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Optimization and Drug Release Studies of Dithranol Loaded Solid Lipid Nanoparticles for its Exploitation as Topical Drug Delivery System

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ABSTRACT

Aim of present study was to encapsulate dithranol into solid lipid nanoparticles to prevent its autooxidation during its storage along with enhancing its skin retention for improved topical application. In addition, attempts were made to perform optimization, characterization and *in vtiro* release pattern of dithranol loaded solid lipid nanoparticles (DTH-SLN). For the preparation of solid lipid nanoparticles biocompatible lipid tristearin, cholesterol and soya phosphotidylcholine were used. The SLNs were stabilized by using steric stabilizer tyloxapol. SLN were prepared by simple liquid crystalline phase technique using self invented hot lipid injector. Hydrogel with carbopol 934 and hydroxypropyl methyl cellulose (HPMC) at a ratio of 2:1 was chosen for vehicle formulation. The percentage skin-drug retention of prepared formulation was found to be much higher than the conventional dithranol gel. When studied for 10 hours DTH-SLN hydrogel provided 20.30 % drug retention in comparison to 9.16 % drug retention for conventional dithranol gel. Release profile of prepared SLN best fitted into Korsmeyer-Peppas (correlation r = 0.9938) and Higuchi equation (correlation r =0.991750 It can be therefore concluded from the studies that dithranol loaded SLN can be successfully used for effective treatment of psoriasis.

KEY WORDS

solid lipid nanoparticle, dithranol, psoriasis, hydrogel, topical.

1. Introduction

Dithranol (also called anthralin, IUPAC nomenclature: 1, 8-dihydroxy-9(10*H*)-anthracenone) is the most effective agent in the treatment of psoriasis still continues to be one of the indispensable topical antipsoriatic agents (Aston et al 1983). Dithranol is a naturally occurring substance found in the bark of the araroba tree in South America. It can also be synthesized from anthrone. Its antipsoriatic action was noted over 100 years ago, when an extract of araroba bark was used to treat psoriasis (Lionel Fry, 2004). Dithranol is an aromatic compound consisting of three benzene rings (anthracene derivative) with two hydroxyl groups at C1 and C8, a carboxyl group at C9, and a methylene group at C10 position (Figure 1). It is easily oxidized at the C10

methylene group by air, light, high temperature and alkali and also quickly oxidized when comes into contact with the skin.

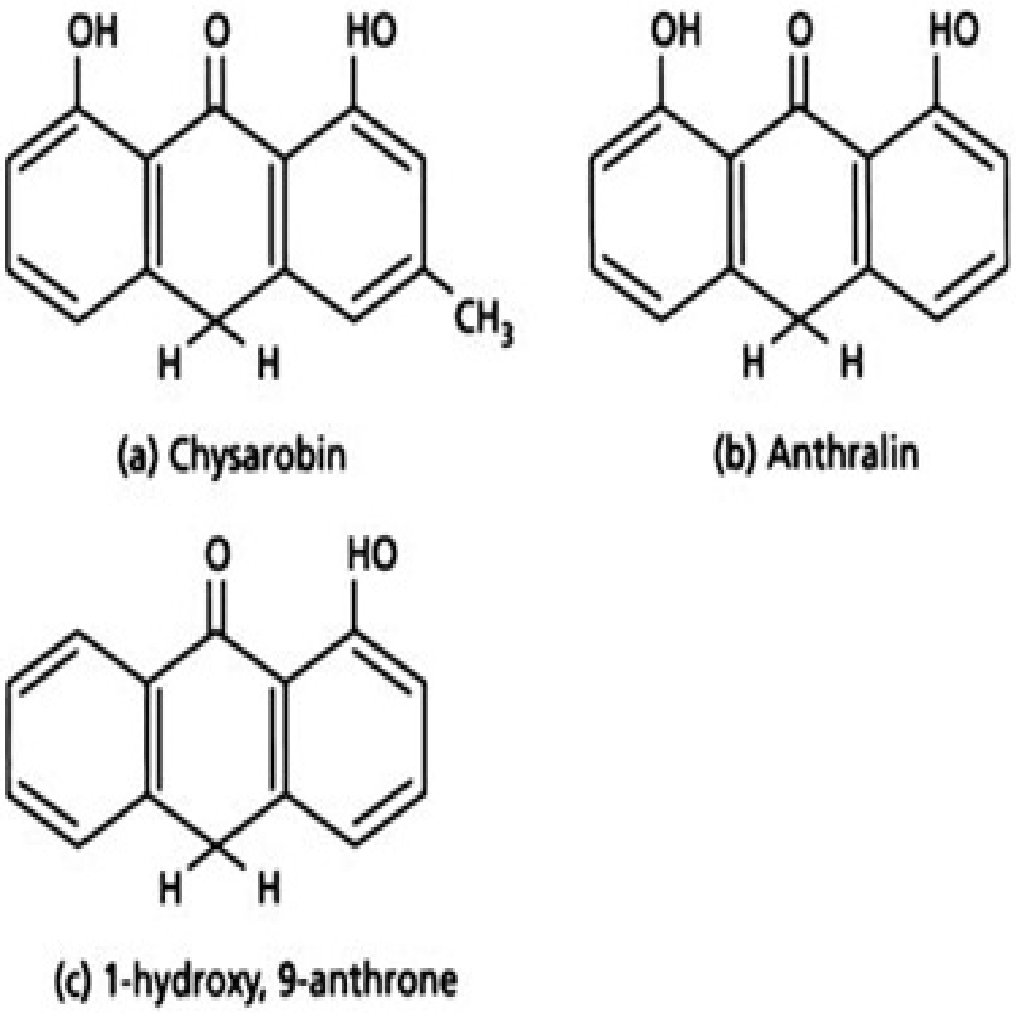

Fig. 1. Chemical structure of antipsoriatic anthrones.

Dithranol (DTH) is a potent agent in treating psoriasis but its adverse effects on intact skin have limited its usage. Its antipsoriatic and related irritation effect is caused by oxygen free radical formation during auto-oxidation (Figure 2).Dithranol further disintegrates into danthrone, dianthrone and anthralin brown. Its decomposition is catalyzed by light, air, oxygen, water, high pH and high temperature (Katare Om Prakash et al, 2008).

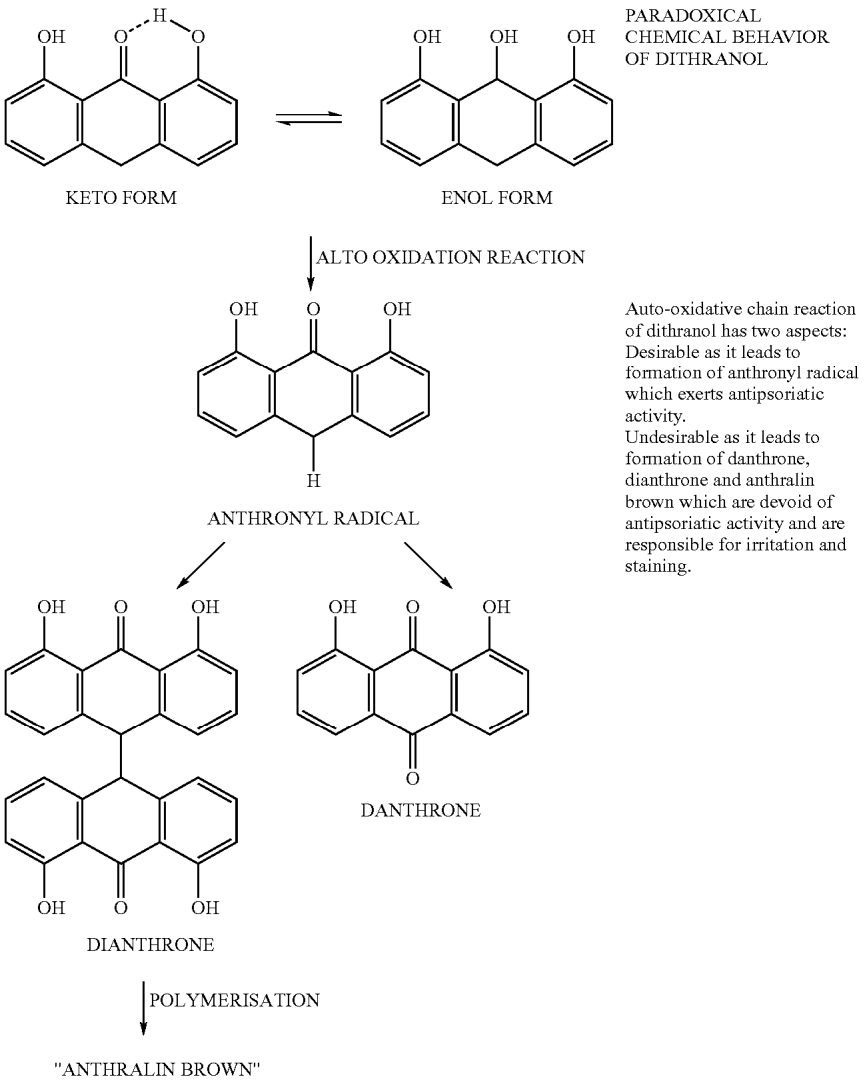

Fig. 2. Autooxidation of dithranol leads to undesirable dithranol dimers.

Solid lipid nanoparticles (SLN) are colloidal carriers, which were developed at the beginning of the 1990s as an alternative system to the existing traditional carriers (emulsions, liposomes and polymeric nanoparticles), especially for the delivery of lipophilic compounds. They are very much like nanoemulsions, differing in lipid nature. The liquid lipid used in emulsions is replaced by a lipid solid at room temperature in SLN including high melting point glycerides or waxes (R.H.Müller, 2003).

Beginning about 4 years ago, attention focused on the use of SLN in topical formulations with not only pharmaceutical, but also cosmetic, applications. Worldwide-registered trade names are SLN® and, for topical products, Lipopearls® (Trademarks by SkyPharma PLC).

In order to retard DTH degradation prior to its skin penetration and to enhance DTH skin retention, drug was encapsulated inside solid lipid nanoparticles. For this purpose tristearin with

high biocompatibility was used as lipid carrier along with cholesterol as membrane rigidizer and soya phosphotidylcholine with tween 80 as emulsifier. Tyloxapol was employed as steric stabilizer. DTH-SLN was prepared by liquid crystalline phase dilution method with microemulsion preparation at high temperature and subsequent solidification at low temperature (Karsten Mäder, 2006). Optimization and characterization of DTH-SLN was performed. DTH release profile from DTH-SLN hydrogel was also investigated.

2. Materials and method

2.1 Materials

Dithranol (DTH 97%) and tristearin (TSN) were purchased from Hi Media Laboratories Pvt. Ltd., India. Tyloxapol (TX) was purchased from Sigma-Aldrich Pvt. Ltd., USA. Cholesterol (CHL) was procured from Qualigens Fine Chemicals, India and Soya Phosphotidylcholine (PC) was kindly received as gift samples from Sun Pharma Advance Research Center, Baroda, India.

All other reagents are of analytical reagent grade and were used as received.

2.2 Method

2.21 Preparation of Solid Lipid Nanoparticles

The procedure for producing solid lipid nanoparticles was a modification to the method called liquid crystalline phase dilution (Karsten Mäder, 2006). Accurately weighed amount of tristearin (TSN) was heated at 75°C temperature then allowed to melt completely with various amount of cholesterol (CHL). Certain amount of DTH was dispersed into melted lipid mixture. Separately, double distilled water was heated to 75°C. Typically various amounts of soya phosphotidylcholine and tyloxapol were added to the water under magnetic stirring and allowed to equilibrate at 75°C.

Next, melted lipid was added to the water-surfactant solution and once again allowed to equilibrate at 75°C.It was found during the preparation that melting point of tristearin is about 67°C and such low melting point really creates some problem regarding its melted delivery on to aqueous phase due to rapid solidification of lipid phase so to facilitate continuous dropping of melted lipid into aqueous phase a device called Hot Lipid Injector was developed.

The emulsion was stirred on a magnetic stirrer for half an hour to form pre-emulsion. The pre-emulsion was sonicated for optimized time.

Rapid solidification of obtained pre-emulsion was performed by pouring the pre-emulsion drop by drop in an ice cooled water-surfactant (tween 80 used as surfactant) solution (4°C). The resulting dispersion was centrifuged at 15000 rpm for 40 minutes and supernatant fluid was collected, sedimentation being discarded. Resulting solid lipid nanoparticle dispersion was frozen at - 20°C for 12 hour and then transferred to freeze-dryer and dried for 24 hour at -55°C using 10%w/v mannitol as cryoprotectant.

2.22 Preparation of Hydrogel vehicle

Hydrogel was prepared using Carbopol 934 and Hydroxypropyl methyl cellulose (HPMC) at a ratio of 2:1. Formulations containing 2 carbopol: 1 HPMC offers a gel of highest viscosity structure and best drug diffusion (Quiñones, D., et al 2008). HPMC (125 mg) was added gradually into 13 ml of water at 90°C while continuous stirring. Double distilled water (12 ml) containing 5%w/w of glycerol was cooled to 5°C and added to the dispersion of HPMC mixture. Carbopol was passed through screen No. 40 and 250 mg of carbopol was dispersed in to HPMC mixture while continuous stirring. Final weight was made up to one gm by adding additional quantity of distilled water. Required quantity of triethanolamine was added to adjust pH 5.8. Prepared mixture was cooled slowly at room temperature. For preparation of 2.0%w/w of dithranol conventional hydrogel, 200 mg dithranol was dispersed in 50 ml of cooled aqueous solution containing 5% w/w of glycerol prior to its adding in HPMC mixture while for equivalent gel of DTH-SLN containing 2.0%w/w dithranol, calculated amount of dithranol loaded SLN was dispersed in 50 ml of cooled aqueous solution containing 5%w/w of glycerol prior to its adding in HPMC mixture.

2.23 Determination of entrapment efficiency and drug loading

The drug content and the encapsulation efficiency (DEE) were expressed as percentage and calculated according to the following equations:

Drug loading capacity = Weight of encapsulated drug/ Weight of SLN recovered X 100

Drug entrapment efficiency= Weight of drug encapsulated in SLN/ Weight of drug used for SLN preparation X 100

Drug entrapment of the antipsoriatic drug, dithranol in Solid Lipid Nanoparticles was determined by using Sephadex mini column (Fry D W, 1978). To prepare Sephadex mini column, firstly one gm of sephadex G-50 was allowed to swell in 0.9% sodium chloride aqueous solution for 8 hr and then the hydrated gel was filled in the barrel of one ml disposable syringe plugged with filter pad. The barrel was centrifuged at 2000 rpm for 2 minutes to remove excess of saline solution to form the sephadex separating column. To separate free drug from formulation, SLN dispersion was applied drop wise on the top of the Sephadex column and then centrifuged at 2000 rpm for 2 minutes to expel and remove void volume containing SLN in to the centrifuged tubes. This eluted SLN dispersion was collected and lysed by disrupting with 5% triton-X100 and then the amount of entrapped drug was analyzed using spectrophotometric method.

3. Optimization of process variables and ingredients.

3.1 Optimization of sonication time with regard to particle size of SLN.

The ultrasonication system employed for SLN preparation consists of a generator, a converter, and a sonotrode. Probe sonicator (Lark innovative Tec knowledge, Chennai) was used to produce pre-nano emulsion for the solid lipid nanoparticle formulation. The energy generated was 2 KW with 25 second pulse rate. Three formulations coded as SLN_{ST2}, SLN_{ST3}, SLN_{ST5} and SLN_{ST7}

were prepared for varying sonication time 2, 3, 5 and 7 minutes respectively.

3.2 Optimization of Tyloxapol and soya phosphotidylcholine with regard to zeta potential of SLN.

Different formulations coded as SLN_{ty01}, SLN_{ty05}, SLN_{ty10} and SLN_{ty15} were prepared using 1.0 %, 5%, 10% and 15% w/w of Tyloxapol.

Similarly different formulations coded as SLN_{PC01} ,$SLN_{PC1.5}$ and $SLN_{PC2.0}$ were prepared using 1.0 %, 1.5% and 2.0% w/v of soya phosphatidylcholine.

Volume of aqueous phase is being kept 40 ml and sonication time 3 minutes.

4. Characterization of DTH-SLN

4.1 Particle size analysis

Particle size analysis of DTH-SLN was performed by Malvern Matersizer 2000 (Malvern instruments UK). The aqueous nanoparticulate dispersion was added to the sample dispersion unit containing stirrer and stirred in order to minimize the interparticle interactions. Particle size by intensity was measured and Z average diameter was calculated.

4.2 Zeta Potential measurement

Zeta potential was analyzed by Malvern Zetasizer 2000. (Malvern instruments UK). Distilled water with dielectric constant 78.5 was chosen as dispersant solvent. Zeta potential as well as zeta deviation in mV was calculated as an indication for stability of SLN dispersion.

4.3 Effect of Tristearin /Dithranol ratio on Drug Entrapment Efficiency

The formulations coded as SLN_{DL2}, SLN_{DL4}, SLN_{DL6} and SLN_{DL8} were prepared keeping the tristearin composition same and varying the tristearin: dithranol ratios 10:2, 10:4, 10:6 and 10:8 respectively. All preparations were subjected to determination of drug encapsulation efficiency.

4.4 Effect of Tristearin /Cholesterol ratio on Drug Entrapment Efficiency

Incorporation of cholesterol with tristearin during SLN preparation is necessary to fill up some negligible gaps created within lipid matrix and to provide rigidity to the outermost crust of SLN. The inclusion of cholesterol as a matrix forming material markedly influences drug entrapment efficiency.

Formulations coded as SLN_{ch0}, SLN_{ch4}, SLN_{ch7} and SLN_{ch1} were formulated by varying lipid-cholesterol ratios 10:0, 9.6:0.4, 9.3:0.7 and 9:1 respectively maintaining overall quantity of matrix forming material same for all formulations. Dithranol entrapment studies were performed for all coded formulations.

4.5 *In vitro* drug release study

In vitro drug release studies were carried out using the HI MEDIA cellophane membrane (12000 molecular cut off weight). Membrane was treated with 5%EDTA solution for half an hour and then in boiling water for one hour prior to use. Optimized DTH-SLN formulation was chosen for *in vitro* drug release profile. 500 mg of SLN formulation was weighed and suspended in 5 ml of distilled water. One ml aqueous suspension was placed inside cellophane membrane and placed in a one liter capacity beaker containing 100 ml phosphate buffer solution (pH 5.8) with 20% PEG 400. Release medium was maintained at 32±1°C with continuous stirring by magnetic stirrer. Aliquots of one ml dissolution medium were removed initially at 30 minutes and then after one hour. Sink condition was maintained by adding equal volume of fresh dissolution medium at each intervals. The samples were filtered and amount of dithranol was estimated using UV spectrophotometer at λ_{max} 254 nm.

4.6 *In vitro* skin retention studies

Samples of human cadaver skin were obtained from Autopsy Department, Government Medical College & Research Centre, Bhopal. Subcutaneous fat was carefully trimmed and skin was immersed in distilled water at 60±1°C for 2 minutes. Stratum Corneum and epidermis (SCE) were removed from dermis using a dull scalpel blade. Epidermal membranes were dried in desiccators at approximately 25% relative humidity. The dried samples were wrapped in aluminium foil and stored at 4°C until use (Marla Sheffer, 2004). Slices of skin were rehydrated by immersion in distilled water for one hour at room temperature and mounted in ten Franz-type diffusion cells. Exposed area was 4.15 cm^2 and receptor volume was of 20 ml. Twenty ml of phosphate buffer (pH 5.8) containing 20% PEG 400 was used as receptor media. Skin slices were carefully mounted in all Franz-type diffusion cells. The experiments were carried out at 32±1°C with continuous stirring by magnetic stirrer. The donor compartments were filled with 500 mg of equivalent gel of SLN containing 2.0% dithranol (DTH-SLN).Similar experiments were conducted for dithranol loaded conventional gel (DTH). Withdrawals of samples were done from different diffusion cells for each time intervals and dithranol content was analyzed. At the end of each experiment, skins were removed from diffusion cells and the excess of formulation on upper part of skin was cleaned with cotton dipped in saline solution and blotted with tissue paper to remove any adhering formulation (Katare O. P et al, 2004). Skin tissue was cut into fragments and transferred into conical flask containing 20ml of chloroform (extractor solvent). Solution mixtures were sonicated for 20 minutes. Contents were filtered and amount of dithranol was finally analyzed (Maria Bernadete et al, 2001).

5. Result and discussion

The aim of present work is to prepare and characterize dithranol loaded Solid Lipid Nanoparticles for effective treatment of psoriasis. For the preparation of solid lipid nanoparticles biocompatible lipid tristearin, cholesterol and soya phosphotidylcholine were used. The SLN

were stabilized by using stearic stabilizer tyloxapol. SLN was prepared using liquid crystalline phase dilution method. Various process variables used for SLN preparation were optimized for smaller lipid nanoparticles, less polydispersity index and optimum zeta potential. The process variables studied were sonication time for optimum particle size and less polydispersity index (PdI). Later on, effect of phospholipid and tyloxapol concentration on zeta potential was studied.

5.1 Effect of Sonication Time

Effect of sonication time was studied for optimum particle size. It was found that sonication of 3 minutes at 2 KW is sufficient enough to provide good spherical shape and particle size of lipid emulsion (151 nm) with PdI 0.229 (Figure 4). Further increase in sonication time might disrupt the steric stabilizer or phospholipids layer necessary to cover and stabilize new smaller particles broken down from lager ones.

Size d.nm	Mean Intensity %	Std Dev Intensity %
0.4000	0.0	
0.4632	0.0	
0.5365	0.0	
0.6213	0.0	
0.7195	0.0	
0.8332	0.0	
0.9649	0.0	
1.117	0.0	
1.294	0.0	
1.499	0.0	
1.736	0.0	
2.010	0.0	
2.328	0.0	
2.696	0.0	
3.122	0.0	
3.615	0.0	
4.187	0.0	
4.849	0.0	

Size d.nm	Mean Intensity %	Std Dev Intensity %
5.615	0.0	
6.503	0.0	
7.531	0.0	
8.721	0.0	
10.10	0.0	
11.70	0.0	
13.54	0.0	
15.69	0.0	
18.17	0.0	
21.04	0.0	
24.36	0.0	
28.21	0.0	
32.67	0.0	
37.84	0.0	
43.82	0.0	
50.75	0.0	
58.77	0.0	
68.06	0.0	

Size d.nm	Mean Intensity %	Std Dev Intensity %
78.82	0.0	
91.28	0.0	
105.7	0.0	
122.4	0.0	
141.8	54.1	
164.2	45.6	
190.1	0.0	
220.2	0.0	
255.0	0.0	
295.3	0.0	
342.0	0.0	
396.1	0.0	
458.7	0.0	
531.2	0.0	
615.1	0.0	
712.4	0.0	
825.0	0.0	
955.4	0.0	

Size d.nm	Mean Intensity %	Std Dev Intensity %
1106	0.0	
1281	0.0	
1484	0.0	
1718	0.0	
1990	0.0	
2305	0.0	
2669	0.0	
3091	0.0	
3580	0.0	
4145	0.0	
4801	0.0	
5560	0.0	
6439	0.0	
7456	0.0	
8635	0.0	
1.000e4	0.0	

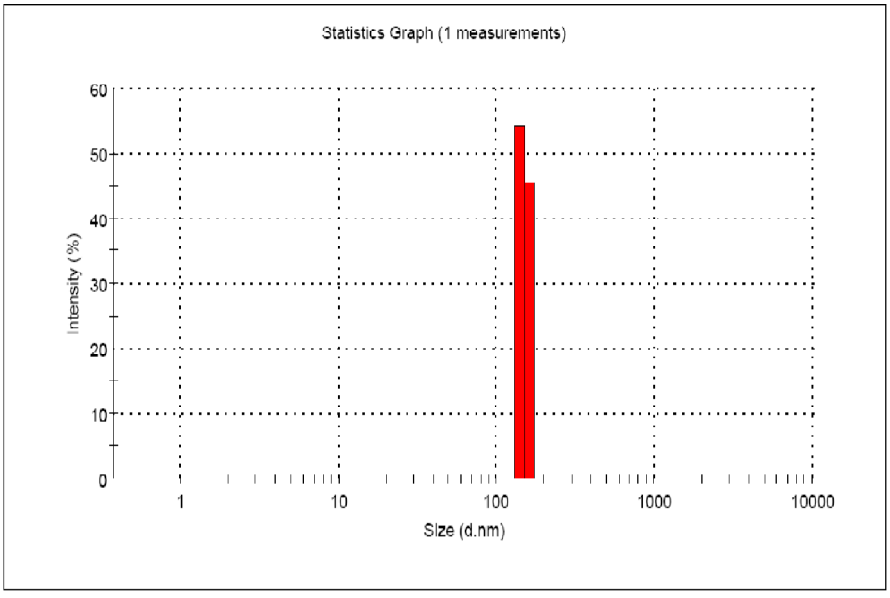

Fig. 4. Statistical graph of particle size distribution of SLN_{ST3} formulation.

5.2 Effect of Steric stabilizer

Different formulations using various concentrations of tyloxapol and phospholipid were prepared and their effects on surface charge measured as zeta potential were studied. Formulation SLN_{ty10} with 10 % w/w tyloxapol (of core forming lipid matrix) resulted into -25.9 mV zeta potential (Figure 8 and 10). Phospholipid concentration of 1.5% w/v for $SLN_{PC1.5}$ resulted in to -26.1 mV zeta potential (Figure 05). Critical value of zeta potential is -60 mV to -30 mV for a stable dispersion. The zeta potential of prepared SLN was found to be just below the critical value. Further stability of prepared SLN might be due to steric stabilization provided by surfactants.

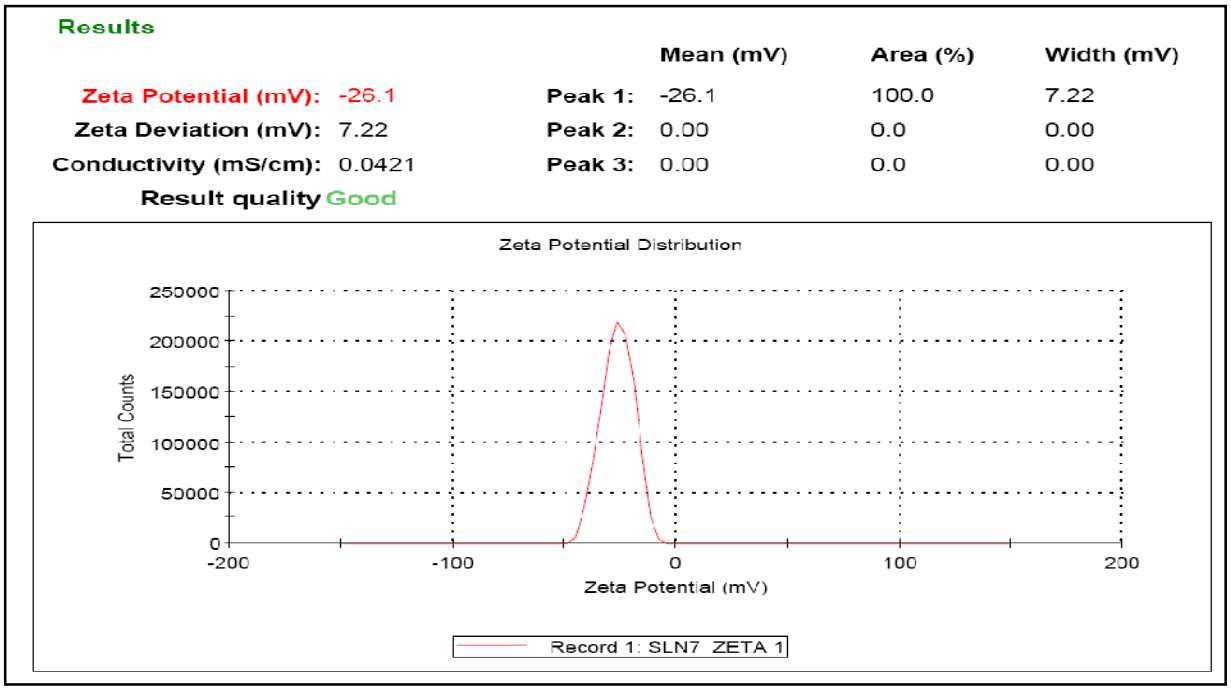

Fig.5. Zeta potential measurement of $SLN_{PC1.5}$ formulation.

5.3 Effect of Tristearin/ Dithranol on drug entrapment efficiency

The effects of dithranol tristearin ratios were studied for % drug entrapment efficiency and % drug loading. It was found that formulation SLN_{DL4} with dithranol tristearin ratio 4:10 provided maximum drug entrapment 85.3±1.9 with 43.6 % drug loading. Further increase in drug composition might caused drug saturation within lipid matrix and hence decrease in both % drug entrapment and drug loading.

5.4 Effect of Tristearin/Cholesterol on drug entrapment efficiency

The inclusion of cholesterol as a matrix forming material markedly influenced drug entrapment efficiency. Highest entrapment efficiency of 88.7±2.3 with 45.2 % drug loading was observed for SLN_{ch7} with tristearin cholesterol ratio 9.3:0.7. Dithranol is highly lipophilic in nature and

more soluble in tristearin than cholesterol. Initial cholesterol inclusion helped filling minute gaps in lipid matrix but further increases in cholesterol ratio resulted in reduction of lipid quantity used for drug dispersion and hence decrease in drug entrapment efficiency.

5.5 *In-vitro* Drug release studies

The *in vitro* release profile of entrapped dithranol was studied using cellophane membrane with 12000 molecular weight cut off. The initial rapid release of dithranol was observed for first few hours (Figure 16). The % cumulative drug release for initial first hour was 4.86±0.4. The sudden burst release may be attributed to surface associated drug. A faster release pattern was observed upto 7 hours with 14.84±2.1% cumulative drug release. SLN prepared from simple melt dispersion techniques provided bi-phasic release pattern. Formation of a drug enriched layer around the core may be attributed to bi-phasic release pattern. Drug enriched layer is near to particle surface and provided initial faster release. After this initial faster release SLN showed a slower release of 24.46±2.3% when studied for 24 hours.

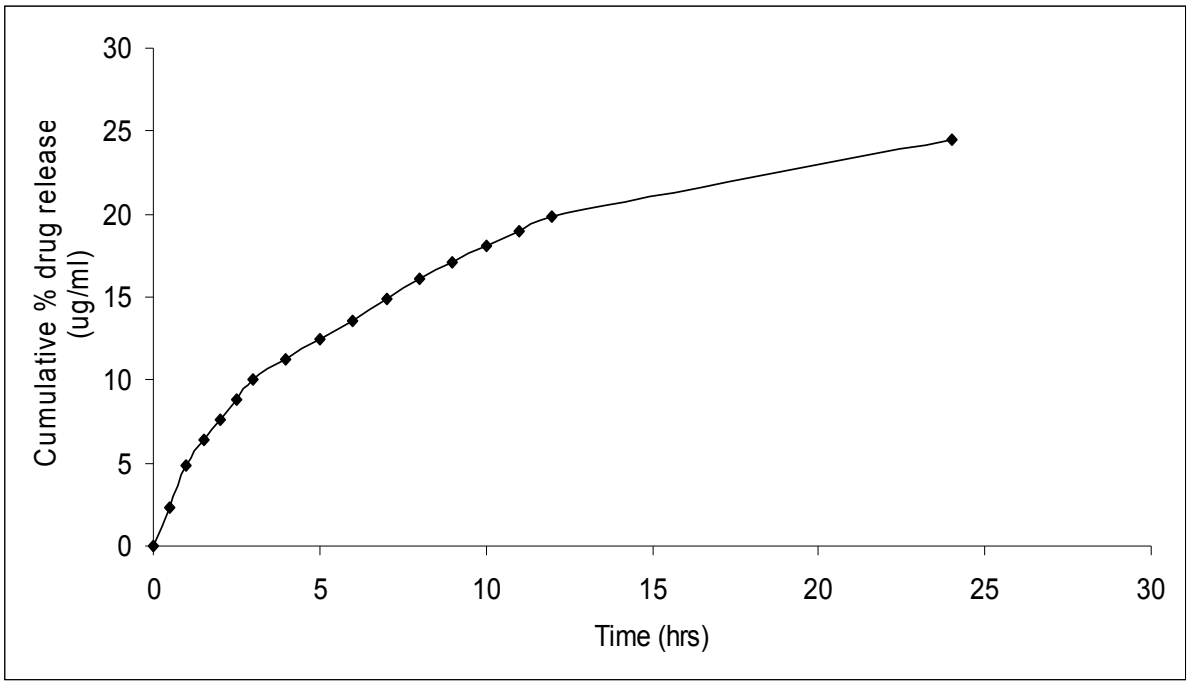

Fig.6. *In-vitro* release profile of $SLNAq_{40}ST_3ty_{10}el_{1.5}DL_4ch_7$ formulation in phosphate buffer pH 5.8.

Drug release profile data were fitted to different drug release kinetic models (Table 1). Release profile of prepared SLN was best fit into Korsmeyer-Peppas and Higuchi equation. The drug release from a matrix system is said to follow Higuchi's release kinetics if the amount of drug released is directly proportional to square root of time (Mohammad Barzegar-Jalali, 2008). A

good correlation of r =0.991750 was observed for prepared SLN. Korsmeyer-Peppas equation predicts whether drug release is diffusional or dissolution. It also gives prediction about fickian and non fickian release kinetics (Mario Grassi, 2005). SLN formulation was well fitted to Korsmeyer-Peppas equation (correlation r = 0.9938). Diffusion release exponent (η) calculated was found to be 0.52 if initial sudden burst release be omitted. The diffusion release exponent for the SLN formulation was found to be near to 0.50 indicating the characteristic fickian release pattern of the prepared SLN formulation. Little difference in value may be due to compact layer of phospholipids or steric stabilizer around lipid core.

Table 1: Non-linear fit analysis for optimized SLN.$aq_{40}ST_3ty_{10}el_{1.5}DL_4ch_7$ formulation.

Function	Equation	Value of correlation (r)
Korsmeyer-Peppas equation	y = 0.5221 x + 0.7248	0.9938
Higuchi equation	y = 5.4983 x - 0.0257	0.9917
Zero-order kinetics	y = 0.9455 x + 6.6207	0.9226
Hixson-crowell cube root law	y = -0.0517 x + 2.3572	0.9195

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