative control tests were also carried out. Positive control test was carried out in order to confirm that the media and the environment provided for incubation were suitable for the

growth of micro organisms. α - haemolytic Streptococci were inoculated into the fluid thioglycollate medium and Clostridium tetani was inoculated into the chopped meat medium and the growth promotion was observed.

Sterility Test

Observation: The positive control tube showed turbidity where as negative control tube showed no turbidity. The negative control tube containing the media appeared clear confirming that the media, area ,apparatus and other accessories used for sterility testing were absolutely free from microorganism. The growth in the positive control which was inoculated with bacteria confirms that the media and the environment were suitable for bacterial growth. The tubes with Niosomal Salbutamol sulphate were clear confirming that they are sterile.

***Stability Studies*:** The formulated niosomes were subjected for stability studies for a period of three months. The formulated niosomes were divided into 3 portions. First portion was kept at refrigeration (40C±10C) temperature. Second portion was kept at room temperature. Third portion was kept at 40^{0}C±2^{0}C, 70% ±5 % RH

3. RESULTS & DISCUSSION

Niosomes are promising vehicle for drug delivery and being nonionic, it is less toxic and improves the therapeutic index of drug by restricting its action to target cells. Hence, in present study salbutamol sulphate was encapsulated in niosomes using non-ionic surfactants like span 40, span 60 and span 80.

The preparation of niosomes was done by Thin film Hydration Technique using Rotary flash evaporator. The formulated niosomes were characterized for optical microscopy, SEM, particle size distribution, entrapment efficiency, *in-vitro* drug release, sterility test, stability studies was compared .

***Optical Microscopy*:** The formulated niosomes were viewed through optical microscope. Less spherical vesicles are formed in F-I containing span 40. Numerous spherical vesicles are formed in F-II containing span 60. F-III containing span 80 showed more spherical vesicles than F-I but less than that of F-II.

Morphological Studies: The formulated niosomes were subjected to microscopic examination by Scanning Electron Microscopy for characterizing size and shape. Microscopic examination revealed spherical small unilamellar vesicles of 80-120 nm, 250- 280 nm and 140-260nm for F-I, F-II and F-III respectively. These results revealed that the vesicle diameter complies within the niosomal size range of 100-300nm.

Particle Size Distrubution of Niosomes: Niosomes were subjected to particle size Analyzer for characterizing size distribution of niosomes. The average mean particle size range was 100nm, 270 nm and 200nm for F-I, F-II and F-III respectively. These values revealed that the mean particle size of all the three formulations complies within the niosomal size range of 100-300nm.

Entrapment Efficiency: The quantity of the drug entrapped in the niosomes is very essential to know before studying the behaviours of this entrapped drug in physical or biological system. The process and formulation variables (cholesterol and surfactant) were altered and optimized to obtain the niosomes with maximum drug entrapment. All the three formulations F-I, F-II and F-III were subjected to percentage drug entrapment. The entrapment efficiency of drug in F II containing span 60 was found to be 76.61% which showed maximum percent drug entrapment where as those containing span 40 and span 80 (F-I and F-III) was found to encapsulate 58.53% and 66.42% respectively. This showed that span- 60 is the more suitable surfactant along with cholesterol for enhancing maximum entrapment for the drug salbutamol sulphate. Further, the percent drug entrapment, is increased by decreasing the sonication time. Therefore, the sonication time was optimized to 15 minutes and further reduction in the size by increasing sonication time was not attempted.

***IN – VITRO* DRUG RELEASE:** The formulated niosomes were subjected to *in vitro* drug release using 0.1N HCl as the medium in sigma dialysis membrane. The amount of salbutamol sulphate diffused was estimated spectrophotometrically at 255nm. The percentage amount of free drug released was 99.04% within 2.5 hours. F-I showed 84.99 % of drug release within 19 hours. F-II showed 93.48 % of drug release within 20 hours where F-III gave 87.97% of drug release within 19 hours. These results showed that niosomal salbutamol sulphate has sustained release upto 20 hours whereas free salbutamol sulphate was released within 2.5 hours. This is because the drug is released slowly for a prolonged period of time in niosomal salbutamol sulphate. Also, F-II containing span 60 showed higher release when compared to F-I containing span 40 and F-III containing span 80.Therefore, F-II is selected for further studies like sterility test, stability studies and pharmacological studies.

4. SUMMARY AND CONCLUSION

Niosomes containing Salbutamol sulphate were formulated using different surfactants such as span 40,span 60 and span 80 and evaluated for various

parameters . From the above studies, it is concluded that Salbutamol sulphate encapsulated in niosomes showed prolonged release and longer duration of action thereby achieving sustained release. The results revealed that niosomal Salbutamol sulphate exhibits better anti asthmatic activity. Thus, the objective of minimizing the dose of Salbutamol sulphate was achieved with niosomal delivery system.

5. REFERENCES

1. Buckton G. Harwood, Interfacial phenomena in Drug Delivery and Targeting. Academic Publishers, Switzerland, 1995; P. 154- 155.
2. Handjani-Vila R.M , Ribier A., Rondot B. and Vanlerberghe G. Dispersions of lamellar phases of nonionic lipids in cosmetic products. Int. J. Cosmetic Sci. 1979;1:303-314.
3. Chandraprakash K.S Udupa N. Pharmacokinetic evaluation of surfactant vesicles containing methotrexate in tumor bearing mice. Int. J. Pharma. 1990; R1-R3: 61.
4. Raja Naresh R.A Chandrashekhar G., Pillai G.K. and Udupa N. Antiinflammatory activity of Niosome encapsulated diclofenac sodium with Tween -85 in Arthritic rats. Int. J.Pharmacol. 1994; 26:46-48 143
5. Cable An examination of the effects of surface modification on the Physiochemical and biological properties of non- ionic surfactant vesicle. Glasgow: Univeristy of Strathclyde, 1989.
6. Blazek-Walsh A.I. and Rhodes D.G. Pharm. Res. SEM imaging predicts Quality of niosomes from maltodextrin-based proniosomes. 2001; 18: 656-661.
7. Khandare , Madhavi G. and Tamhankar B.M. Niosomes-novel drug Delivery system. The Eastern Pharmacist. 1994; 37: 61-64.
8. Parthasarathi , Udupa N., Umadevi P. and Pillai G.K. Niosome Encapsulated of vincristine sulfate: improved anticancer activity with reduced toxicity in mice. J. Drug Target. 1994; 2(2): 173-182
9. Namdeo , Mishra P.R., Khopade A.J. and Jain N.K. Formulation and evaluation of noisome encapsulated indomethacin, Indian Drugs. 1999; 36 (6) 378- 380.
10. Yoshida , Lehr, CM., Kok W., Junginger H.E., Verhoef J.C. and Bouwistra J.A. Niosomes for oral delivery of peptide drugs. J.Control Rel. 1992; 21:145-153.
11. Eadie MJ, Bladin PF (2001), A Disease once sacred: a History of the Medical under standing of Epilepsy.
12. Cascino GD. “Epilespy; contemporary Perspectives on evaluation and treatment Mayo clinic Proc 1994; 69: 1199-1211

Conflict of Interest: None

Source of Funding Nil