PREPARATION AND CHARACTERIZATION OF TOLTERODINE TARTRATE PRONIOSOMES

ABSTRACT

The present work deals with the preparation of Tolterodine tartrate proniosome formulations by ether injection method by using different surfactants i.e. span 60 and tween 40 in different ratios. The prepared proniosomal formulation were evaluated for vesicle size analysis, rate of spontaneity, encapsulation efficiency, angle of repose, drug content etc.

In vitro release study conducted for 12 hrs indicated that, increases in liphophilicity of surfactants decreases release of Tolterodine tartrate from proniosomal formulations.

Stability studies performed at optimized formulation PG4, indicate that, the prepared formulations remained more stable at room refrigeration temperature than oven temperature.

KEY WORDS: Tolterodine tartrate, proniosome, span 60, tween 40, *in vitro* release.

INTRODUCTION

At present scenario vesicular systems have been receiving a lot of interest as a carrier for advanced drug delivery[1]. Encapsulation of the drug in vesicular structures is one such system, which can be expected to prolong the duration of the drug in systemic circulation[2].

Proniosomes are water soluble carrier particles that are coated with surfactants and can be hydrated to form niosomal dispersion immediately before use in hot aqueous media. Proniosome is a dry free flowing, granular product that could be hydrated immediately before use and would avoid many of the problems associated with aqueous noisome dispersions and problem of physical stability[3]. Proniosome technology offers novel solution for poorly soluble drugs.

Proniosomes avoid many of the problems associated with aqueous niosome dispersions, and problems of physical stability (aggregation, fusion, leaking) could be minimized. The additional convenience of the transportation, distribution, storage, and dosing would make 'dry niosomes' a promising industrial product[4].

Tolterodine is used for the treatment of overactive bladder with symptoms of urge urinary in continence, urgency and frequency[5]. Use of Tolterodine tartrate is associated with side effects like dry mouth and other side effects like constipation, headache, stomach pain and blurred vision, often leading to discontinuation of therapy[6].

MATERIALS AND METHODS:

Tolterodine tartrate was obtained as gift sample from Churchbells Pharma Nigeria Limited. Span 60, tween 40 and cholesterol were procured from Drugfield Pharmaceuticals Limited, Nigeria. Diethyl ether was procured from Interpharma Industries Nigeria Limited. All other reagents used were of analytical grades.

Preparation of Proniosomal Gel:

Ether injection process

Proniosomes formulations containing Tolterodine tartrate were prepared by taking cholesterol, span 60, tween 40 and lecithin in a 50 ml beaker. The mixture was dissolved in diethyl ether and the solution was slowly injected into a beaker containing Tolterodine tartrate in phosphate buffer saline (pH 7.4). The temperature maintained during the injection was 40-60^{o}C. The differences in temperature between phases cause rapid vaporization of ether resulting in spontaneous vesiculation[7].

Table 1: Composition of Tolterodine tartrate pronisomal gel formulations.

S.N.	Code	Drug (mg)	Span 60 (mg)	Tween 40 (mg)	Diethyl ether (ml)	Lecithin (mg)	Cholesterol (mg)
1	PG1	100	1500	-	10	900	200
2	PG2	100	-	1500	10	1800	400
3	PG3	100	-	1500	10	900	200
4	PG4	100	1500	-	10	1800	400

Evaluation of proniosome formulations

Vesicle size analysis:

Hydration of Tolterodine tartrate proniosomal gel (100 mg) was done by adding saline solution (0.9% solution) in a small glass vial with occasional shaking for 10 min. The dispersion was observed under optical microscope at 45 x magnification. The sizes of 200-300 vesicles were measured using a calibrated ocular and stage micrometer (Erma, Tokyo) fitted in the optical microscope[8].

Rate of spontaneity:

Approximately 10 or 20 mg of Tolterodine tartrate proniosomal gel was transferred to the bottom of a clean stoppered glass bottle and spread uniformly around the wall of the glass bottle with the help of a glass rod. At room temperature, 2 ml of phosphate saline (0.154 M NaCl) was added carefully along the walls of the glass bottle and left in a test-tube stand After 20 minutes, a drop of this saline solution was withdrawn and placed on Neubauers Chamber (Marienfeld) to count the number of vesicles. The number of niosomes eluted from proniosomes was counted[9].

Encapsulation efficiency:

To evaluate the loading capacity of proniosomal systems for Tolterodine tartrate gel (100 mg) was dispersed in distilled water and warmed a little for the formation of niosomes. Then the dispersion was centrifuged at 18000 rpm for 40 min the clear fraction was used for the determination of free drug at 224 nm spectrophotometrically. The percentage encapsulation efficiency was calculated from following equation[10].

$$\% \text{ Encapsulation Efficiency} = \frac{\text{Total drug} - \text{ Free drug / Total drug}}{\text{Total drug}} \text{X } 100$$

pH and Viscosity

Accurately weighed gel was taken and then diluted with the pH 7.4 phosphate buffer and checked the pH by using pH meter and Brook field viscometer is used to determine the viscosity of the gel[11].

Zeta potential analysis

Zeta potential analysis was determining for the colloidal properties for a prepared formulations of Tolterodine tartrate. The proniosomes derived from noisome dispersion will be determined using zeta potential analyser based on the Electrophoretic light scattering and laser Doppler Velocimetery method. The temperature was set at 25°C and measures the charge on vesicles and means zeta potential values[12].

***In vitro* release study:**

In vitro release studies on proniosomal gel of Tolterodine tartrate were performed using locally manufactured Franz-diffusion cell. The capacity of receptor compartment was 15 ml. The area of donor compartment exposed to receptor compartment was 1.41cm^2. The dialysis cellophane

membrane (MMCO 14KDC) was mounted between the donor and receptor compartment. A weighed amount of proniosomal gel was placed on one side of the dialysis membrane. The receptor medium was phosphate saline buffer pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±1^{o}C. Samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically at 281 nm[13].

Stability Studies:

The ability of vesicles to retain the drug was assessed by keeping the proniosomal gel at three different temperature conditions, i.e., refrigeration temperature (4-8^{0}C), room temperature (25±2^{0}C) and oven (45±2^{0}C) for 12 weeks.Throughout the study, proniosomal formulations of Tolterodine tartrate were stored in aluminium foil-sealed glass vials. The samples were withdrawn at different time intervals over a period of one month and drug leakage from the formulations was analyzed for drug content spectrophotometrically[14].

RESULTS AND DISCUSSION:

Results of vesicle size of Tolterodine tartrate proniosome formulations are presented in (Table2), which indicated that vesicle formed with Span 60 is smaller in size than vesicle formed with tween 40. The reason for it may be grater hydrophobicity of spans as compared to tweens[15]. As hydrophobicity increases, surface energy of surfactants decreases, resulting in smaller vesicle size. Size of vesicle was reduced when dispersion was agitated. The reason for this is the energy applied in agitation which results in breakage of larger vesicles to smaller vesicles[16]. The size range was found to be 15.28±0.33 to 16.43±0.22 µm. Rate of spontaneity lies in between 12.20±0.43 to 14.44±0.76.

Table 2: Characterization of the proniosomal formulations of Tolterodine tartrate.

Batch Code	Mean particle size µm	Rate of spontaneity mm^{3}x1000	Encapsulation efficiency (%)	Angle of repose	% Drug content	pH	Viscosity (cp)
PG1	15.28±0.33	13.16±0.33	77.2±0.45	34.32 ^{o} ±0.43	95±0.32	7.12	7244
PG2	8.34±0.45	12.20±0.43	79.4±0.39	36.12 ^{o} ±0.78	88±0.12	7.34	8247
PG3	7.27±0.67	14.44±0.76	81.2±0.48	38.21 ^{o} ±0.27	90±0.77	7.42	9314
PG4	16.43±0.22	13.66±0.57	88.3±0.55	36.35 ^{o} ±0.06	99±0.47	7.11	7642

(Mean± S.D., n=3)

Encapsulation efficiency was found to be higher in case of proniosome prepared with Span60 than proniosome prepared with Tween 40. This may be due to more hydrophobic nature of span 40, as compared to tween 60, which act as solid at room temperature and showed higher phase transition temperature (Tc), low HLB value and long alkyl chain length[17].

Drug content is important parameter to maintain the minimum effective concentration and it is also used to estimate the drug release profile. The percent drug content was higher for PG4 that is 99±0.47 % and lower for PG2 (88±0.12%).

In vitro release studies (figure 1) are often performed to predict how a delivery system might work in an ideal situation as well as give some indications of its *in vivo* performance since drug release dictates the amount of drug available for absorption.

The amount of drug released from different proniosomal formulations was found in the order of PG4 > PG3 > PG2> PG1. *In vitro* release study performed on different proniosome formulations shows maximum release for formulations of batch PG4 (87.45 %), and minimum for formulations of batch PG4 (50%), during the study of 12 hrs. It was found that *in-vitro* release

from proniosomal formulations prepared with Tween 40 is slower as compared to proniosomal gel formulation prepared with span 60. This was expected due to the larger size of the vesicles[18].

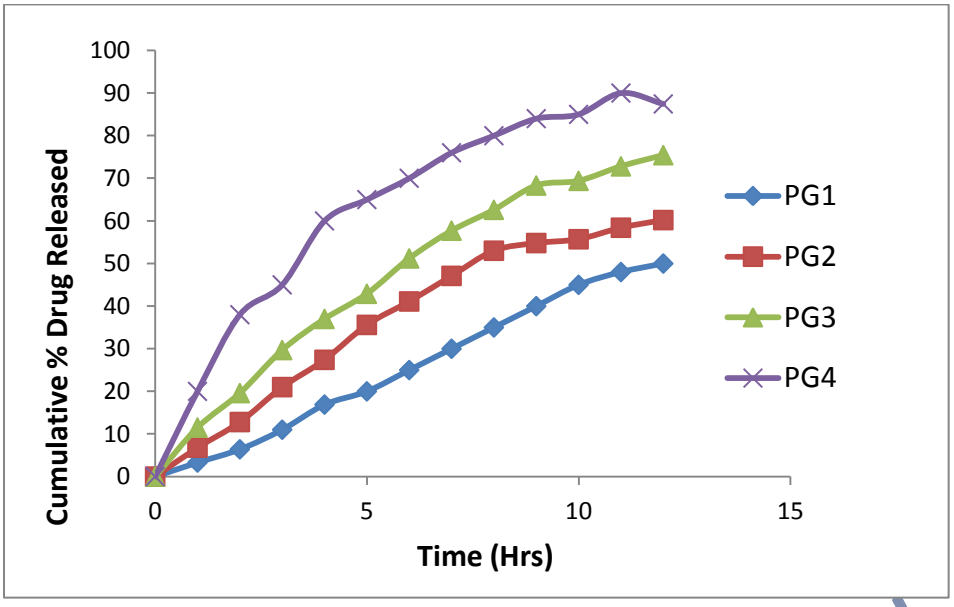

Figure 1: Comparative *in-vitro* release study of different proniosome formulations of Tolterodine tartrate

Stability studies performed on optimized formulations PG4 shows 96.74% drug content at refrigeration condition, 94.74% drug content at oven condition and 99.59% drug content at room temperature during the studies performed for 12 weeks on the formulations (figure 2). Hence it is concluded from the obtained data that the optimum storage condition for proniosomes was found to be room temperature.

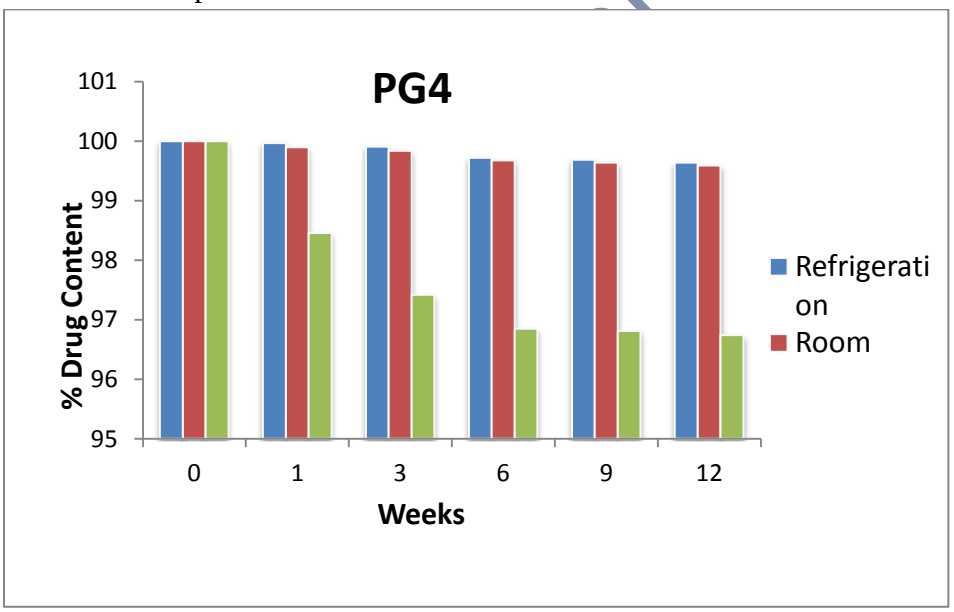

Figure 2: Stability study of optimized gel formulation (PG4) at different temperature conditions.

CONCLUSION

The results of investigation demonstrated that proniosomes offers an alternative colloidal carrier approach. The results obtained from the present study clearly revealed that Tolterodine tartrate proniosome formulations prepared by using ether injection method are capable of releasing drug for the extended period of time. Results of the present work have shown that surfactant type affect the encapsulation efficiency and drug release rate from proniosomes. Based on different parameters formulation of batch PG4 was considered as an optimum formulation.

REFERENCES:

1. Kakkar R, Rao R, Dahiya NK, Nanda S. Formulation and characterization of valsartan proniosomes. Maejo Int J Sci Technol. 2011; 5(01):146-158.
2. Mahmoud Mokhtar, Omaima A. Sammour, Mohammed A. Hammad, Nagia A. Megrab. Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes. Int j of pharm. 2008, 361, 104–111.
3. Gamal M. Mahrous. Pronisomes as a drug carrier for transdermal delivery of Meloxicam. Bull. Pharm. Sci. Assiut University. 2010, 33 (2), 131-140.
4. Chavan P, Jain B, Jain P. Proniosomal gel: a novel approach for transdermal drug delivery-a review. Int. J Pharm. Res and Dev. 2012; 4(03), 158-170.
5. Shaik RP, Srinivasa BP, Kothapalli CB, Zayed B, Challa BR. A validated LC–MS/MS method for the determination of tolterodine and its metabolite in rat plasma and application to pharmacokinetic study. J of Pharm Anal. 2013; 3(6):489–499.
6. Zhang B, Zhang Z, Tian Y, Xu F. High performance liquid chromatography-electrospray ionization mass spectrometric determination of tolterodine tartrate in human plasma. J Chromatography. B, Analyt Technol Biomed Life Sci. 2005, 25; 824(1-2):92-8.
7. Ammara HO, Ghorab M, El-Nahhas SA, Higazy IM. Proniosomes as a carrier system for transdermal delivery of tenoxicam. Int j of pharm. 2011, 405, 142–152.
8. Trupti AU, Wankhade VP, Latika MI, Atram S, Tapar KK. Proniosomes- a novel approach to vesicular drug delivery system. Int J of Pharm and Pharm Sci Res. 2013: 3(1), 1-6.
9. Ibrahim MM, Sammour OA, Hammad MA, Megrab NA, *In vitro* evaluation of proniosomes as a drug carrier for flurbiprofen, AAPS Pharm Sci Tech. 2008, 9(3), 782–790.
10. Thakur R, Anwer MK, Shams MS, Ali A, Khar RK, Shakeel F, Taha EI. Proniosomal transdermal therapeutic system of losartan potassium: development and pharmacokinetic evaluation. J Drug Target. 2009, 17(6), 449.
11. Azarbayjani AF, Tan EH, Chan YW, Chan SY. Transdermal delivery of haloperidol by proniosomal formulations with non-ionic surfactants", Biol. Pharm. Bull. 2009, 32, 1453-1458.
12. Melike U, Benek C. Design of hydralazine hydrochloride matrix tablets based on various polymers and lipids. Indian J of Pharm Ed and Res. 2012, 46(1).
13. Varshosaz J, Pardakhty A, Mohsen S, Baharanchi H. Sorbitan monopalmitate-based proniosomes for transdermal delivery of chlorpheniramine maleate. Informa Health Care. 2005; 12(2): 75-82.
14. Hazel G, Dubey A, Prabhakara P, Kamath J. Development and evaluation of norfloxacin loaded maltodextrin based proniosomes.Int Res J Pharm. 2012;3(6):176-79.
15. Csoka G, Marton S, Zelko R, Otomo N, Antal I. Application of sucrose fatty acid esters in transdermal therapeutic systems, Eur. J. Pharm. Biopharm. 2007, 65(2), 233–237.
16. Varshosaz J, Pardakhty A, Baharanchi SM, Sorbitan monopalmitate-based proniosomes for transdermal delivery of chlorpheniramine maleate, Drug Deliv. 2005, 12(2), 75–82.
17. Pardakhty A., Varshosaz J., Rouholamini A., *In vitro* study of polyoxyethylene alkyl ether niosomes for delivery of insulin, Int. J. Pharm. 328(2), 2007, 130–141.
18. Kumar K, Rai AK. Development and Evaluation of Proniosomes as a promising drug carrier to improve transdermal drug delivery, Int Res J Pharm. 2011, 2 (11), 71-74.