FORMULATION AND CHARACTERIZATION OF SOLID LIPID NANOPARTICLES FOR TRANSDERMAL DELIVERY OF TESTOSTERONE

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Abstract

Present study deals with the formulation and characterization of testosterone bearing solid lipid nanoparticles for transdermal drug delivery. Solid lipid nanoparticles of testosterone were prepared by ether injection method. Solid lipid nanoparticles were prepared by testosterone, glyceryl mono stearate, brij 35, propylene glycol and double distilled water. Four different formulations i.e. F1 to F4 were prepared in different quantity of brij 35. All the solid lipid nanoparticles formulations were characterized on the basis of electron microscopy, particle size by zeta sizer, polydispersity index, encapsulation efficiency, viscosity and *in vitro* drug release study. Average size and polydispersity index of developed formulation F3 found to have 298 nm and 0.328 respectively, determined by zeta sizer. Encapsulation efficiency of formulation F3 was found to have 54.16%. Viscosity of formulation was also enough to handle for transdermal application. All the formulations followed zero order release profile.

Keywords: Solid lipid nanoparticles, transdermal, gel, testosterone.

Introduction

In last decade, significant attempt has been made to develop nanotechnology for drug delivery system, as it offers a appropriate means of delivering drugs whether small molecules or large molecules such as proteins, peptides or genes to cells and tissues and prevents them against enzymatic degradation[1]. The feature of nanoparticles as drug delivery systems is that they are non-toxic, non-irritant, biodegradable, and biocompatible[2].

Nanoparticles are solid colloidal particles ranging from 10 to 1000 nm (1.0 μm), in which the bioactive materials are incorporated in such a ways as either dissolved, entrapped, and/or to which the bioactive is adsorbed or attached [2].

Solid lipid nanoparticles (SLN) have emerged as a new drug delivery system with potential applications in pharmaceutical field, diagnosis, cosmetics, treatment and other allied sciences. In recent times, increasing attention has been focused on these SLN as colloidal drug carriers for incorporating lipophilic or hydrophilic drugs. Proteins, peptides and antigens intended for therapeutic purposes may be incorporated or adsorbed onto SLN, and further administered by parenteral and transdermal routes or other alternative routes such as nasal and pulmonary. SLN may be useful with conventional chemotherapy to overcome[3].

Solid lipid nanoparticles are nanosize articles composed of mainly solid lipid and active drug. They were proposed as novel colloidal carrier in 1990s[4] and they considered as a new generation of submicron sized emulsion or vesicles where the liquid lipid is replaced with solid lipid[5]. SLN combine the advantages of and avoiding some disadvantage of other colloidal vesicular systems[6]. SLN has advantage such as excellent physical stability, protection of labile drugs from degradation, controlled drug release at site, good tolerability and site specific targeting. Disadvantages of SLN are insufficient drug loading, drug leakage on storage and relatively high water content of dispersion[7]. In general, the drug can be located in between the chains of fatty acids or in between the lipid layers and also in imperfections.

SLN considered as one of the successful vesicular system. Advantages of SLN include application of biodegradable physiological lipids which decreases the danger of acute and chronic toxicity and avoidance of organic solvents in production methods[8] and improved bioavailability of poorly water soluble molecules[9]. They can be use as site specific delivery of drugs, enhanced drug penetration into the skin via dermal application and possibility of scaling up[10-12]. SLN are useful in protection of chemically labile agents from degradation in the

gut and sensitive molecules from outer environment and enhance the bioavailability of entrapped bioactive and chemical production of labile encapsulated drug[13,14]. SLN have better stability compared to liposomes. SLN offer many advantages however, they also have some disadvantages[15,16]. SLN has poor encapsulation capacity, drug leakage after polymeric transition during storage and relatively high water content of the dispersions (70-99.9%)[3, 17].

Method of preparation of SLN includes high shear homogenization, ultrasonication, microemulsion based SLN preparation, supercritical fluid technology, spray drying, solvent emulsification/evaporation, solvent injection technique and solvent emulsification-diffusion[3]. Among these solvent displacement technique considered as one of the simple and economic. This technique was first described for the preparation of liposomes and polymeric nanoparticles. Recently, this technique has also employed to prepare lipid nanoparticles[18]. This process is based on the precipitation of lipid dissolved in solution. In this method solvent displacement takes place and lipid precipitate in the same time. Solvent removal is necessary and can be performed by distillation or other method if do not remove under given condition. The lipid nanoparticles formation takes place after evaporation of the water immiscible organic solvent. Particle size is depends on the various parameters such as amount to be injected, concentration of lipid, temperature, stirring, type of organic solvent and emulsifier[19].

Testosterone is used as a supplement therapy in men suffering from hypogonadism. Testosterone associated with extensive first-pass metabolism after oral administration. Its conventional treatment for hypogonadal men comprises slow release intramuscular injections or oral administration. Recently, formulations of testosterone have been developed for the treatment of hypogonadism. Transdermal delivery system[20], topical spray[21], sublingual tablets[22], and subcutaneous implants[23]. These systems are associated with certain drawback except transdermal system which is considered to be safer and more effective than other preparations[24].

Materials and methods

Testosterone was generously supplied as gift sample by SPARC, Baroda, India. Glyceryl monostearate, brij35, propylene glycol and chloroform were procured from CDH India. All other ingredients were of analytical reagent grade. Distilled water was used in this study.

Method of preparation

Solid lipid nanoparticles of testosterone were prepared by solvent injection method given by Muller Goymann and Schubert[25], with some modification. Glyceryl monostearate was melted and then testosterone, propylene glycol and chloroform were mixed and dissolved with slight heating. Separately, brij35 solution in an aqueous phase was prepared at the same temperature. Then, organic phase was quickly injected into aqueous phase at the same temperature with constant stirring using magnetic stirrer. Now, chloroform was evaporated with continuous stirring. SLN were formed and subjected to centrifugation at 9000 r/m for 50 min. This semisolid dispersion of SLN was used for the purpose of application on the skin as transdermal delivery of drug.

Evaluation of solid lipid nanoparticles

Electron microscopy of solid lipid nanoparticles

Solid lipid nanoparticles were observed by transmission electron microscopy. Samples of SLN were diluted to ten time and then mounted on gold plate. The mounted plates were dried and examined under a transmission electron microscope (Philips Morgangni 268D, Netherland) without using any kind of stain. The CCD camera and soft image system was employed with the transmission electron microscope to visualize SLN[26]. The photomicrograph of solid lipid nanoparticles is given in Figure 1.

Zeta potential of solid lipid nanoparticles

Zeta potential of SLN formulations were determined by Zetasizer (Malvern Instruments, UK). Samples were appropriately diluted with deionized water to obtain 50 and 200 Kcps for the measurements. Samples were placed in the cubit available for instrument and zeta potential measured directly[27].

Particle size and Polydispersity index of solid lipid nanoparticles

The average particle size and polydispersity index of SLN formulations were measured by Zetasizer DTS (Malvern Instrument, UK). The samples of SLN dispersions were diluted with deionized water[27]. The results of average particle size and polydispersity index were obtained from instrumental based calculation system and data reported in Table no. 2.

Encapsulation efficiency of solid lipid nanoparticles

Amount of testosterone encapsulated in solid lipid nanoparticles were calculated as encapsulated efficiency (EE). Solid lipid nanoparticles were kept in dialysis tube (molecular weight cut off 12 KD, HiMedia Laboratories Pvt. Ltd., India) and dialyzed. Thirty milliliter of 30% v/v PEG 400 in phosphate buffer (pH-6) solution was used as dialyzing medium[28]. Dialysis of solid lipid nanoparticles was performed for two hour. The one hundred miligram of dialyzed solid lipid nanoparticles were taken from dialysis bag and analyzed for drug content by high performance liquid chromatography (HPLC), (Shimadzu, Japan) at 254 nm. The samples were

suitably diluted and filtered through Millipore membrane filter (0.2 µm; Millipore Corporation, USA), an aliquot of 20 µl was injected into HPLC column and assayed for drug content.

$$EE\% = \frac{\text{Amount of testosterone found in solid lipid nanoparticles}}{\text{Amount of testosterone added during preparation of solid lipid nanoparticles}} \text{ X } 100$$

Viscosity of solid lipid nanoparticles

Viscosity of testosterone containing solid lipid nanoparticles was measured by Brookfield viscometer (DV-E viscometer, Brookfield, USA) using spindle no 63 at 30 r/m in ambient condition. The spindle speed no 63 was fixed in viscometer nobe and maximum torque was measured before observing viscosity. Viscosity of testosterone containing solid lipid nanoparticles was measured directly from the viscometer digital display[29]. The averages of three determinations were given in Table no. 2.

***In vitro* release study of solid lipid nanoparticles**

The *in vitro* drug release study of testosterone containing solid lipid nanoparticles was performed by locally fabricated Franz diffusion type cell. The study was performed at 30±2°C temperature. Receptor compartment of diffusion cell contained 30 ml 30% v/v PEG 400 in phosphate buffer (pH-6) solution and was constantly stirred by a magnetic stirrer (Expo India Ltd., Mumbai, India) at 50 r/m. Dialysis membrane (molecular weight cut off 12 KD, HiMedia Laboratories Pvt. Ltd., India) was employed as release barrier in between receptor and donor compartment which was previously was with distilled water and soaked with 30% v/v PEG 400 solution. Time to time 5 ml samples was withdrawn through the sampling port of the diffusion cell in intervals one h, over 8 h. Same amount of 30% v/v PEG 400 solution was replaced immediately. The collected samples were suitably diluted and analyzed by HPLC (Shimadzu, Japan) at 254 nm[30].

Results and discussion

Present study deals with the formulation and characterization of testosterone bearing solid lipid nanoparticles for transdermal drug delivery. Solid lipid nanoparticles of testosterone were prepared by ether injection method. Solid lipid nanoparticles were prepared by testosterone, glyceryl mono stearate, brij 35, propylene glycol and double distilled water. Four different formulations i.e. F1 to F4 were developed in different quantity of brij 35. All the solid lipid nanoparticles formulations were characterized on the basis of electron microscopy, particle size by zeta sizer, polydispersity index, encapsulation efficiency, viscosity and *in vitro* drug release study.

Presence of solid lipid nanoparticles was observed under transmission electron microscopy[26]. Samples of SLN were diluted to ten times and then mounted on gold plate. The mounted plates were dried and examined under a transmission electron microscope (Philips Morgangni 268D, Netherland) without using any kind of stain. The CCD camera and soft image system was employed with the transmission electron microscope to visualize SLN and suitable photomicrograph of SLN were taken[26]. The photomicrograph of solid lipid nanoparticles is given in Figure 1.

Zeta potential of SLN formulations were determined to observed net surface charge on SLN and surrounding system. Zeta potential of SLN was determined to understand stability aspect and quality control measure. Samples were appropriately diluted with deionized water to obtain 50 and 200 Kcps for the measurements of zeta potential and placed in the cubit available for instrument and zeta potential measured directly on monitor and data recorded[27]. Zeta potential of all formulations was found from -26.14 mv to -32.83 mv. This says zeta potential increases on increasing brij 35 quantities. brij 35 is useful for solubilisation and stabilization hence its quantity can utilize for getting appropriate zeta potential. However, higher quantity may be harmful for skin[31].

The average particle size and polydispersity index of SLN formulations were measured by Zetasizer DTS (Malvern Instrument, UK) as the same method used for zetapotential[27]. Results of average particle size and polydispersity index were obtained from instrumental based calculation system and data reported in Table no. 2. Average particle size of SLN was found from 286 to 321 nm. Polydispersity index of SLN found from 0.263 to 0.371. Both the parameter associated with the cooling, stirring, heating, type of solid lipid and surfactant. Therefore it is a sum of these entire variables.

In general EE of SLN found less than 50% however; lipophilic drug may show on higher side EE. Solid lipid nanoparticles were kept in dialysis tube (molecular weight cut off 12 KD, HiMedia Laboratories Pvt. Ltd., India) and dialyzed. Thirty milliliter of 30% v/v PEG 400 in phosphate buffer (pH-6) solution was used as dialyzing medium[28] to make testosterone solubility in dialysis medium. The samples were suitably diluted and filtered through Millipore membrane filter (0.2 µm; Millipore Corporation, USA), an aliquot of 20 µl was injected into HPLC column and assayed for drug content determination. Encapsulation efficiency data were reported in Table no. 2. Encapsulation efficiency of SLN formulations were found from 41.93 to 55.32%. EE for formulation F1 and F2 is relatively less of in general average results however, F3 and F4 it is good and can be consider for further study.

Viscosity of testosterone containing solid lipid nanoparticles was measured by Brookfield viscometer (DV-E viscometer, Brookfield, USA) using spindle no 63 at 30 r/m in ambient condition[29]. The averages of three determinations were given in Table no. 2. Viscosity of testosterone containing solid lipid nanoparticles was found from 2850 to 3390 cp. Handling and spreading point of view it may be consider an acceptable viscosity.

The *in vitro* release study of solid lipid nanoparticles was performed by locally fabricated Franz diffusion type cell. The study was performed at 30±2°C temperature. Receptor compartment of diffusion cell contained 30 ml 30% v/v PEG 400 in phosphate buffer (pH-6) as diffusion medium[32]. Dialysis membrane (molecular weight cut off 12 KD, HiMedia Laboratories Pvt. Ltd., India) was employed as release barrier in between receptor and donor compartment. The study was performed for 8 hour and samples were withdrawn in one hour interval. The collected samples were suitably diluted and analyzed by HPLC (Shimadzu, Japan) at 254 nm[30]. *In vitro* release pattern of formulation F1 to F4 was found from 36.49 to 48.63 cumulative percentage drug releases in 8 h Figure 2. Formulation F3 found to have lowest release and formulation F1 found relatively faster release. However, all the formulation showed controlled release patter which could enough for 24 h to get controlled release once a day formulation.

In vitro release data applied to determined release mechanism. Release data of formulations were treated for different models like zero order, first order, Higuchi matrix and Peppas-Korsmeyer and data given in Table 3. Formulation F1, F2 and F4 followed order release kinetics and formulation F3 followed Peppas-Korsmeyer[33]. Zero order achievement is to develop a controlled formulation. However, Peppas-Korsmeyer release also appears in the formulation where some hindrance found. Similarly in F1 formulation it may be due to travelling of drug in aqueous environment after release from vesicle. Therefore formulation F3 considered as appropriate and developed formulation.

Conclusion

Solid lipid nanoparticles were prepared by ether injection method which was found to have simple and economic. Ingredients used in this study were economic and safe. Characterization of SLN reveals a good kind of product which could be reproduced for commercial purpose. Entrapment efficiency and viscosity were good and upto acceptable range.

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Table 1. Composition of solid lipid nanoparticles formulation containing testosterone

Ingredients	**F1**	**F2**	**F3**	**F4**
Testosterone (mg)	100	100	100	100
Glyceryl monostearate (gm)	5	5	5	5
Brij 35 (gm)	2	3	4	5
Propylene glycol (ml)	1	1	1	1
Chloroform (ml)	10	10	10	10
Distilled water (ml) q.s.	50	50	50	50

Table 2. Characterization of solid lipid nanoparticles containing testosterone

Formulation code	**Zero order**	**First order**	**Higuchi Matrix**	**Peppas-Korsmeyer**
	r^2	**r^2**	**r^2**	**r^2**
F1	0.9823	0.7965	0.8825	0.8842
F2	0.9751	0.8182	0.8968	0.9134
F3	0.9714	0.7622	0.8142	0.9837
F4	0.9867	0.7643	0.8280	0.8946

TABLE 3. THE REGRESSION COEFFICIENTS FOR *IN VITRO* RELEASE STUDY OF ACYCLOVIR FROM SOLID LIPID NANOPARTICLES

Formulation Code	Zeta potential (mV)	Particle Size nm	Polydispersity index	Encapsulation Efficiency %	Viscosity cp
F1	-26.14	321	0.263	41.93	2850
F2	-28.67	313	0.371	46.79	2930
F3	-31.26	298	0.328	54.16	3310
F4	-32.83	286	0.349	55.32	3390

Figure 1. Electron photomicrograph SLN formulation

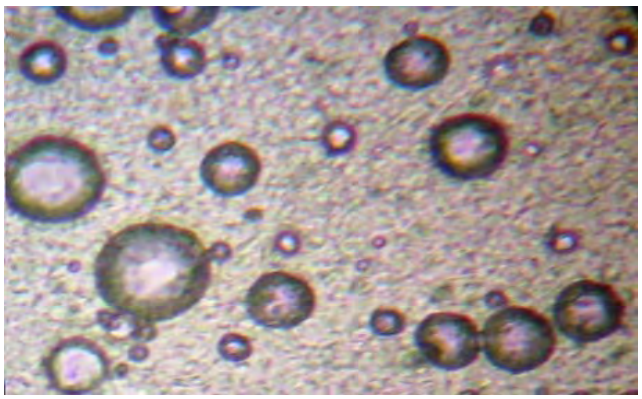

Figure 2. *In vitro* release study SLN formulations