PREFORMULATION STUDIES AND PREPERATION OF DITHRANOL LOADED SOLID LIPID NANOPARTICLES

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ABSTRACT

Dithranol belongs to the keratolytic category, which is widely used drug in the treatment of psoriasis. The drug is practically insoluble in water. Many conventional dosage forms for psoriasis treatment have been have been formulated earlier, but they did not show good results. Hence in the present study, it was attempted to formulate dithranol in the form of solid lipid nanoparticle. Solid lipid nanoparticles of dithranol were obtained by adaption of lipid dispersion method. Preformulation studies were performed to check the compatibility of drug and excepient for the preparation of formulation by DSC and no interaction was found. Solubility study, partition coefficient determination, UV analysis, HPLC study, FTIR study were also performed. After the preformulation studies Dithranol loaded solid lipid nanoparticles was also prepared. Hence it was concluded that solid lipid nanoparticle of dithranol could be formulated.

Keywords: Dithranol, Psoriasis, Solid Lipid Nanoparticle, Preformulation Studies

1. Introduction

Topical therapy is the mainstay of treatment for mild to moderate psoriasis and serves as a useful adjunct support to systemic therapy in severe disease. However, efficacy and compliance to topical therapy in psoriasis have been a major concern. Approximately, 70% of the psoriasis patients in a survey were found to be unsatisfied or moderately satisfied with their current treatment.

Lack of effective delivery of drugs and undesirable skin interactions of the topical treatments are the main reasons for patient noncompliance. Nevertheless, newer developments in the formulation approaches have raised hopes in making topical therapy more useful and acceptable.[1] In the present paper Psoriasis treatment by dithranol loaded sln has been discussed.

Solid lipid nanoparticles (SLN) are a new pharmaceutical delivery system or pharmaceutical formulation. These are made of solid lipids which remain solid at room temperature.

Advantages of SLN are the use of physiological lipids, the avoidance of organic solvents, a potential wide application spectrum (dermal, per os, intravenous) and the high pressure homogenization as an established production method. Additionally, improved bioavailability, protection of sensitive drug molecules from the outer environment (water, light) and even controlled release characteristics were claimed by incorporation of poorly water soluble drugs in the solid lipid matrix. SLNs do not show biotoxicity as they are prepared from physiological lipids. [2]

Dithranol used to treat skin diseases such as psoriasis, eczema and chronic dermatoses.[3] It is a drug of keratolytic category[7]. Solid lipid nanoparticles of dithranol will result in better delivery of drug to the site of action.

In present investigation, dithranol loaded solid lipid nanoparticles were prepared by lipid dispersion method.

2. Materials and Methods (Experimental)

Dithranol was obtained as a gift sample from Agon Pharma Pvt Ltd, Pune. Tristearin was obtained from HiMedia Laboratories Pvt. Ltd. Mumbai. Cholesterol was obtained from Oxford Laboratory Mumbai. Soya lecithin 30% was obtained from HiMedia Laboratories Pvt. Ltd. Brij 35 was obtained from Oxford Laboratory Mumbai. Tween 80 was obtained from Oxford Laboratory Mumbai.

2.1 UV Analysis

2.1.1 Sample: Dithranol reference substance was supplied by Agon Pharma Pvt Ltd Pune.

2.1.2 Solvent: Chloroform was of analytical grade.

2.1.3 Instrumentation: Spectral and absorbance measurements were made with a SHIMADZU - 1800 UV-VIS spectrophotometer at 254nm.

2.2 Procedure

2.2.1 Absorption maxima of dithranol: The light absorption in the range 230 to 360 nm of 1mg/ml solution of dithranol in chloroform exhibited three maxima at about 254, 287, and

354 nm, absorbances at the maxima 0.55, 0.5 and 0.45 respectively [4] (fig1)

2.2.2 Dithranol reference standard: Solutions of the dithranol reference standard (1000 µg.ml^{-1}) were prepared by accurately weighing 100 mg dithranol reference substance into 100 ml chloroform in a volumetric flask. Aliquots were drawn and making concentration of 10; 20; 30; 40; 50 µg.ml^{-1}. Absorbance was taken at 254 nm[5].(table1, fig2)

2.3 High Performance Liquid Chromatography (HPLC): Dithranol was analyzed by using reverse phase Phenomenex C18 column (4.6mm x 25cm, 5microns) with mobile phase consisting of acetonitrile: glacial acetic acid: water (62:8:30). The flow rate was set 1.5ml/min and the analysis was performed at wavelength 254nm using Photo Diode Array (PDA) detector at 25°C temperature. . The retention time for dithranol was around 5.32 minutes.(table3, fig3)

2.3.1 Chemicals and reagents: Dithranol standard (Standardized known purity of Dithranol) Acetonitrile, Water , glacial Acetic acid .

2.3.2 Instrumentation: The HPLC system consisted of a Shimadzu equipped with solvent delivery module in a quaternary gradient mode and PDA detector. Data acquisition was performed by LC solution software. Analysis was carried out at 254nm with a reversed phase phenomenex C18 column (250x4.6mm, 5ìm) at 25°C temperature with mobile phase consisting of acetonitrile: glacial acetic acid:water (62:8:30). The mobile phase was degassed and filtered through 0.45ìm membrane filter before pumping into HPLC system. Injection volume 5ul,detection at 254nm, run time 40mins.

2.3.3 Preparation of drug stock solution: Weighed 5.00 mg of Dithranol drug substance in to 10ml volumetric flask and dissolved in diluent. This solution was injected onto the chromatographic system[6].

2.4 Fourier Transform Infrared Spectroscopy (FTIR): Triturate the solid substance (drug) with dry, finely powdered potassium halide (potassium bromide IR); the proportion of substance to the halide should be about 1 to 200. The amount taken should be such that the weight of substance per area of the disc is about 5-15 µg per mm2. Insert a portion of the mixture in a special die and subject it under vacuum to a high pressure. Mount the resultant disc in a suitable holder. Several factors, for example, inadequate or excessive grinding, moisture or other impurities in the halide carrier, may give rise to unsatisfactory discs. Unless its preparation presents particular difficulties, a disc should be rejected if visual inspection shows lack of uniformity or if the transmission at about 2000 cm-1 (5 µm) in the absence of a specific absorption band is less than 75% without compensation. Identification was done by comparing the obtained spectrum to reference spectrum [7]. (fig4, table4)

2.5 Differential Scanning Calorimetry (DSC): The interaction between dithranol ,tristearin and soya lecithin has been investigated in the solid state. The interaction in the solid state was studied by differential scanning calorimetry (DSC).Instrument used was Jade Pyris DSC. The extent of complexation between the substances was poor, as indicated by the low value of the slope of the linear part of the solubility curve. A phase diagram was constructed by measuring the thermal behaviour of various physical mixtures of dithranol, tristearin, and soya lecithin. The results show that there was no interaction between drug and excepient[8]. (fig5)

2.6 Melting Point Determination: Melting point determination was done by capillary method.

2.6.1 Sample Preparation:, Dithranol loaded into a melting point capillary was:

1. Fully dry
2. Homogeneous
3. In powdered form

Firstly, the open end of the capillary tube was sealed by heating. Then Capillary tube was filled with drug sample. The powder is then pushed to the bottom of the tube by repeatedly pounding the bottom of the capillary against a hard surface. A sample height between 2.0 mm to 3.0 mm was kept for optimum results and reproducibility.

The sample tubes were inserted into one of the sample position slots located on top of the instrument. A thermometer was placed into another slot. And instrument was set on. The temperature at which dithranol started melting was recorded. it was found to be 169^{o}c[9].

2.7 Partition Coefficient: Determination of partition coefficient was done by shake flask method.

Preperation

n-Octanol: The determination of the partition coefficient was carried out with high purity analytical grade reagent.

Water: distilled water was used

Procedure:10mg of drug was added to 25 ml of distilled water and 25 ml of n- octanol. It was shaken saperately for half an hour. Both phases were then mixed together in a separating funnel and shaken for 4 hrs on a mechanical shaker and then let them stand long enough to allow the phases to separate.

Analysis: For the determination of the partition coefficient, it is necessary to determine the concentrations of the test substance in both phases. This may be done by taking an aliquot of each of the two phases from each tube for each test condition and analyzing them by the chosen procedure. The total quantity of substance present in both phases should be calculated and compared with the quantity of the substance originally introduced. Uv analysis was used to determine the concentration[10].

Log p was found to be 1.99 (table 5)

2.8 Solubility Analysis: Dithranol samples were examined in various solvents.

Procedure : 10 mg of dithranol was dissolved in 10 ml of different solvents i.e. water, ethanol, glacial acetic acid, ether, acetone, benzene, chloroform. Solubility was determined on the basis of physical appearance. Dithranol was found to be insoluble in water, slightly soluble in ethanol, glacial acetic acid and ether, soluble in acetone, benzene, and chloroform.[11](table 6)

3. Preparation of Dithranol loaded solid lipid nanoparticles: For the formation of lipid phase 90% of tristearin was taken and to it 10% of cholesterol was added. It was melted about at 75ºc. Then 10% of Brij 35 surfactant was added to it. Then stirring was done for half an hour. For the formation of aqueous phase 40 ml of distilled water was taken, at 4ºc, to it 1gm of soya lecithin was added. Stirring was done for 1 hr. Then tween 80 was added to it again stirring was done for 1hr. Drug was added into the lipid phase. Lipid phase was added to aqueous phase drop by drop. Stirring was done for 6 hrs. Pre emulsion was formed. It was then sonicated by probe sonicator. Ultracentrifugation was done at 1500 rpm at -20 ºc. Supernatant was collected and passed through polycarbonate membrane filter. Solid lipid nanopaticles were obtained fig6.[12]

4. Result

4.1 UV Analysis of Dithranol: Preformulation studies were done to choose a correct drug and excepients for the formulation. The light absorption in the range 230 to 360 nm of 1mg/ml solution of dithranol in chloroform exhibited three maxima at about 255, 287, and 354 nm, absorbances at the maxima 0.55, 0.5 and 0.45 respectively (FIG 1).

Standard Curve of Dithranol: Various dilutons of ditranol were prepared and absorbance was determined at 254nm (table1) and standard curve was obtained (fig. 2). Slope was found to be 0.000797, and regression coefficient was 0.998428 (table 2)

4. 2 High Performance Liquid Chromatography (HPLC): Reverse Phase Hplc of dithranol was done with Acetonitrile: glacial acetic acid: water as solvent in the ratio 62:8:30. Its flow rate was1.5 ml/min.(Table 3) .And chromatogram was obtained (fig 3) with a retention time of 5.32.

4.3 FOURIER TRANSFORM INFRA RED SPECTROSCOPY (FTIR): FTIR was done for the identification of dithranol and principal peaks at wavenumber 1605.67, 1453.93, 1165.13, 1280.49, 1220.23, 1165.13cm^{-1}(KBr Disc) were obtained (Fig 4). Each peak represents a functional group (table 4).

4.4 Differential Scanning Calorimetry (DSC): Drug –excepient interaction was determined with Jade Pyris DSC between mixtures of dithranol, tristearin, and soya lecithin and thermogram was obtained (fig 5) showing that there was no interaction between drug and excepient.

4.5 Melting Point Determination: Melting point of dithranol by capillary method was found to be 179ºc.

4.6 Partition Coefficient: Partition coefficient (n-octanol/water) of Dithranol was determined by shake flask method. Amount of drug in organic phase was found to be 9.9 Amount of drug in aqueous phase was found to be 0.1. Partition coefficient was 99. log p was found to be 1.99 (table 5)

4.7 Solubility Analysis: Solubility analysis of ditnranol was done with various solvents and results are shown in table 6. It was found to be soluble in acetone, benzene, and chloroform. Dithranol was found to be insoluble in water; it was slightly soluble in ether, glacial acetic acid, and ethanol.

5. Discussion

Fig 1. Absorption maxima of Dithranol

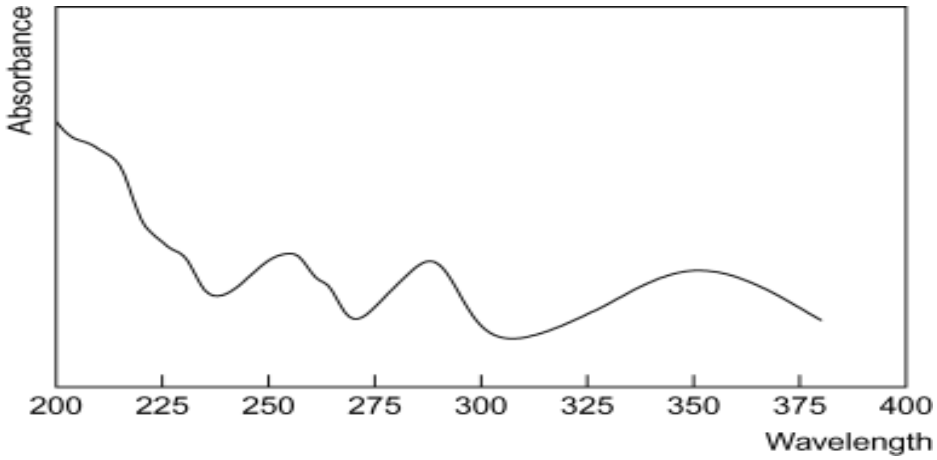


Fig 2. Standard curve of Dithranol

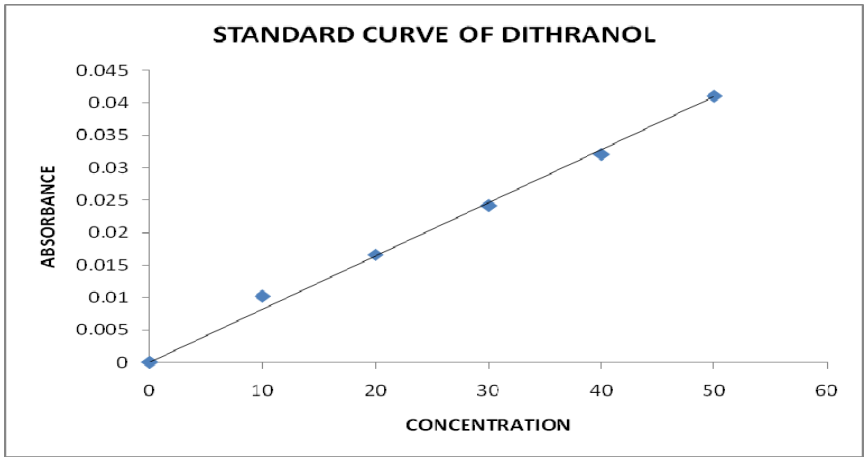


FIG 3. High Performance Liquid Chromatography (HPLC)

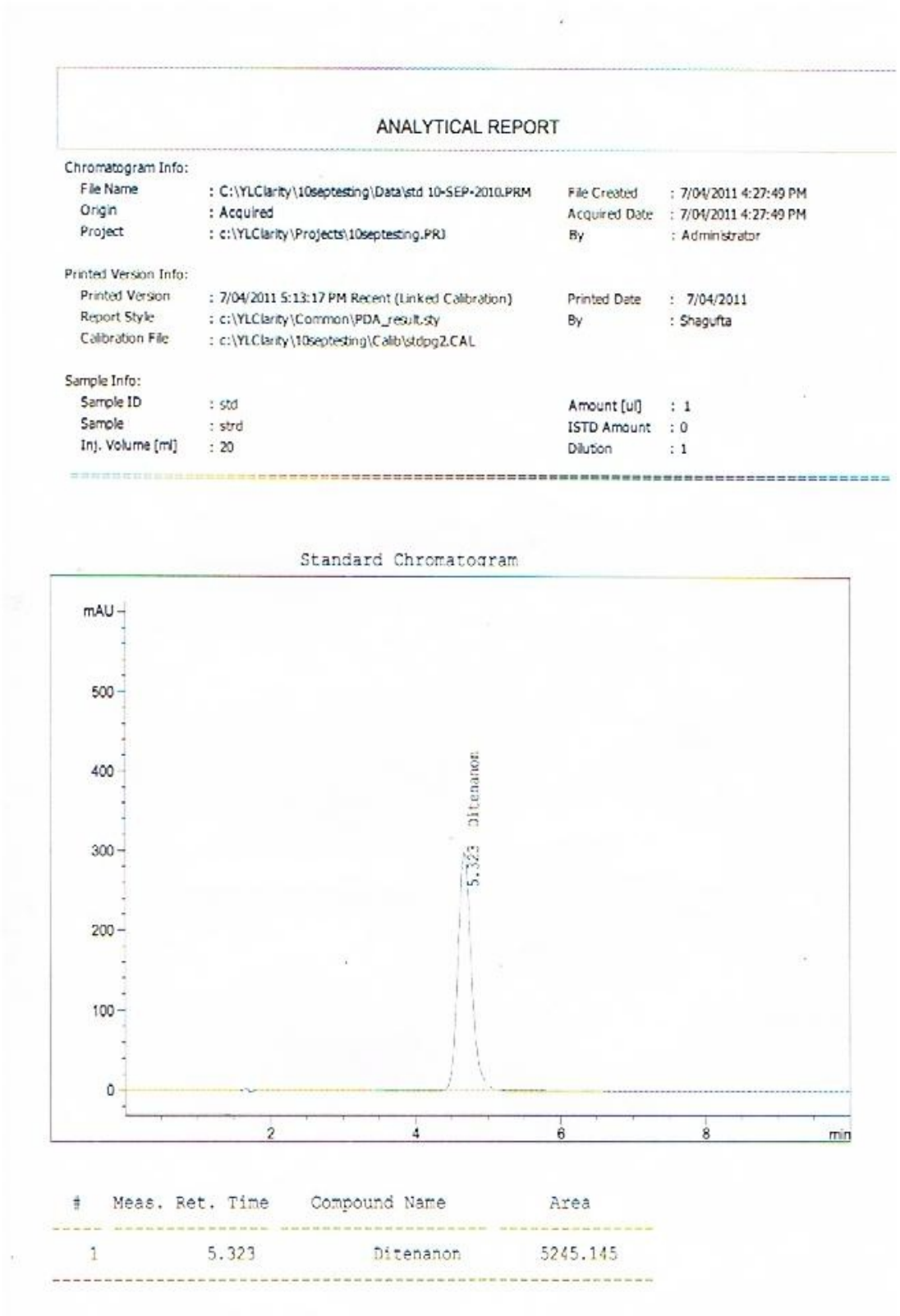
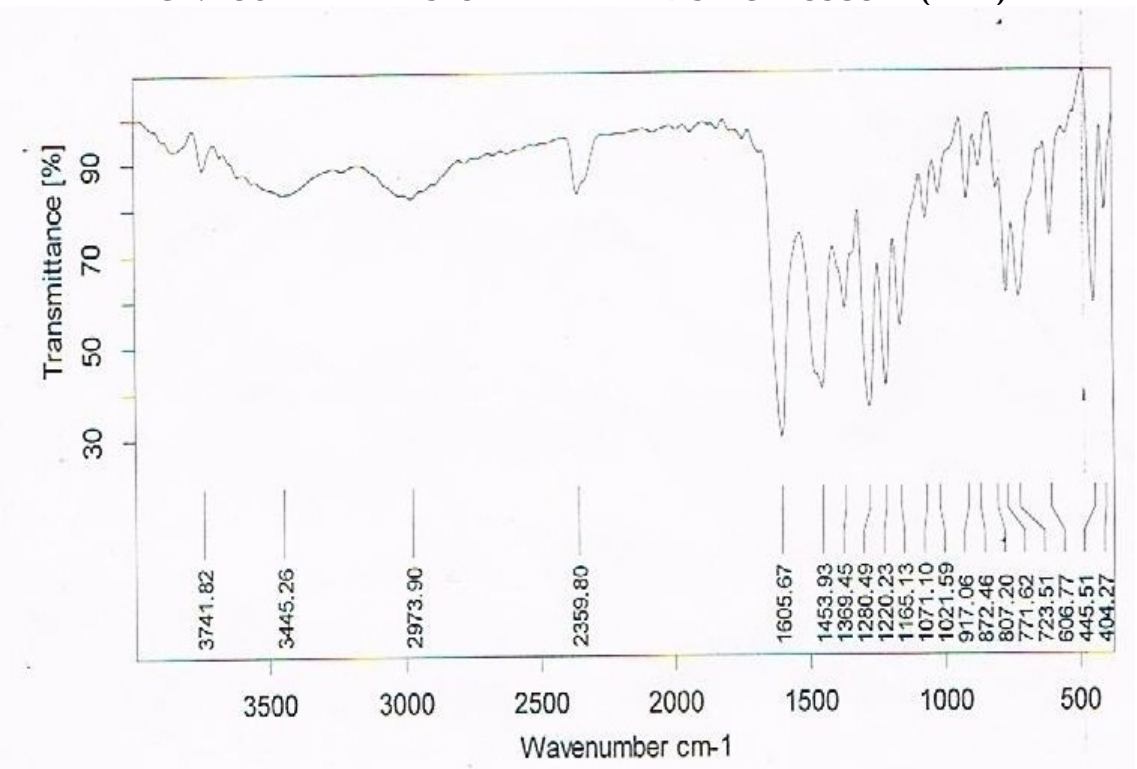


FIG 4. FOURIER TRANSFORM INFRA RED SPECTROSCOPY (FTIR)



Wavenumber	Abs. intensity	Rel. intensity	Width	Found if threshold <	Shoulder
3741.8176	0.888	0.064	33.3524	7.083544	0
3445.2594	0.832	0.099	3248.8827	7.990080	0
2973.8969	0.821	0.173	895.9605	21.111628	0
2359.8015	0.830	0.125	62.6271	15.470757	0
1605.6732	0.300	0.764	78.8539	88.209679	0
1453.9247	0.403	0.355	68.7626	42.437424	0
1369.4457	0.579	0.169	48.6714	16.589523	0
1280.4891	0.363	0.440	45.8900	53.587963	0
1220.2291	0.409	0.279	28.0676	33.154682	0
1165.1328	0.540	0.200	28.8606	23.481911	0
1071.0986	0.771	0.106	23.4990	11.845084	0
1021.5849	0.825	0.091	27.5174	8.745053	0
917.0568	0.812	0.180	26.4217	22.257854	0
872.4580	0.884	0.086	25.2238	8.515137	0
807.1973	0.836	0.055	1223.3642	2.432112	0
771.6177	0.609	0.205	741.3032	18.658991	0
723.5066	0.600	0.430	101.4405	50.342949	0
606.7687	0.734	0.228	25.3991	25.395399	0
445.5054	0.586	0.468	31.4872	50.255352	0
404.2678	0.786	0.191	19.3528	23.272444	0

Sample Name DITRANOL PURE

Fig 5. Differential Scanning Calorimetry (DSC)

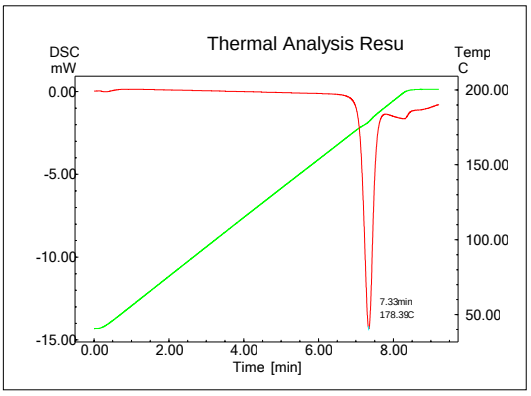


Fig 6. TEM of solid lipid nanoparticles

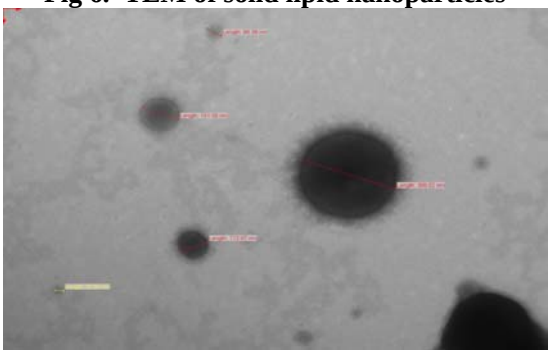


Table 1. Standard Curve of Dithranol

Concentration (µg/ml)	Absorbance
0	0
10	0.0102
20	0.0166
30	0.0242
40	0.0321
50	0.0411

Table 2.

Slope	0.000797
Regression coefficient	0.998428

Table 3. High Performance Liquid Chromatography (HPLC)

Solvent	Acetonitrile: glacial acetic acid: water
Ratio	62 : 8 : 30
Flow Rate	1.5 ml/min
Column	Reverse Phase
Retention Time	5.32

Table 4. Fourier Transform Infra Red Spectroscopy (FTIR)

Wave number CM^{-1}	Functional Group
1605.67	Aldehyde C-H streaching
1453.93	C=C str(aromatic)
1280.49	C=O str
1220.23	O-H bending (phenol)
1165.13	O-H bending (alcohol)

Table 5. Partition Coefficient

Amount of drug in organic solvent	9.9 mg
Amount of drug in aqueous solvent	0.1 mg
Partition coefficient	99
Log P	1.99

Table 6. Solubility Analysis

Solvent	Solubility
Water	Insoluble
Ethanol	Slightly soluble
Glacial acetic acid	Slightly soluble
Ether	Slightly soluble
Acetone	Soluble
Benzene	Soluble
Chloroform	Soluble

Conclusion

Solid lipid nanoparticle of dithranol was successfully formulated. DSC Studies have concluded that there is no interaction between drug and excepient. Partition coefficient has revealed that Dithranol is a lipophilic drug. Drug is stable at high temperature. Dithranol was found to be soluble in various organic solvents. FTIR spectroscopy, UV analysis, hplc studies showed that drug sample used was pure.

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