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STUDIES ON REVERSE MICELLE SOLUTION TRANSFORMATION INTO LAMELLAR LIQUID CRYSTALLINE SYSTEM OF PROPRANOLOL

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Abstract - The purpose of this research was to develop and evaluate transformation type liquid crystalline transdermal drug delivery system of propranolol in a form of gel. The reverse micelle lamellar transformation liquid crystals were prepared from lecithin, isopropyl myristate and propranolol. Prepared liquid crystalline transdermal gel was evaluated for anisotropy, vesicular size, polydispersity index, encapsulation efficiency, viscosity and *in vitro* drug release study. Formulations were observed under polarized microscopy and found to have liquid crystals on the basis of the presence of birefringence. Vesicular size of the liquid crystals formulation was found from 784 to 968 nm and polydispersity index was found from 0.51 to 0.78 among all developed five formulations. Entrapment efficiency and viscosity was found from 64.54 to 83.24% and from 1090 to 1420 cp respectively in all five formulations. *In vitro* release of all the formulations was performed and data were treated for release mechanism. All the formulations exhibited controlled release property and formulation PLC2 followed Peppas-Korsmeyer release pattern. It was concluded that final formulation may offer controlled transdermal delivery of propranolol as compared conventional therapy.

Keyword - Reverse micelle, transformation, liquid crystals, transdermal, drug delivery

1. INTRODUCTION

Nobel drug delivery system is gaining its importance worldwide due to their therapeutic advantages and patients compliance. Similarly, Transdermal drug delivery system (TDS) has many advantages over conventional drug delivery. It avoid hepatic first-pass metabolism and improves patient compliance. TDS also offers controlled drug release, protection from gastric environment, convenient and self administration of dosages forms [1], [2]. Recent development has been shown that transdermal pathway is a potential mood of delivery of lipophilic drugs in systemic circulation [3]. TDS releases drug at a zero order rate or near zero order which is now in most cases considered as an ideal system for maintaining constant drug levels. Transdermal drug delivery system offers wide scope in controlling rate of drug delivery [4], [5]. This technology has been successfully utilized in the development of nitroglycerine [6], ephedrine [7], Ketoprofen [8], fentanyl [9], testosterone [10], [11], nicotine [12], glipizide [13], glibenclamide [14] etc. transdermal drug delivery system.

Liquid crystalline systems are intermediate state of matter which exists between solid crystals and liquid. The can also be termed as fourth phase of matter. The properties of liquid crystals are characteristic of solids and liquids. They possess both structural ordering and mobility. Liquid crystals, when viewed under polarizing light microscope, intense color bands and birefringence are observed. Liquid crystals are a condensed state of matter formed by anisotropic organic molecules. While not all anisotropic molecules can form liquid crystals, all liquid crystals are formed by anisotropic molecules. These molecules are termed mesogens and are either of rod-like or less commonly disc-like shape. On the basis of method of preparation liquid crystals are classified as thermotropic liquid crystals and lyotropic liquid crystals [15]. Lyotropic liquid crystals are prepared in presence of water or other solvent. They are formed by molecules called mesogens that are not the molecules themselves but hydrates or solvates as well as by associates of hydrated or solvated molecules

[16]. While in case of lyotropic liquid crystals, when the surfactant crystals are in contact with water or solvent and the temperature is increased to Kraft temperature, the lipophilic chains are transformed into a disordered liquid state and the water penetrates between the polar hydrophilic layers to form a lyotropic liquid crystalline (called neat phase) when mixture is cooled below the Kraft point, the hydrocarbon chains will crystallize and arrange themselves in a lattice with water still present between the polar groups this is referred to as a gel phase. Lyotropic mesophases are always strongly birefringent, although their physical nature may vary widely from that of a waxy substance to that of a clear gel. The liquid crystalline phase is thermodynamically stable and represents a state of incomplete melting [17]. Unlike lyotropic liquid crystals, thermotropic liquid crystals form with or without solvent. They are prepared on heating above Kraft temperature and cooling in a particular manner.

During the past decade, there has been great interest in liquid crystalline systems as drug delivery systems in the field of pharmacy. The reasons for this interest includes the extensive similarity of these colloid systems to those in living organism and their advantageous properties over those of traditional semisolid dermal dosage forms. Their formation is explained by the spatial organization of aggregates of nonionic surfactant molecules, at lower and higher concentrations. These lyotropic mesophases are usually formed from water and one or two surfactant and possibly co surfactants as very definite proportions with low energy input or by means of spontaneous structural organization. Their production is relatively simple and energy saving. They are thermodynamically stable and can be stored unchanged for long periods of time without phase separation. Depending on the concentration of the solvent (generally water or an aqueous solution) and on the polarity of the solvated mesogen, these systems can undergo various phase transition. As a consequence, their consistency can be changed systematically [18], [19]. These drug bearing liquid crystals were utilized as transdermal drug delivery system.

Propranolol is a non selective β blocking drug, therapeutically used in the management of mild to moderate hypertension. It absorbed from gastro intestinal tract after oral administration of conventional dosages forms and undergo extensive hepatic first-pass metabolism [20]. It is also associated with gastro intestinal tract side effect due to this alternate mode of controlled drug delivery system proposed as reverse micelle solution transformation into lamellar liquid crystalline system of propranolol in this study.

2. MATERIALS AND METHODS

Gift sample of propranolol hydrochloride was generously supplied by Lupin Lab. Pvt. Ltd.; Aurangabad, India. Soya lecithin purchased from HiMedia Laboratories Pvt. Ltd., India, isopropyl myristate was procured from E. Merck, Mumbai. All other ingredients unless otherwise specified were of analytical reagent grade. Double distilled water was used throughout the experiment which was boiled and cool to room temperature to remove dissolved gases.

2.1. Modification of propranolol hydrochloride to propranolol

The propranolol hydrochloride was converted to propranolol base by taking 5 g of propranolol hydrochloride and it was dissolved in 100 ml of double distilled water. In this solution, 5 % w/v aqueous solution of sodium hydroxide was added gradually. Precipitate of propranolol base drug was formed which was filtered on Whatman filter paper number 01 and washed with double distilled water until the washings were free from any traces of chloride in the precipitate [21].

2.2. Preparation and characterization of transformation type liquid crystals

The reverse micelle lamellar transformation based liquid crystals (LC) were prepared by the

method reported by Goymann and Hamann [22] with a slight modification. Weighed amount of soya lecithin was dissolved in isopropyl myristate at 60°. After cooling to room temperature the solution remain clear and transparent upto a phospholipids content of 60% (w/v). A small amount of water was added to the oily solution of phospholipids with the help of syringe. Solubilization took place immediately without stirring leading to the formation of reverse micelle. For the preparation of drug loaded reverse micellar solution propranolol was mixed with isopropyl myristate.

2.3. Optical microscopy

The presence of liquid crystals were observed using the polarized light microscope (Nikon HFX, Japan) attached with digital camera in bright field and between crossed polar of light. Transformation type liquid crystals were examined immediately after preparation and at frequent intervals. Photomicrograph of liquid crystalline systems was also taken in plane and polarized light [23], [24].

2.4. Average vesicular size, polydispersity index

Average vesicular size and polydispersity index were determined laser particle size analyzer (DTS 5.03, Melvern Instruments Ltd., U. K.). An aliquot of liquid crystals was diluted with 5 ml of deionized water and diluted sample of liquid crystals was filled in cuvette. Particle size, and polydispersity index were observed in display screen at ambient temperature [25].

2.5 Entrapment efficiency

Entrapment efficiency of drug in a particular vesicular system is defined as the percent of total drug entrapped in liquid crystals or ratio of entrapped drug and un-entrapped drug in liquid crystals. Drug, which is not encapsulated in the liquid crystals, is termed as free drug. This free drug from liquid crystals dispersion was removed by placing the 5 g of liquid crystals dispersion in dialysis tube (Spactrapore, membrane size 5000-7000 molecular weight cut off), thickness 25 μm, (Los Angle, USA) and dialyzing exhaustively against small amount of methanol in centrifuge apparatus. Un-encapsulated drug comes outside the dialysis tube; it was separated, dissolved in methanol and analyzed spectrophotometrically at 290 nm using Shimadzu double beam spectrophotometer [26].

2.6. Viscosity

Resistance of flow of liquid crystalline system was observed by Brookfield viscometer (DV-E viscometer, Brookfield, USA). Samples were kept in a wide glass tube at ambient condition and viscosity measured using spindle no 62 of viscometer supplied along with viscometer. The spindle speed of viscometer was fixed at 30 rpm and viscosity directly noted from the digital viscometer display [27]. All the measurements were taken as average of three determinations.

2.7. *In vitro* drug release study

Locally fabricated Franz diffusion type cell was employed for *in vitro* release study of propranolol from liquid crystalline gel [28], [29]. Synthetic semipermeable membrane was employed as permeation barrier (Spactrapore, membrane size 5000-7000 molecular weight cut off), thickness 25 μm, (Los Angle, USA). This commercially available semipermeable membrane was fixed on the receptor compartment of the diffusion cell and liquid crystalline formulation equivalent to 5 mg of propranolol was applied on the outer side of the membrane. The receptor compartment filled with 50 ml of the 40 % polyethylene glycol 400 and isotonic buffer (pH-6) solution at 32°C. Five milliliter samples were withdrawn at different time interval and the same was replaced with 5 ml of the fresh media solution in order to maintain the sink condition. The withdrawn samples were filtered and diluted with methanol. The samples were analyzed spectrophotometrically at 290 nm using (Shimazdu, Japan) double beam spectrophotometer [30].

TABLE 1: Composition of liquid crystalline formulation of propranolol

Ingredients	Formulation Code				
	PLC1	PLC2	PLC3	PLC4	PLC5
Propranolol (mg)	50	50	50	50	50
Soya lecithin (mg)	1	1	1	1	1
Isopropyl myristate (ml)	0.5	1.0	1.5	2.0	2.5
Double distilled water q.s. (ml)	10	10	10	10	10

TABLE 2: Evaluation of liquid crystalline formulation of propranolol

Formulation code	Zero order	First order	Higuchi Matrix	Peppas-Korsmeyer
PLC1	0.9154	0.8651	0.7974	0.8867
PLC2	0.8943	0.7667	0.9265	0.9853
PLC3	0.9639	0.8732	0.8990	0.8836
PLC4	0.9942	0.8879	0.9176	0.8789
PLC5	0.9832	0.8781	0.9157	0.8947

3. RESULTS AND DISCUSSION

Propranolol hydrochloride was converted to propranolol base. This conversion of hydrochloride salt of drug to free base increases the lipophilicity, decreases ionization, and size of the permeant molecules. All these three property are advantageous in the development of good transdermal system. Partition coefficient i.e. logP value of propranolol free base is reported about 1.2 and its pKa is 9.5, which indicates the relative affinity of the free propranolol base toward the nonpolar phase. However, propranolol hydrochloride is Polar in nature and has low skin permeability which makes it unsuitable for transdermal application. Because of high permeability, favorable logP value, and greater pKa, free base was selected for the development of transformation type liquid crystals transdermal drug delivery system [20].

Liquid crystals are intermediate state of matter which exists between solid crystals and liquid. The properties of liquid crystals are characteristic of solids and liquids. They possess both structural ordering and mobility. Liquid crystals, when viewed under polarizing light microscope, intense color bands and birefringence are observed. Propranolol absorbs from gastro intestinal tract after oral administration of conventional dosages forms and undergo extensive hepatic first-pass metabolism. Propranolol is also associated with gastro intestinal tract side effect such as nausea, vomiting etc. due to this, alternate mode of controlled drug delivery system proposed as reverse micelle solution transformation into lamellar liquid

crystalline system of propranolol in this study.

Transformation type liquid crystals were prepared from lecithin, isopropyl myristate, propranolol and double distilled water and evaluated for microscopy/ anisotropy, size and size distribution, polydispersity index, viscosity, entrapment efficiency and *in vitro* release study. In the preparation of transformation type liquid crystals the increase of water content causes a change in shape and size and finally a phase transformation from the reverse micellar solution into a lamellar liquid crystals [23]. Formulation and composition of liquid crystals formulation is given in Table 1. Anisotropy and birefrigerence of the system seen in polarized light microscope which proved the presence of liquid crystals. Photomicrograph of transformation type liquid crystals were taken at 40X in plane and polarized light as given in Figure 1.

Average vesicular size and polydispersity index were determined laser particle size analyzer (DTS 5.03, Melvern Instruments Ltd., U. K.). Both are useful parameter for quality control parameter, stability and release kinetics study. In general, particle size, and polydispersity index should be uniform for quality control purpose. Mixture of smaller and larger vesicles are good at certain level, higher polydispersity index may leads to instability in system. Particle size, and polydispersity index were observed in display screen of particle size analyzer at ambient temperature [25]. Vesicular size of the liquid crystals formulation was found from 784 to 968 nm. Polydispersity index was found from 0.51 to 0.78 which reflect almost uniform average mix-up of different vesicles. The data of average vesicular size and polydispersity index is given in Table 2.

Entrapment efficiency of drug is important aspect for the determination of drug content in dosages form and amount to be incorporate to fix the dose. It is determined by dialyzing non-entrapped using dialysis tube (Spactrapore, membrane size 5000-7000 molecular weight cut off), thickness 25 μm, (Los Angle, USA) and dialyzing exhaustively against small amount of methanol in centrifuge apparatus. Unencapsulated drug comes outside the dialysis tube; it was separated and analyzed spectrophotometrically at 290 nm using Shimadzu double beam spectrophotometer. Entrapment efficiency was found from 64.54 to 83.24% in different formulation. Entrapment efficiency more than 75% in vesicular system thought to be good. Select formulation PLC2 has 83.24% entrapment efficiency which is higher as compared to all other formulation as given in Table 2. This higher entrapment efficiency in PLC2 may be due to right combination of lecithin and isopropyle myristate which has formed compact bilayer of vesicle.

Viscosity is a significant parameter for semisolid material and also important for transdermal gel for proper spreading on the skin. It was determined with the help of Brookfield viscometer (DV-E viscometer, Brookfield, USA)[27]. Higher viscosity takes more energy to apply to the skin and also may be difficult to make thin film. Therefore sufficient viscosity that can apply without extra effort and make a thin film is desired in a developed formulation. Viscosity was found from 1090 to 1420 cp in all the formulation (Table 2). Formulation PLC2 had 1420 cp viscosity, this is sufficient viscosity for handling point of view and considered as selected formulation.

In vitro release study of propranolol from liquid crystalline gel was performed by using locally fabricated Franz diffusion type cell [28], [29]. In this study, synthetic semipermeable membrane was employed as permeation barrier (Spactrapore, membrane size 5000-7000 molecular weight cut off), thickness 25 μm, (Los Angle, USA). The receptor compartment of diffusion cell was filled with 50 ml of the 40 % polyethylene

glycol 400 and isotonic buffer (pH-6) solution at 32°C. Five milliliter samples were withdrawn at different time interval and the same was replaced with 5 ml of the fresh media solution in order to maintain the sink condition. The withdrawn samples were filtered and diluted with methanol. The samples of *in vitro* release study were analyzed spectrophotometrically at 290 nm using (Shimazdu, Japan) double beam spectrophotometer [30]. The release study was performed for eight hour and samples were withdrawn in an interval of one hour. Formulation offered cumulative release of drug from 46.33 to 62.38% in eight hours. The release profile of all the formulations is given in Figure 2, showing cumulative drug release versus time in hour.

The data of *in vitro* release profile were studied for various kinetic models like zero order, first order, Higuchi matrix and Peppas-Korsmeyer Table 3. Formulation PLC1, PLC3, PLC4 and PLC5 showed zero order release profile however formulation PLC2 showed Peppas-Korsmeyer release pattern [32], 33].

TABLE 3. Regression coefficients value of *in vitro* release study of propranolol from liquid crystal formulation

Film Code	Average vesicular size (nm)	Polydispersity index	Viscosity (cp)	Entrapment efficiency (%)
PLC1	784	0.66	1240	78.56
PLC2	843	0.58	1420	83.24
PLC3	968	0.51	1380	81.72
PLC4	893	0.72	1210	72.18
PLC5	876	0.78	1090	64.54

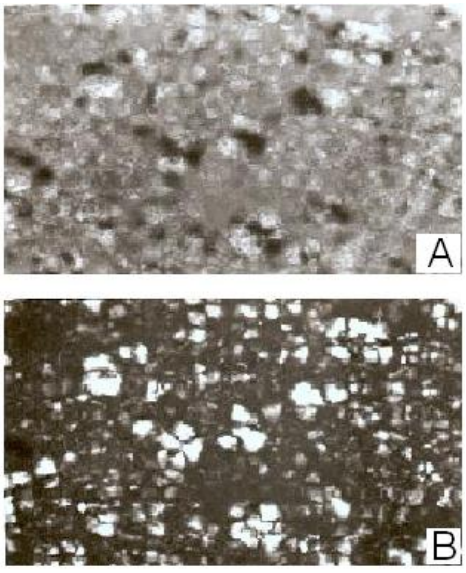

FIGURE 1: Photomicrograph of transformation type liquid crystals formulation of propranolol at 40X under (A) Plain (B) Cross polarizer microscope

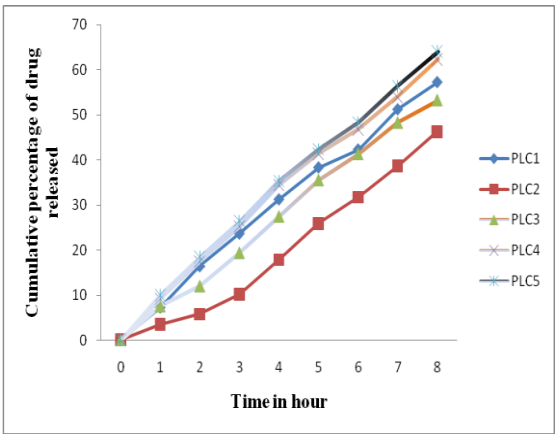

FIGURE 2: *In vitro* cumulative percentage of drug released vs time of propranolol from liquid crystals formulations

All the formulation showed controlled release property and formulation PLC2 showed mixed pattern i.e. anomalous type which may

be due to the combination provided by the formulation offered compactness in the bilayered membrane structure. Due to this, it is presumed that formulation could hold higher encapsulation efficiency and viscosity also. Therefore, PLC2 selected as developed formulation.

4. CONCLUSION

It concluded that method of preparation of transformation type liquid crystals is simple and does not require any specific equipment. Encapsulation efficiency is also higher and viscosity of all the formulation found in the acceptable range. *In vitro* release profile of the drug offered controlled release of drug that can be utilized for once in a day application. Therefore, on the basis of method of preparation and evaluation, further it can be concluded that formulation may be simple, economic and easy to apply for once a day application in the management of mild to moderate hypertension.

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