www.sphinxsai.com

ChemTech
International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.1, No.3 , pp 567-573, July-Sept 2009

FORMULATION AND EVALUATION OF ACECLOFENAC LOADED MALTODEXTRIN BASED PRONIOSOME

Tank Chintankumar J.[1]*, Borkhataria Chetan H[2]., Baria Ashok H[3]., Patel Rakesh P.[4], S. Tamizharasi[5], Dipen K. Sureja[6] ,Sandip D. Patel[7] ,Ghanshyam R. Parmar[8]

[1]Pharmaceutics Dept,Nandha College of pharmacy,Koorapalayam "piruvu",Erode-638 052,Tamilnadu, India.

[2]Pharmaceutics Dept,Shree H.N. Shukla Inst. of Pharmaceutical Edu. and Research,Amargadh, Bhichri, Rajkot.GUJARAT, INDIA.

[3]S. J. Thakkar College of pharmacy,Near NRI Banglow,Rajkot. GUJARAT, INDIA.

[4]Pharmaceutics Dept,S. K. Patel College of Pharm. Edu. & Res.,Ganpat University, Kherva., GUJARAT, INDIA.

[5]Nandha College of pharmacy, Koorapalayam "piruvu",Erode-638 052, Tamilnadu, India.

[6]Chemistry department,Shree H.N. Shukla Inst. of Pharmaceutical Edu. and Research,Amargadh, Bhichri, Rajkot.,GUJARAT, INDIA.

[7]Pharmaceutics Dept, Shree H.N. Shukla Inst. of Pharmaceutical Edu. and Research,Amargadh, Bhichri, Rajkot.,GUJARAT, INDIA.

[8]Pharmacognosy Dept,Shree H.N. Shukla Inst. of Pharmaceutical Edu. and Research,Amargadh, Bhichri, Rajkot.,GUJARAT, INDIA.
E-mail- chintan_tank@yahoo.com, Ph: +919979573227

ABSTRACT: Aceclofenac loaded maltodextrin based proniosome were prepared by slurry method with different surfactant to cholesterol ratio. The proniosome formulation was evaluated for FT-IR study, angle of repose and scanning electron microscopy. The niosomal suspensions were further evaluated for entrapment efficiency, *In-vitro* release study, Kinetic data analysis, Stability study, *In-vivo* anti-inflammatory Study. The result from SEM analyses has showed smooth surface of proniosome. The formulation F_4 which showed higher entrapment efficiency of 83.24 ± 1.34 and *in-vitro* releases of 97.122% at the end of 24hr was found to be best among the all 7 formulation. Release was best explained by the zero order kinetics. Kinetic analysis shows that the drug release follows super case II transport diffusion. Proniosome formulation has showed appropriate stability for 90 days by storing the formulation at refrigerator condition.

KEYWORDS: Aceclofenac, Proniosome, Maltodextrin, Span-60

INTRODUCTION

Proniosome are dry product which could be hydrated immediately before use would avoid many of the problems associated with aqueous niosome dispersions and problems of physical stability (aggregation, fusion, leaking) could be minimized.[1] These dry formulations of surfactant-coated carrier can be measured out as needed and rehydrated by brief agitation in hot water.[2] They are water-soluble carrier particles that are coated with surfactant and can be hydrated to form a niosomal dispersion immediately before use on brief agitation in hot aqueous media. Reported methods for preparation of proniosomes are the spraying of surfactant on water-soluble carrier particles and the slurry method.[3] This dry, free-flowing, granular product which, upon addition of water, disperses or dissolves to form a multilamellar niosome suspension suitable for administration by oral or other routes.

Aceclofenac is considered to be the first-line drug in the symptomatic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. The successful treatment of arthritis depends on the maintenance of effective drug concentration level in the body, for which a constant and uniform supply of drug is desired. The short biological half-life (about 4 h) and dosing frequency more than once a day as well as two-third (70-80%) of dose is excreted by renal transport make aceclofenac an ideal candidate for sustained release.

The objective of the present study was to prepare proniosomes of aceclofenac in order to sustain the release of aceclofenac in upper GIT, which may decrease the side effect of GI disturbance by maintaining the concentration of the drug in the blood and decrease the renal excretion as well as frequency of dosing.

MATERIAL AND METHOD

Aceclofenac obtained as a gift sample from Novartis Limited, India. Maltodextrin was procured from Hi-media, Mumbai. Cholesterol and span-60 were purchased from Loba Chem Pvt. Ltd., Mumbai. All other reagents used were of analytical grade.

Preparation of Proniosome [1, 4]:

Proniosome were prepared by the slurry method. For ease of preparation, a 250µmol stock solution of span-60 and cholesterol was prepared in chloroform: methanol (2:1). The required volume of span-60 and cholesterol stock solution and drug dissolved in chloroform: methanol (2:1) solution was added to a 100ml round bottom flask containing the maltodextrin carrier. Additional chloroform: methanol solution added to form slurry in the case of lower surfactant loading. The flask was attached to a rotary flash evaporator to evaporate solvent at 60 to 70 rpm, a temperature of 45 ± 2ºC, and a reduced pressure of 600mmHg until the mass in the flask had become a dry, free flowing product. These materials were further dried overnight in a desiccator under vacuum at room temperature. This dry preparation is referred to as 'proniosomes' and was used for preparations and for further study on powder properties. These proniosome were stored in a tightly closed container at refrigerator temperature until further evaluated.

Preparation of niosomes from proniosomes[2]:

Proniosomes were transformed to niosomes by hydrating with phosphate buffered saline pH 6.8 at 80ºC and by gentle mixing. The niosomes were sonicated twice for 30sec using sonicator and then evaluated for further studies.

Measurement of Angle of repose[1]:

The angle of repose of dry proniosomes powder and maltodextrin powder was measured by a funnel method (Lieberman et al 1990). The maltodextrin powder or proniosomes powder was poured into a funnel which was fixed at a position so that the 13mm outlet orifice of the funnel is 5cm above a level black surface. The powder flows down from the funnel to form a cone on the surface and the angle of repose was then calculated by measuring the height of the cone and the diameter of its base.

Particle size analysis and Surface Morphology[1-4]:

Particle size of proniosomes is very important characteristic. The surface morphology (roundness, smoothness, and formation of aggregates) and the size distribution of proniosomes were studied by Scanning Electron Microscopy (SEM).

Microscopy[4]:

The vesicle formation by the particular procedure was confirmed by optical microscopy in 400x resolution. The niosome suspension placed over a glass slide and fixed over by drying at room temperature, the dry thin film of niosome suspension observed for the formation of vesicles. The photomicrograph of the preparation also obtained from the microscope by using a digital SLR camera.

Entrapment efficiency [5]:

Niosome entrapped aceclofenac was estimated by dialysis method. The calculated amount of prepared niosomes was placed in the dialysis bag 50 (presoaked for 24 hrs). Free aceclofenac was dialyzed for 30 minutes each time in 100 ml of phosphate buffer saline pH 6.8. The dialysis of free aceclofenac always completed after 12-15 changes, when no aceclofenac was detectable in the recipient solution. The dialyzed aceclofenac was determined by finding out the concentration of bulk of solution by UV spectrophotometer at 275 nm. The samples from the bulk of solution diluted appropriately

before going for absorbance measurement. The free aceclofenac in the bulk of solution gives us the total amount of un-entrapped drug. Encapsulation efficiency is expressed as the percent of drug trapped.

$$\text{Percent Entrapment} = \frac{\text{Total drug - Diffused drug}}{\text{Total drug}} \times 100$$

***In vitro* release study[6]:**

In vitro release pattern of niosomal suspension was carried out in dialysis bag method. 10 mg equivalent of aceclofenac niosomal suspension was taken in dialysis bag (Hi media) and the bag was placed in a beaker containing 75 ml of 0.1 N HCl. The beaker was placed over magnetic stirrer having stirring speed of 100 rpm and the temperature was maintained at 37 ± 1^0C. 5 ml samples were withdrawn periodically and were replaced by fresh buffer. After two hours, 25 ml of 0.2 M tribasic sodium phosphate was added to change the pH of test medium to 6.8, and the test was continued for a further 22 hours. The sink condition was maintained throughout the experiment. The withdrawn samples were appropriately diluted and analyzed for drug content using U.V. spectrophotometer at 275nm keeping phosphate buffer pH 6.8 as blank. All the determinations were made in triplicate.

Drug Release Kinetic Data Analysis:

The release data obtained from various formulations were studied further for their fitness of data in different kinetic models like Zero order, Higuchi's and peppa's.

Stability Study[7]:

Physical stability study was carried out to investigate the degradation of drug from proniosome during storage. Best three (F_4, F_5, F_6) of the optimized aceclofenac proniosome formulation composed of span-60 and cholesterol sealed in glass vials and stored in refrigerated temperature (2-8^{0}C) for a period of 3 months. Samples from each batch were withdrawn after the definite time intervals and converted into noisome and the residual amount of drug in the vesicles was determined. Stability data of three formulations were further analyzed for significant difference by paired t-test.

***In-Vivo* Anti - Inflammatory Study[8,9]:**

The *in-vivo* study was carried out for the best formulation F_4 by measuring anti-inflammatory activity in adult male wistar albino rats using cotton pellet granuloma method.

The study was approved by institutional animal ethical committee (proposal no. NCP / IAEC / PG / 11 / 2008-2009).

To evaluate the anti-inflammatory activity of prepared aceclofenac niosomes, the male wistar albino rats of average weight 180-220 gm were taken and divided in to three groups, each group consisting of six animals. One group served as control (2.5 ml of 0.5% sodium carboxymethylcellulose orally), second group received standard (10mg/kg of aceclofenac in two divided dose orally), while third group received niosomes containing aceclofenac by orally (10 mg/kg of aceclofenac niosomes orally) once daily during experiment. Rats with an average weight of 200g were anaesthetized with ether and the lumbar region shaved and disinfected with 70% ethanol. An incision will be made in this lumbar region. By a blunt forceps Sub-cutaneous tunnel will be formed and a sterilized cotton pellet of known weight will be placed on both sides in the scapular region. The animals will be treated for seven days. The granuloma will be removed after seven days, under ether anesthesia, and the wound will be closed by suturing. All the animals rehabilitated by giving ampicillin 10mg/kg i.p. for one week

RESULT AND DISCUSSION

FT-IR Spectra of Aceclofenac, maltodextrin, physical mixture of drug: carrier and F_4 formulation were recorded. The aceclofenac present in the formulation F_4 was confirmed by FT-IR spectra. The characteristic peaks due to pure Aceclofenac shows IR absorption at 717.47 cm^{-1}(C-H bending), 1282.57 $cm^{-1}$and 1344.29 cm^{-1} (C-N Stretching), 1417.58 cm^{-1} (O-H bending in carboxylic acid), 1716.53 cm^{-1} (C= O Stretching). All these peaks have appeared in pure aceclofenac, physical mixture and formulation indicating no chemical interaction between aceclofenac and carrier. It also confirmed that the stability of drug during formulation.

Angle of repose of maltodextrin powder compared with proniosome formulation by fixed funnel method. Results of measurements of the angle of repose of proniosome powder and pure maltodextrin are summarized in Table no. II and indicate that the angle of repose of dry proniosome powder is smaller than that of pure maltodextrin. If the proportion of surfactant in the formulation decreases, the angle of repose of dry proniosome powder decreases slightly. It indicate that the fluidity of proniosome dry powder is equal to or better than that of maltodextrin powder, so further processing of proniosome powder as a beads, tablets, or capsules is possible.

Shape and surface characteristic of proniosome were examined by Scanning Electronic Microscopy analysis. Scanning electron microscopy shows the porous surface of the pure maltodextrin particles, this makes them effective carrier and provides more surface area for the coating of the surfactant mixture. Surface morphology illustrates the smooth surface of proniosome formulation.

The prepared vesicles were studied under 400x magnifications to observe the formation of vesicles. Some unevenness of vesicles that observed under the study may be due to drying process under normal environment condition. The particles found to be uniform in size and shape.

Entrapment efficiency was studied for all the seven formulations to find the best in terms of entrapment

efficiency. Higher entrapment efficiency of the vesicles of span 60 is predictable because of its higher alkyl chain length. The entrapment efficiency was found to be higher with the formulation no. F_4 (83.24%), which may have an optimum surfactant cholesterol ratio to provide a high entrapment of aceclofenac. The niosomal formulations having high surfactant concentration (F_2, F_3 and F_4) have the higher entrapment efficiency which might be due to the high fluidity of the vesicles. Very low cholesterol content (F_1) was also found to cause low entrapment efficiency (56.42%), which might be because of leakage of the vesicles. The higher entrapment may be explained by high cholesterol content (~50% of the total lipid). There are reports that entrapment efficiency was increased, with increasing cholesterol content and by the usage of span-60 which has higher phase transition temperature. It was also observed that very high cholesterol content (F_6, F_7) had a lowering effect on drug entrapment to the vesicles (42.73%). This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bi-layered structure leading to loss of drug entrapment. The larger vesicle size may also contribute to the higher entrapment efficiency. Entrapment efficiency showed by various formulations is specified in Table no. I.

The release study was conducted for all the seven formulations as shown in the Figure I. Most of the formulations were found to have a linear release and the formulations were found to provide approximately 90% release within a period of 24 hours. The formulations which have high cholesterol ratio (F_6, F_7) were found to sustain the drug release. Cholesterol, which has a property to abolish the gel to liquid transition of niosomes, this found to prevent the leakage of drug from the niosomal formulation. The slower release of drug from multilamellar vesicles may be attributed to the fact that multilamellar vesicles consist of several concentric sphere of bilayer separated by aqueous compartment. The above specified three best formulations F_3, F_4, and F_5, were found to give a cumulative release of 97.86 %, 97.28 % and 92.26 % respectively over a period of 24 hrs, the higher release from the formulation F_3 may be because of its low cholesterol content. Formulations F_6 and F_7 having the highest cholesterol content showed the lowest release over 24 hours, they provide a release of 85.65 % and 81.20 % respectively.

The zero order plots showed the zero order release characteristics of the formulation, which was confirmed by the correlation value which found to be nearer to one. Correlation value of Higuchi's plot revealed that the mechanism of drug release is diffusion. The *in vitro* kinetic data subjected to log time log drug release transformation plot (peppa's model), all the value ranges from 1.008 to 1.112 revealed the fact that the drug release follows a super case II transport diffusion.

Physical stability of proniosome formulation is as shown in the Figure II. The percentage of aceclofenac retained in the span-60 vesicles after a period of three months were 98.83%, 98.72% and 97.52% respectively for formulations F_4, F_5 and F_6. Also the results indicate that more than 90% of aceclofenac was retained in the niosomal formulation for a period of 90 days. From this it can be concluded that proniosomes are stable to store under refrigeration temperature with least leakage. The leakage of drug from F_6 may be due to its lower surfactant content and higher cholesterol which formed a leaking vesicle. The stability data analyzed for significant difference between retention patterns of drug in three different niosomal formulations on storage. The test value showed no significant difference ($P>0.05$) between the stability data of formulations from each other.

Anti inflammatory activity of F_4 formulation was measured by cotton pellet granuloma method, in which inflammation and granuloma developed during period of 7 days. The effects of aceclofenac treatment on the mean rate of granuloma are shown in Table no. III**.** The weight of the aceclofenac niosomal formulation treated granuloma was less as compare to control and standard. This indicates that prepared noisome formulation of aceclofenac exhibited better efficacy than standard preparation. It can be consider that the niosomal formulation has better therapeutic efficacy as compare to the aceclofenac solution.

On conclusion, this novel drug delivery system i.e. proniosome as compared to liposome or niosome suspensions, represent a significant improvement by eliminating physical stability problems, such as aggregation or fusion of vesicles and leaking of entrapped drugs during long-term storage. Proniosomes derived niosomes are superior in their convenience of storage, transport, and dosing as compare to niosomes prepared by conventional method. By these facts study can be concluded by saying proniosomes based niosomes formed from span 60 and cholesterol in the ratio 150:100 (in μmol) where 1gm of carrier used per one mMol of surfactant, is a promising approach to sustain the drug release for an extended period of time and by that reducing the side effects related to GI irritation.

ACKNOWLEDGEMENT:

I am acknowledging my personnel thanks to Nandha college of pharmacy and Shree HN Shukla Institute of Pharmaceutical Education and Research.

Table I: Compositions of proniosome batches of aceclofenac and entrapment of different formulation

Formulation No.	Formulation Code	Ratio(µmol) (surfactant: cholesterol)	Entrapment efficiency* %
1	F_1	225:25	56.42 ± 1.15
2	F_2	200:50	68.14 ± 0.65
3	F_3	175:75	77.91 ± 0.26
4	F_4	150:100	83.24 ± 1.34
5	F_5	125:125	64.27 ± 0.81
6	F_6	100:150	54.32 ± 0.67
7	F_7	75:175	42.73 ± 0.94

1 g of Carrier per 1 m mole of surfactant
Drug content used 100 mg per batch
***Average of three formulations, ± S.D.**

Table II: Angle of repose of uncoated maltodextrin and proniosome formulation

Preparation	Angle of Repose*
Maltodextrin powder	45°.19' ± 0.43
F_1	37°.22' ± 0.18
F_4	34°.91' ± 0.06
F_7	29°.37' ± 0.23

- **Average of three Preparation ± SD.**

Table III: Result of anti inflammatory activity measurement

S. No	Treatment	Dose (mg/kg)	Weight of dry cotton pellet granuloma (mg)		Weight of dry granuloma (mg)	Percentage Decreases in granuloma (%)
			Before	After*		
1.	Control	-	10.0	94.28 ± 8.92	84.28 ± 8.92	-
2.	Standard	5	10.0	75.41 ± 7.53	65.41 ± 7.53	22.39 ± 8.93
3.	F_4 formulation	5	10.0	53.93 ± 7.59	43.93 ± 7.59	47.88 ± 9.03

***Average of three preparation**

Figure I: *In-vitro* release of all formulation

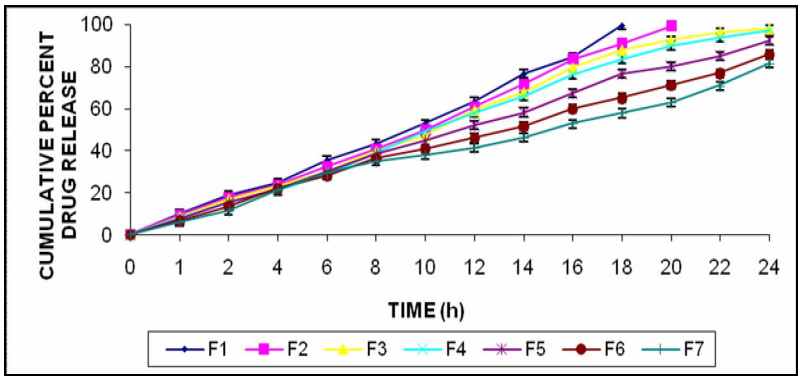
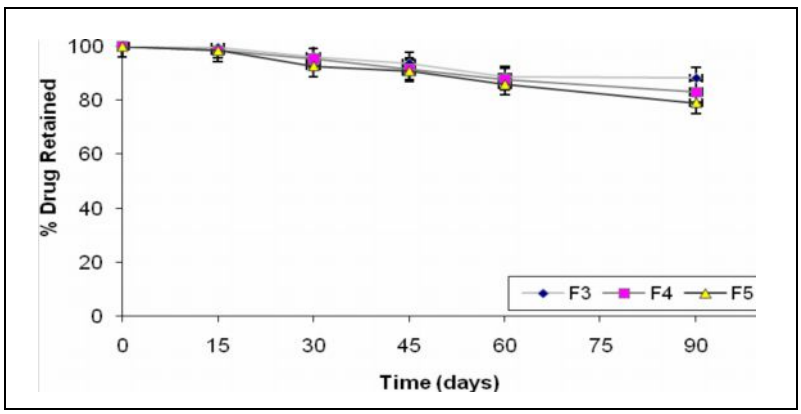


Figure II: Percentage of aceclofenac retained in the niosome formulations after storage at refrigeration temperature



REFERENCES

1) Rhodes D.G. and Chengjiu H., Proniosomes: A Novel Drug Carrier Preparation, Int. J. Pharm., 1999, 185, 23–35.

2) Almira I., Blazek W. and Rhodes D.G., Maltodextrin-Based Proniosomes, AAPS PharmSciTech., 2001, 3(1), E1.

3) Almira I., Blazek W. and Rhodes D.G., SEM Imaging Predicts Quality of Niosomes from Maltodextrin-Based Proniosomes, Pharmaceutical Research., 2001, 18(5), 656-661.

4) Solanki A.B., Parikh J.R. and Parikh R.H., Formulation and Optimization of Piroxicam Proniosomes by 3-Factor, 3-Level Box-Behnken Design, AAPS PharmSciTech. 2007, 8(4), E1-E7.

5) Goopi N.D., Prakash S.R., Ravi D., Apte, S.S., Rao B.R. and Rambhav D., Release studies on Niosomes containing fatty alcohols as bilayer stabilizers instead of cholesterol, Journal of colloids and interface science, 2002, 251, 360-365.

6) Aggarwal D. and Kaur I.P., Improved pharmacodynamics of timolol maleate from a mucoadhesive niosomal ophthalmic drug delivery system, Int. J. Pharm. 290, 155–159.

7) Guinedi. A.S., Mortada N.D.., Mansour S. and Hathout R.M., Preparation and evaluation of reverse-phase evaporation and multilamellar Niosomes as ophthalmic carriers of acetazolamide. Int. J. Pharm, 2001, 306, 71–82.

8) Mutalik S., Naha A., Usha A.N., Ranjith A.K., Musmade P., Manoj K., Anju P. AND Prasanna S., Preparation, *In Vitro,* Preclinical and Clinical Evaluations of Once Daily Sustained Release Tablets of Aceclofenac. Arch Pharm Res, 2007, 30(2), 222-234.

9) Rao C.G. and Vijayanarayana K., Nimesulide Incorporated lipid microspheres: an Approach to Targeting and controlled release, Indian J. Pharm. Sci, 2005, 67(3), 292-295.

*****