Analysis of surfactant micelles and their interaction with antimicrobial drug itraconazole for potential pharmaceutical application

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In partial fulfilment of the requirements for the award of degree of

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By

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CERTIFICATE OF ORIGINALITY

This is to certify that the work submitted in this thesis entitled. **"Analysis of surfactant micelles and their interaction with antimicrobial drug itraconazole for potential pharmaceutical application"** submitted by **Anubhav Jamwal** and **Anmol Bansal** in partial fulfilment of the requirements for the award of degree of Bachelors of Technology in Biotechnology, of Jaypee University of Information Technology, Solan, has been carried out under the supervision of Dr. Poonam Sharma. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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(Anubhav Jamwal
Anmol Bansal)

ABSTRACT

Drug surfactant interaction have received an increased attention in the recent past because of wide-spread applications of surfactants in pharmaceutical field. The physiochemical interaction of drugs with surfactant micelles can be visualised as an approximation for their interactions with biological tissues at the molecular level is their ability to bind to membrane resulting into drug action. This thesis investigates the physico-chemical properties to obtain the critical micelle concentration (CMC) and thermodynamic properties i.e the values of ΔH°m, ΔG°m, ΔS°m of surfactants i.e anionic surfactant sodium dodecyl sulfate (SDS), cationic surfactant cetyl trim ethyl ammonium bromide (CTAB), non-ionic surfactant TWEEN 20 in presence of anti microbial drug i.e itraconazole, at different temperatures 25ºc, 30ºc and 35ºc. Further topical gel formulation have been formed and its physical & analytical evaluation (physical appearance &spreadability) studies with the help of optimum concentration of drug/surfactants have been evaluated.

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Anmol Bansal Anubhav Jamwal Dr. Poonam Sharma
(Signature of students) (Signature of Supervisor)

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1.INTRODUCTION

Drug surfactant interaction have got attention in the recent past because of widespread applications of surfactants in pharmaceutical field. The physiochemical bonding of drugs against surfactant micelles can be seen having interactions with biological tissues at the molecular level is their ability to bind to membrane resulting into drug action [1]. As many biological processes occur at the ionisable surface of membranes or along their hydrophobic region, a comparative study of the drug interaction with cationic, zwitterionic, anionic and neutral surfactants is suggested to provide useful information on the nature of drug-membrane interactions.

Micellar systems can soluble poorly soluble drugs, increasing their bioavailibilty and therefore, may be used as a drug carriers by encapsulation of the drugs, in order to ensure the transport to specific sites of action, to minimise drug degradation and loss, to prevent harmful side effects, thus improving the treatment efficacy[2,3].

When surfactant molecules are added to water , they have tendency to dissolve and exist in solution in their monomeric state. The further increase in the surfactant concentration, the surfactant molecules aggregates to form an organised assembly of colloidal clusters known as micelles. This phenomenon of aggregation is known as micellization. This micellization is result of the delicate balance between various repulsive and attractive forces present in their solutions[4]. The polar head of the surfactant in the micelle entity are hydrophilic in nature and are oriented towards and in contact with water molecules and the hydrocarbon tail which are hydrophobic in nature are oriented away from water phase and in contact wit one another thus forming a hydrocarbon core region.
Topical delivery is an important delivery route that delivers prescribe amount of drug through skin for systematic action. improved methods of drug delivery for bio pharmaceuticals are important for two reasons drugs represent rapidly growing portion of new therapeutics are most often given by injections discovery of new drug delivery system have not only been enabled the successful implementation of nobel pharmaceutical but also permitted the development of new medical treatment so for this technology topical gel has been proven the efficient method[5,6].

There are three potential pathways in topical gel delivery for drug penetration (i) through skin to viable tissues (ii) through hair follicles with associated sebaceous glands (iii) through sweat ducts : diffusion through the stratum corneum principally via lipidic intercellular pathway (the rate limiting steps for most compounds)

Topical route offer several potential advantages over conventional routes like avoidance of first pass metabolism, predictable and extended duration of activity, minimising side effects, utility

of short half life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter and intra patient valuation and most importantly it provides patient convince[7].

Topical gel could be administered in limited manner due to limitations of drug delivery through harmful environment in body thus limited mobility reduces the effectiveness of administered drugs progress came with development of biomaterial carriers which could be encapsulated or immobilised with drugs allowing with safely reach into target site.

Topical gel are soft semisolid in nature these consist atlas two components one is liquid that is present in substantial quantity and another is macromolecule uniformly dispersed with no apparent boundary among the drug delivery system topical gels are most preferred choice forth local and systematic admistiration of drugs topical gels characteristics such as non flowing material adhesion and low viscosity for easy and rapid room temperature dispensing no heat generate during cure and low toxicity.

But one of the major problems in topical drug delivery is low penetration rates through the outermost layer of skin specially the hydrophobic drugs [8]. Hence in this project we have tried to increase the penetration rate via the details knowledge of drug and developing the most stable and feasible system for topical drug delivery.

1.1 Surfactants

SDS: **Sodium dodecyl sulfate**, synonymously **sodium lauryl sulfate** (or *laurilsulfate*; **SDS** or **SLS**, respectively), is a synthetic organic compound with the formula $CH_3(CH_2)_{11}SO_4Na$. It is an anionic surfactant used in many cleaning and hygiene products. The sodium salt is of an organo sulfate class of organics. It consists of a 12-carbon tail attached to a sulfategroup, i.e. it is the sodium salt of *dodecyl hydrogen sulfate,* the ester of dodecyl alcohol and sulfuric acid.

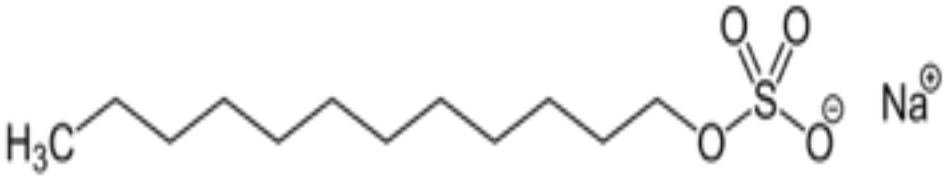

Fig 1. Structural representation of SDS

CTAB: **Cetrimonium bromide** [$(C_{16}H_{33})N(CH_3)_3Br$; cetyltrimethylammonium bromide; hexadecyltrimethylammonium bromide; CTAB] is a quaternary ammonium surfactant .It is one of the components of the topical antiseptic cetrimide . The cetrimonium (hexadecyltrimethylammonium) cation is an effective antiseptic agent against bacteria and fungi. It is also one of the main components of the buffer for the extraction of DNA. It has been widely used in synthesis of gold nanoparticles (*e.g.*, spheres, rods, bipyramids), mesoporous silica nanoparticles

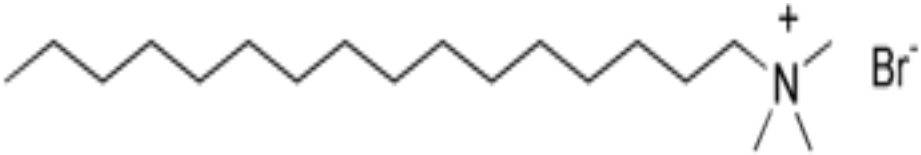

Fig. 1.1 Structural representation of CTAB

Tween 20: **Polysorbate 20** (common commercial brand names include **Scattics**, **Alkest TW 20** and **Tween 20**) is a polysorbate-type nonionic surfactant formed by the ethoxylation of sorbitan before the addition of lauric acid. Its stability and relative nontoxicity allows it to be used as a detergent and emulsifier in a number of domestic, scientific, and pharmacological applications. As the name implies the ethoxylation process leaves the molecule with 20 repeat units of polyethylene glycol; in practice these are distributed across 4 different chains leading to a commercial product containing a range of chemical species

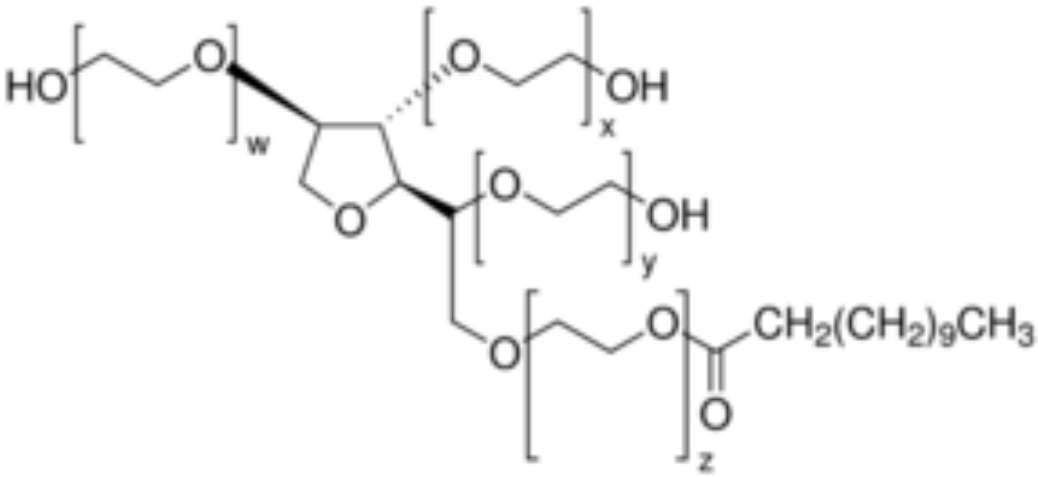

Fig. 1.2 Structural representation of Tween 20

1.2 Drug Itraconazole :

Drugs are rarely administered in their pure forms. So they have to be necessarily admixed with various kinds of adjusts resulting in their transformation into so called ‘dosage form’ or ‘drug delivery systems’. Although the physical forms of medication have not changed dramatically, the attitude of the scientist has been changed in developing the drug into a formulation. In this regard itraconazolehad been chosen for the present study. Itraconazole ($C_{35}H_{38}Cl_2N_8O_4$) is a triazolefungistatic agent used for it is active against *Aspergillus*, which fluconazole is not.

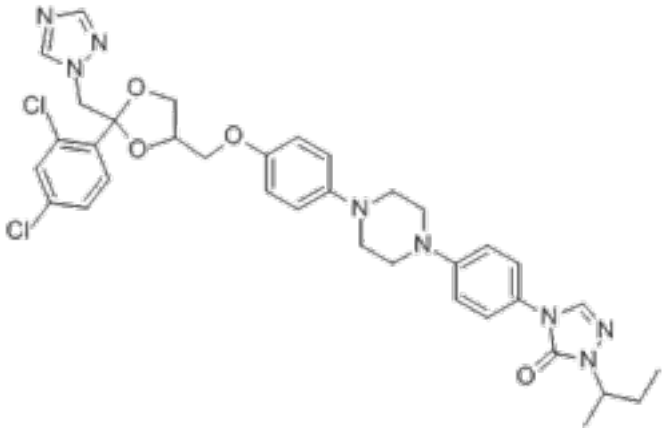

Fig 1.3 Structure of Itraconazole

Itraconazole is an anti-fungal drug in the same class of drugs as fluconazole (Diflucan),miconazole (Micatin, Monistat) and ketoconazole (Nizoral). It prevents growth of several types of fungi by preventing the fungi from producing the membranes that surround the fungal cells. Itraconazole is used for the treatment of fungal infections in both HIV- and non-HIV-infected individuals. It is active against fungal infections such as aspergillosis, blastomycosis, histoplasmosis, and candidiasis, as well as fungal infection localized to the toenails and fingernails (onychomycosis).

1.3 MECHANISM OF ACTION OF AZOLES

- Itraconazole class of azoles group derivatives is used.
- Itraconazole is a triazole anti-fungal agent prescribed to patients with fungal infections.
- Properties : Antifungal, Anti-cancer agent
- Azoles inhibit the fungal cytochrome P-450 3-A dependent enzyme 14-alpha demethylase which is needed to synthesize ergosterol
- This leads to depletion of ergosterol in the cell membrane and accumulation of toxic intermediate sterols leading to increased membrane permeability and inhibition of fungal cell growth.

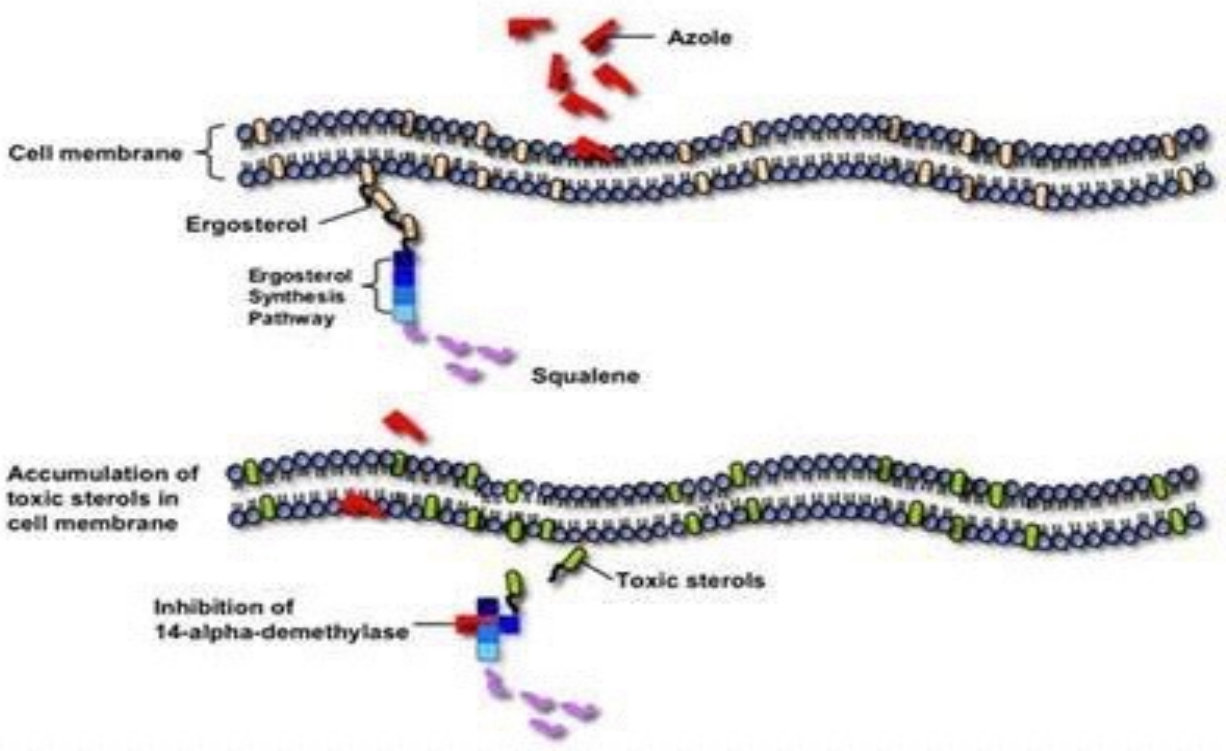

Fig 1.4 Mechanism of action of azoles

1.4 MECHANISM OF ANTIFUNGAL RESISTANCE

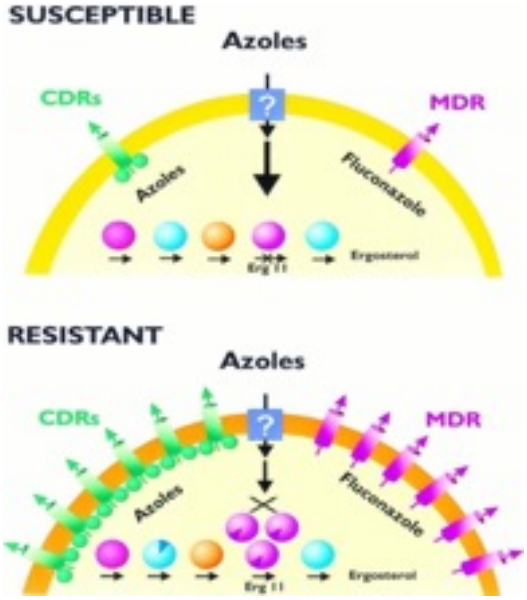

Fig 1.5 Mechanism of anti fungal resistance

- Target site changes itself
- Efflux pumps
- Target site amplified
- Surfactant micellization is important parameters for studying drug release, drug uptake and improvement in biological availability & profile of drug.
- Increasing surfactant conc. : Wetting effect
- Surfactant aggregation: Micellization
- Wetting effect &Micellization property of surfactant are of greater significance in pharmaceutical industries as their ability to act as drug delivery vehicle and disperse bioactive molecules at site of action.

Drug-Surfactant interaction system is needed to study and explore interaction between drug with different additives including surfactants.

Micelles play important role in drug delivery system as they have ability to increase the solubility of poorly soluble or insoluble drug in water, hence increase their bioavailibity

- Specific conductance at different temperature are taken and their effect with change in concentration is studied.
- Critical micelle concentration at different temperature are calculated

- Thermodynamic parameters i.e ΔG, ΔH,ΔS were calculated
- Surface tension versus concentration is studied

Surfactant micelle formation is used for better drug release, drug uptake and improve in bioavailability and bioprofile of drugs. Poorly soluble drugs due to increase of presence of surfactants decreases the aggregation of drugs particles. However lowering the surface tension may results in penetration of water into drug mass results in wetting effect at lower concentration. Above CMC increase saturation solubility of drug substance by solubilizing of drug substance in surfactant micelles can result in more rapid drug dissolution, hence increases the rate of drug into blood stream.

2. **Literature review**

In this section, the relevant representative studies on topical gel, itraconazole, surfactant and physiochemical interaction that appeared in literature during the recent past have been summarised.

Jodi Lestner *et al.* [1] Fungal infections are a major source of global morbidity and mortality. Itraconazole is a triazole antifungal agent that is widely used for the prevention and treatment of fungal infection. Itraconazole is an orally bioavailable agent with broad-spectrum antifungal activity. Itraconazole remains a useful drug for the management of allergic and invasive mycoses worldwide. This article provides a summary of the pharmacodynamics, pharmacokinetics and clinical uses of itraconazole. Additionally, the authors summarise the safety and recently described toxicodynamics and discuss the value of therapeutic drug monitoring (TDM) with itraconazole. The following search criteria were constructed in order to identify relevant literature using Ovid-MEDLINE and PubMed: itraconazole, triazole, pharmacokinetics, pharmacodynamics, toxicodynamics and TDM. Relevant abstracts and articles identified from reviewing secondary citations were additionally retrieved and included if relevant. Itraconazole is an important agent in the treatment and prevention of fungal infection. Itraconazole has a broad-spectrum of activity and is available in both an oral form and intravenous making long-term use in chronic mycoses practical. Endemic fungal infections is treated with the use of itraconazole. Clinically important drug and pharmacokinetic variability interactions make TDM of itraconazole an important consideration.

Jerry M. Zuckerman*et al.* [2] The azole antifungal agents represent a major advance in the management of superficial and systemic fungal infections. Itraconazole appears to have a broad spectrum of in vitro activity and is the first azole antifungal agent to have activity against Aspergillus species. Itraconazole, results in a defective fungal cell membrane with altered permeability and function by acting primarily by impairing the synthesis of ergosterol. It is effective for a wide variety of mycotic infections and some fungal meningeal infections. Most adverse effects have been relatively minor and do not lead to discontinuation of therapy.

John Martin Wishart. *et al.* [3] another form of itraconazole which is orally active triazole, which can also be used to estimate it dosage as compared to ketaconazole. There are different prescribed amounts of itraconazolefor different patients depending upon the the fungal infection site, stage and growth period of bacteria and also keeping in mind that a particular strain of bacteria does not develop resistance towards a particular drug. Dosage also depends upon from person to person. Synergy of drugs generally results in effective results as compared to individual drug. Combination

of two broad spectrum drugs can effectively kill major population of fungal organisms and should be prescribed in a form which is more bioavailable to body, generally oral intake is suggested.

David Lange *et al.* [4] Thirty-three healthy individuals participated in an open-label, randomized, three-way crossover study designed to compare the bioavailability of a single 200-mg oral dose of itraconazole when administered alone or after treatment with ranitidine, both with and without coadministration of a cola beverage. Each treatment phase was separated by a 2-week washout period. Participants pretreated with ranitidine were required to have a gastric pH of at least 6.0 before receiving itraconazole. An analysis of the area under the curve (AUC) and peak plasma concentration (Cmax) data indicated that the bioavailability of itraconazole was significantly reduced when the gastric pH was increased by pretreatment with ranitidine but showed that this effect was counteracted by the coadministration of an acidic solution (e.g., a cola beverage) that transiently reduced the gastric pH. These findings suggest that the coadministration of an acidic beverage with itraconazole may be an effective approach in improving the bioavailability of itraconazole in patients who are hypochlorhydric or who are taking gastric acid suppressants.

Herman Van Cauteren *et al.* [5] Several pharmacologic studies of itraconazole, an orally active antifungal triazole, were conducted in humans and animals. In dogs and rats, no significant toxic effects were seen at doses of up to 40 mg/kg. Endocrinologic studies demonstrated that itraconazole, unlike ketoconazole, does not significantly affect human testicular and adrenal steroidogenesis. Pharmacokinetic studies indicated that itraconazole has a high tissue affinity and a longer half-life than ketoconazole. These pharmacologic observations suggest that itraconazole has a broader spectrum of activity than ketoconazole and a lower potential for producing adverse effects.

Amit Gupta *et al.* [6] Doctors prescribe solids to semisolids form of gel used for skin care and topical treatment of dermatological disease, High molecular weight water soluble polymers of Hydroxypropyl methylcellulose (HPMC), Carbapol 934P, Sodium these all possess very high viscosity, transparency, film forming properties at low concentration, and considered useful in formation of gel. In recent time investigation on Diclofenac sodium gels were done for topical drug delivery by using different concentration of HPMC, Sodium alginate, Carbapol 934P, with an objective to increase transparency and spreadability. From the observations concluded from experiment HPMC gel containing Diclofenac sodium shows good consistency, homogeneity, spreadability and stability and has many applications for topical preparations as compared to Sodium alginate based gel form.

Song et. al [7] Novel carrier was studied for delivery of itraconazole for enhanced skin, Profile was created using diffusion method and was testedon hairless mouse skin and was compared

with the method using conventional liposome, Highly potential ethosomal carrier were employed and skin recovery in tested mouse model. Hence, the results shows that lower vesicle size and higher encapsulation efficiency is considerable. Efficient suppression for allergic reaction can be done using topical gels.

Touitou et al. [8] For enhanced skin delivery the system is composed of phospholipid ethanol and water. These syatems were more efficient at delivering fluorescent probe to skin in terms of quality and depth either liposomes or phospholipids micellar solution. These were measured by dynamic light scattering, was modulated by altering the composition which showed that these had high entrapment capacity for molecules of various lipophilicities.

Gradzielski et al. [9] Investigated propertied and phase behavior of systems which is composed of medium chain alcohol and anionic surfactant, Formation of vesicle gel that increase and ionic strengthincreases as it strongly binds the counter ion to charged amphiphilic interphase. Therefore this gel was strongly stabilized so that counter ion has minor effect. As a function of co surfactant diffrences were observed for phase behavior at high content and was remarkably similar to that of experiment performed with drug at lower concentration.

Modha&co worker [10] Developed the polymorph of itraconazole which improved the bioavailibilty of the gel in the body and leads to higher dissolution rate, Itraconazole polymorphs were prepared by crystallization methods and was characterized by many different methods. These different methods were X ray diffraction, Infrared absorption spectrum, differential scanning calorimetry, melting point and particle size determination, The result of this overall process indicated that the identification, characterization and selection of discriminatory dissolution medium could be useful to the development of itraconazole dosage form.

Randy Mellaerts *et al.* [11] Drugs which are available in market are mostly only partially soluble in water. Partially soluble form is not that much effective to that of completely soluble form of drug. Hence oral bioavailability can be increased by pharmaceuticals formulations that target the formation of supersaturated form. But, there is very little known about evolution of supersaturated intestinal media. The study confirms that itraconazole, which is poorly water soluble drug, its phase transition occur in simulated intestinal fluid. Electron spin resonance probes shows that itraconazole is solubalized when present in supersaturated form in hydrophobic core of mixed micelles which is made of lecithin and bile salt. In supersaturated state, itraconazole leads to leads to formation of nano fibres. Nano fibres produced by itraconazole in supersaturated state reduces transepithelial transport.

Henning Birkedal-Hansen *et al.* [12] Specific different detergents have different effects on body. SDS, on its exposure with latent collagenase results in its activation. Activity of SDS with collagenous was observed after removal of SDS by Triton-X. Latent enzyme is also activated by pre incubation with trypsin and leads to its disappearance. The relationship between precursor-product is observed between double bonds of two sets.

Michael V. Martin *et al.* [13] Most of fungal infections in human are caused by Candida albicans. For its proper treatment, combination of itraconazole along with fluconazole can be used. Fluconazle is first line management option for its treatment. Benefits of using fluconaole over itraconazole is that its predictable pharmokinetics and is suitable for most cases of patients. The increase in use of fluconazole nowadays leads to emergence of strain which are resistance towards fluconazole. Hence other option of treatment is use of itraconazole. Only problem with itraconazole is that its poor solubility and absorption, hence solution formulation is needed to be prepared. Itraconazole in some cases of diseases eg HIV have proven to be more efficient than fluconazole in case of first line treatment.

De Doncker P *et al.* [14] In this study, comparision between itraconazole and terbinafine drug is done and their results are checked with patients creating superficial fungal infection.efficiacy and safety have been increased with treatments using terbinafine and itraconazole. The results shows that both drugs treatment were of same efficiacy and was well tolerated by patients. The only difference between these two treatments are that terbinafine has to be given continuosly for several more time than itraconazole but as the results incomplete treatment can also lead to development of resistance against these drugs.

Van de Velde VJ *et al.* [15] In many pharmacokinetics studies, different studies shows that dose of 6.14mg/kg in every 24hrs leads to inhibition of fungal organism in most of cases. To determine the minimum inhibition concentration , different concentration of drug were prepared and different animal species were treated with it. Results shows that different concentrations of drugs results in difference in effectiveness of drug. Hence while prescribing a dose for a patient certain criteria must be considered for its complete effectiveness.

2.1 OBJECTIVES

- To analyse physiochemical interaction of drug with three different classes of surfactants (SDS, CTAB, Tween-20)
- To formulate surfactant aided drug in carbapol 40 based topical gel.
- Physical & analytical evaluation (Physical appearance &spreadability)

3. EXPERIMENTAL

3.1 Material

3.1.1 Water: Water being one of the major solvent in the study which is also employed in calibration of instruments or apparatus was obtained by double distillation process.

3.1.2 Solvents: Absolute alcohol i.e methanol was obtained from Merck Chemicals with purity > 99.9%. Other solvents such as acetone, labolene were also obtained from Merck Chemicals.

3.1.3 Pharmaceutical Ingredients: Itraconazole was received from Anglo-French Drugs & Industries Ltd. (Bengaluru). Polymer; Carbopol 940 was purchased from Himedia and triehanolamine was purchased from Merck Chemicals for formulation of topical gels. Anionic surfactant; sodium dodecyl sulphate (SDS) was obtained from Merck Chemicals. Cationic surfactant; cetyltrimethyl ammonium bromide (CTAB) was also obtained from Merck Chemicals. Non-ionic surfactant; Tween- 20 was obtained from Sigma-Aldrich Chemicals company.

3.2 Methodology

3.2.2 Conductivity measurements:

Specific Conductance (SC) was measured as a function of SDS, CTAB and Tween-20 concentration in the presence of itraconazole, having fixed concentration using digital conductivity meter Cyber Scan CON-510. The entire experiment were carried out at three temperatures viz. 25, 30 and 35°C. Circulating water from thermostat through a double walled vessel containing the solution was employed to maintain the temperature constant at 0.1°C.

Specific Conductance (SC) is a measure of how well water can conduct an electrical current. Conductivity increases with increasing amount and mobility of ions. These ions, which come from the breakdown of compounds, conduct electricity because they are negatively or positively charged when dissolved in water.

3.2.3 Critical micelle concentration (CMC) is defined as the concentration of detergents above which micelles are spontaneously formed. The CMC is important in biology because at concentrations above it the detergents form complexes with lipophilic proteins. Below this borderline, detergents merely partition into membranes without solubilising membrane proteins.

3.2.4 Thermodynamic studies

$\Delta H°m = - RT2(2-\alpha) (d \ln Xcmc) / dt$

$\Delta S°m = (\Delta H°m - \Delta G°m) / T$

$\Delta G°m = RT (2-\alpha) (\ln Xcmc)$

α denotes the degree of ionization of surfactant , ΔG°m is the standard Gibbs free energy change of micellization. , ΔH°m is the standard Enthalpy for micellization., ΔS°m is the standard Entropy of micellization.

3.2.1 pH Measurements

The pH of different gels was tested using Cyber scan 2500 pH meter.

3.3 Surface Tension Studies

Surface tension : The formation of the droplets, which flows freely from the capillary, is dependent on the surface tension of the liquid. This phenomenon is used to determine the surface tension using stalagmometric method (method of counting drops). Stalagmometer is a device made up of a glass bulb with marked above and below the bulb indicators designating specific volume of liquid, ended capillary. To measure the surface tension of the test liquid fill of stalagmometer and allow it to flow freely. During the formation of the drop in volume continuously increases. Thereby growing its weight Q. At a time where the weight of drops of exceeds values of forces of surface tension (F) along a drop detached from a capillary. Begins the process of forming the next drop.

Density of liquid (ds) = (wt. of solvent/wt. of water) × Density of water

nw (no. of drops of water), dw (density of water at temp.), ns (no. of drops of solvent).

3.3.1 Surface Tension

Stalagmometer is the device used for measuring surface tension. It consists of capillary tube with bulb shape middle section which reslts in flow of water drop wise. It is used to calculate the number of drops of water with respect to a particular reference liquid. The application of this instrument is to measure liquids surface tension and is mainly used by physicists and chemists

The method of using stalagmometer is to vertically suspending it and adding liquid. Then water is allowed to flow down drop wise. Smaller drops will flow for liquids having lower surface tension and larger drops for liquid having larger surface tension. When weight of liquid in drop reaches its equilibrium state in surface tension, it is the maximum value for liquid.

General calculations:

Density of liquid (ds) = (Wt. of solvent/Wt. of water) * Density of solvent

=(W3-W1/W2-W1)*dw gm/cm3

(b) To calculate the surface tension of liquid

γs = (dw. nw/ dw. ns)*γw dyne/cm

Temperature of water = TºC

Density of water at this temperature= dw gm/lt

Number of drops of water = nw

Surface tension of water = γw dyne/cm

Where, the density of liquid (ds)

Weight of empty bottle = W1

Weight of empty bottle + water =W2

Weight of empty bottle + solvent = W3

3.3.2 Viscosity Studies

Viscometer is a device used to measure the viscosity of the liquid with a known density. The method of determining viscosity with this instrument consists of measuring the time for a known volume of the liquid (the volume contained between the marks A and B) to flow through the capillary under the influence of gravity.

STEPS TO PERFORM OSTWALD VISCOMETER 1. Prepare solution of itraconazole at different concentrations in distilled water. 2 Set the temperature at desired point 3. Properly adjust viscometer so that it does not get disturbed and fill the viscometer with desired volume of distilled water as much as required. 4. Fill the beaker with each solution as mentioned in step 1. 5 For taking readings at different temperature heat is provided by keeping viscometer in beaker on magnetic stirrer. 6.Carefully add solution with the help of syringe from arm 1 that column and then pull the liquid or solution through mouth or syringe till liquid reaches to upper mark C and then note the time taken by liquid to reach to lower mark D from upper mark C 7. After completition of sample 1 rinse the viscometer with pure water and waste with acetone and dry up to perform for next sample. 8. Repeat the measurement for each sample at different temperatures and take the average of the time.

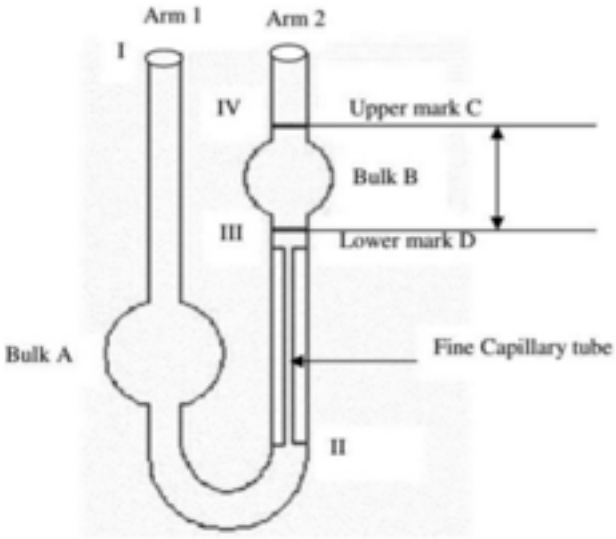

Fig. 3. Structure of Ostwald Viscometer

General calculations:

Density of liquid (ds) = (Wt. of solvent/ Wt. of water)* Density of water

=(W3-W1/W2-W1)*dw gm/cm3

(b) To calculate the viscosity of liquid

nw/ns = (dw.tw/ds.ts)

OR ns = (ds.ts/ dw.tw) * nw centipoise

Temperature of water = TºC

Density of water at this temperature = dwgm/lt

Time of flow of water = tw

Viscosity of water = nw centipoises

(a) Where, the density of liquid (ds)

Weight of empty bottle = W1

Weight of empty bottle + water =W2

Weight of empty bottle + solvent = W3

3.4 Formulation of gel

1.Dispersion of SDS aided itraconazole in Carbopol 940 based topical gel.

2. Dispersion of ctab aided itraconazole in Carbopol 940 based topical gel.

3. Dispersion of Tween 20 aided itraconazole in Carbopol 940 based topical gel.

4. Dispersion of itraconazole incarbopol 940 based topical gel**3.5 Preparation of gel**

Gel was prepared by dispersing carbopol 940 distilled water using dispersion method. After kept for stirring for two hours measured quantity of carbopol 940 is mixed with distilled water. Continuously stirring is done with triehanolamine. the composition of gels aresummarised in table below

3.5 Preparation of gel

Gel was prepared by dispersing carbopol 940 distilled water using dispersion method. After kept for stirring for two hours measured quantity of carbopol 940 is mixed with distilled water. Continuously stirring is done with triehanolamine. the composition of gels are summarised in table below

Table 3 Different measures taken for preparation of Carbopol 940 gel

S. No.	Drug	SDS	CTAB	Tween 20	Carbopol
1	0.1 gm	0.255 gm			0.5 gm
2	0.1 gm	0.227 gm			0.5 gm
3	0.1 gm	0.210 gm			0.5 gm
4	0.1 gm		0.300 gm		0.5 gm
5	0.1 gm		0.296 gm		0.5 gm
6	0.1 gm		0.292 gm		0.5 gm
7	0.1 gm			0.399 ml	0.5 gm
8	0.1 gm			0.383 ml	0.5 gm
9	0.1 gm			0.429 ml	0.5 gm
10	0.1 gm				0.5 gm

Fig 3.1. Formulation of Carbopol 940 gel

3.6 Evaluation of gels

Homogeneity

All the gel formulation were tested for homogeneity by visual inspection after the gel have been set in container they were tested for there appearance and presence of any aggregates

Grittiness

All the formulations were evaluated microscopically for presence of particles and very few particulate were seen under microscope. Hence gel preparation fulfills the requirement of freedom from particular matter and from grittiness desired for any topical preparation

pH measurement

pH of gel formulations were also determined one gram of gel was dissolved in 100 ml of distilled water and stored for two hours. Then the measurement of pH for each formulation was done and average value were calculated

Spreadability

Criteria for a gel to meet the ideal quantities is that it should possess good spreadability. The therapeutic efficiacy also depends upon its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel placed in between the slides under the direction of certain load, lesser the time taken for separation of two slides better the spreadability.

It is calculated by using the formula **S=M. L / T**

Where ,**M** = weight tied to upper slide

L = length of glass slides

T= time taken to separate slides

4. RESULTS & DISCUSSION:

4.1 Conductivity studies and determination of CMC

The experimentally determined specific conductance values of various surfactants i.e SDS, CTAB and Tween 20 in the presence of itraconazole were measured which were then used to determine the CMC for each surfactant i.e SDS, CTAB and Tween 20 in the presence of drug. CMC values have been further used to calculate ΔH°m, ΔG°m, ΔS°m values which have been summarized for itraconazole in **Table 4- 4.2**. The negative value of ΔH°m ,ΔG°m and positive value of ΔS°m shows the drug – surfactant interaction.

The influence of temperature on degree of micellization of the various surfactants was also evaluated with drug in Table **4- 4.2.** The value of CMC was found to fluctuate with different temperatures (25, 30, 35°C) and with changing concentration of the surfactant for each surfactant (SDS, CTAB, Tween 20) in presence of itraconazole..

Table 4. Itraconazole with SDS at T=25, 30, 35°C

temp	cmc	Xcmc	T^2	LnXcmc	dLnX-cmc	ΔhoM	Δgo	Δso
25	0.0088	0.000158305	625	-4.733	0.022	-0.11432	-0.98375	0.034777
30	0.0076	0.000136721	900	-4.87961	0.022	-0.16462	-1.21707	0.035082
35	0.0071	0.000127727	1225	-4.94766	0.022	-0.22406	-1.43972	0.034733

Table 4.1. Itraconazole with CTAB at T=25, 30, 35°C

temp	cmc	Xcmc	T^2	L n X-cmc	d L n X-cmc	ΔhoM	Δgo	Δso
25	0.0106	0.00019068	625	-4.5469	0.022	-0.11432	-0.94507	0.03323
30	0.0055	9.89467E-05	900	-5.20301	0.022	-0.16462	-1.29773	0.037771
35	0.0042	7.5561E-05	1225	-5.47267	0.022	-0.22406	-1.59249	0.039098

Table 4.2 Itraconazole with Tween-20 at T=25, 30, 35°C

temp	cmc	Xcmc	T^2	L n X-cmc	d L n X-cmc	ΔhoM	Δgo	Δso
25	0.0056	0.000100746	625	-5.18499	0.022	-0.11432	-1.0777	0.038535
30	0.0042	7.5561E-05	900	-5.47267	0.022	-0.16462	-1.36499	0.040013
35	0.0078	0.000140319	1225	-4.85363	0.022	-0.22406	-1.41236	0.033951

4.1 Thermodynamic studies

The thermodynamic parameters ΔH°m, ΔG°m, and ΔS°m were evaluated using CMC values and have been summarized for itraconazole in Table 4- 4.2. Fig. 4 shows the post micellar region, pre micellar region and CMC which is evaluated using specific conductance.

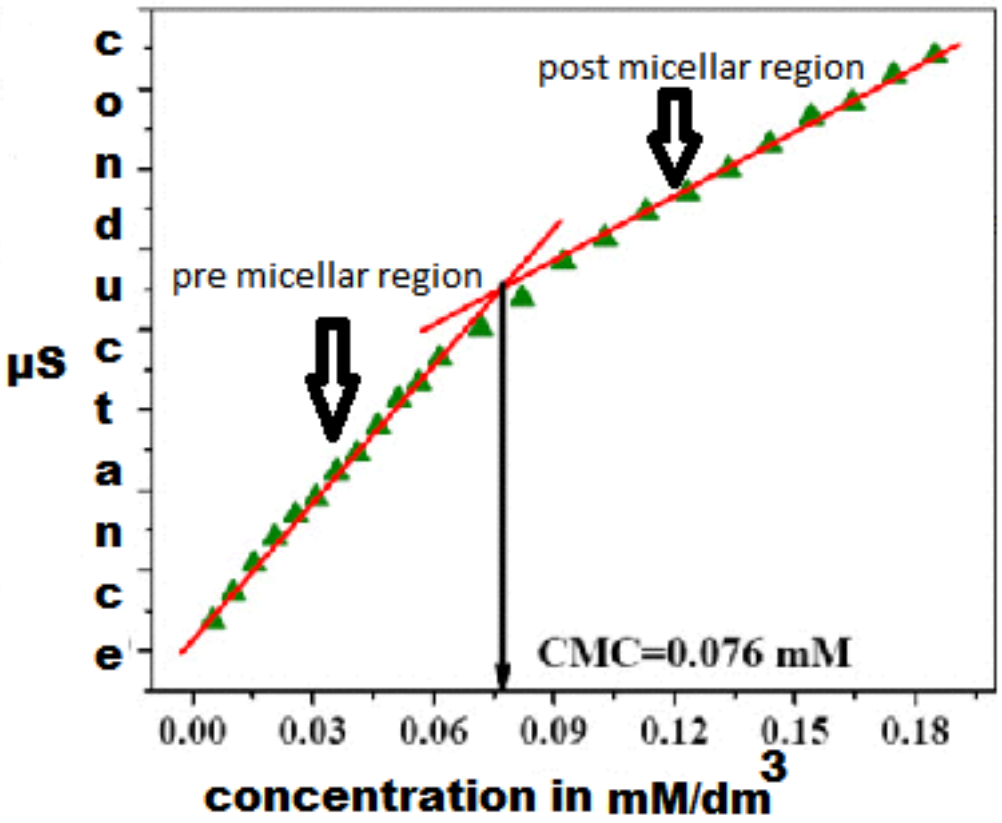

Fig. 4 Plot showing the post micellar region, pre micellar region and CMC.

The experimentally determined CMC values of SDS in the presence of itraconazole shown in the **Fig. 4.1** were found to lie in the range of (4.2 - 10.6) mM. **Fig. 4.2** CMC values of CTAB in the presence of itraconazole were found to lie in the range of (7.1 - 8.8) mM. The influence of temperature on degree of micellization of SDS was also evaluated in**(Table 4- 4.3)** The value of CMC was found maximum at a temperature of 35ºC for SDS and 25ºC for CTAB and for Tween-20 the value of CMC was found to maximum at a temperature of 35ºC. With increase in temperature the value of CMC was found to decrease and then increase.

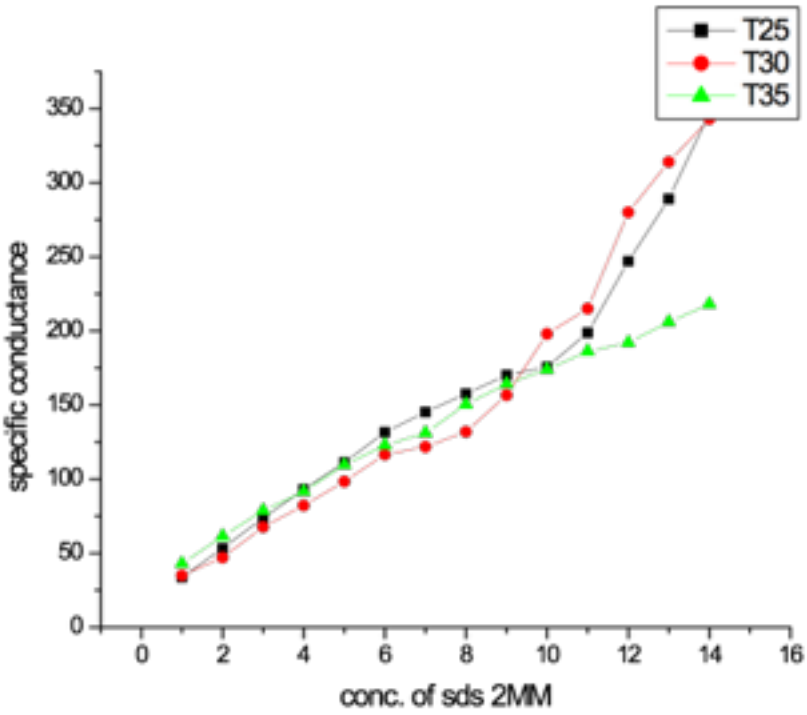

Fig 4.1 The plot of specific conductance versus SDS concentration in solution of Itraconazole at temperatures T=25, 30, 35°C.

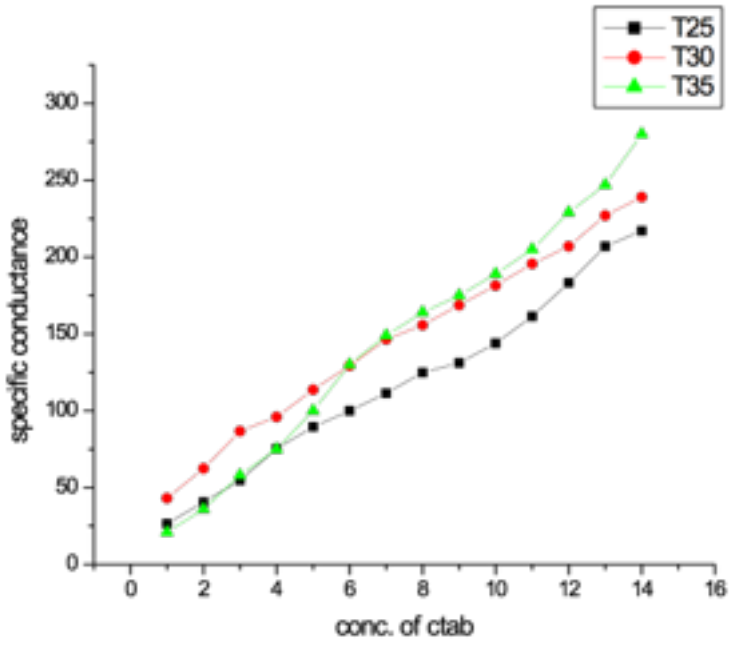

Fig 4.2 The plot of specific conductance versus CTAB concentration in solution of Itraconazole at temperatures T=25, 30, 35°C.

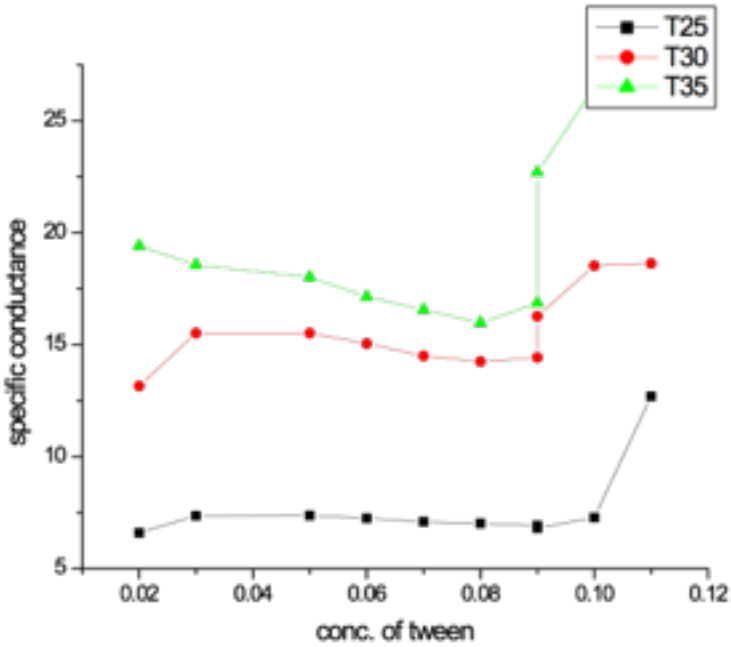

Fig 4.3 The plot of specific conductance versus Tween concentration in solution of itraconazole at temperatures T=25, 30, 35°C.

4.2 SURFACE TENSION STUDIES

The phenomenon is used to determine the surface tension using stalagmometric method (method of counting drops). To measure the surface tension of the test liquid fill of stalagmometer and allow it to flow freely. During the formation of the drop in volume continuously increases. Thereby growing its weight Q. At a time where the weight of drops of exceeds values of forces of surface tension (F) along a drop detached from a capillary. Begins the process of forming the next drop. The cross-section drops its place throat is a circle with a circumference of radius r l = 2πr.

The no. of drops are recorded for each sample of itraconazole with SDS, CTAB and Tween 20 at different temperatures of 25, 30 and 35°C. The density for each sample at a specific conc. and temperature is calculated in gm/cm3 which was further used to calculate the surface tension of the sample at that conc. theoretical values of density of water and viscosity of water at a particular temperature was also used in the above calculations.

From the **Fig.(4.4-4.6)**the variation of each surfactant SDS, CTAB, Tween20 is seen. The **Fig. (4.4 – 4.6)**also shows the variation of each surfactant SDS, CTAB, Tween20 at different conc. with different temperatures (25,30 and 35°c)in **Fig (4.4 – 4.6)**variation is seen in the presence of itraconazole. From the above result we can conclude that surface tension is decreasing with the increase in concentration of SDS, CTAB, Tween-20 at all different temperatures.

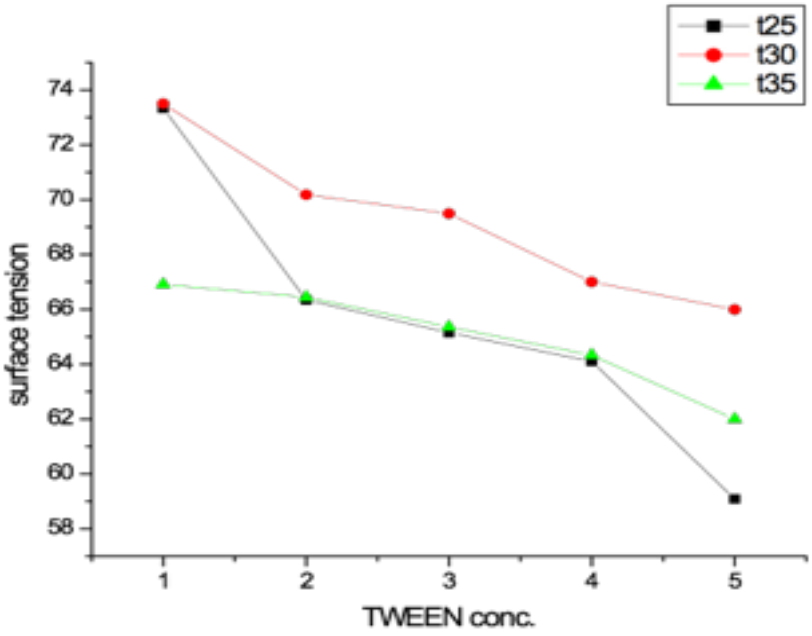

Fig. 4.4 The plot of surface tension versus Tween 20 concentration in solution of Itraconazole at temperatures T=25, 30, 35°C

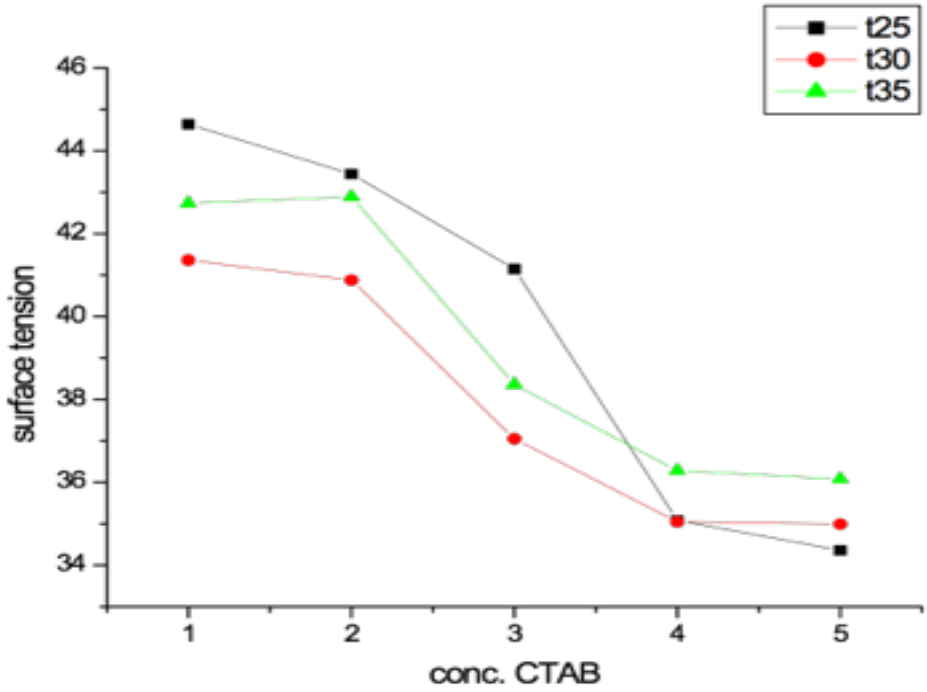

Fig. 4.5. The plot of surface tension versus CTAB concentration in solution of Itraconazole at temperatures T=25, 30, 35°C.

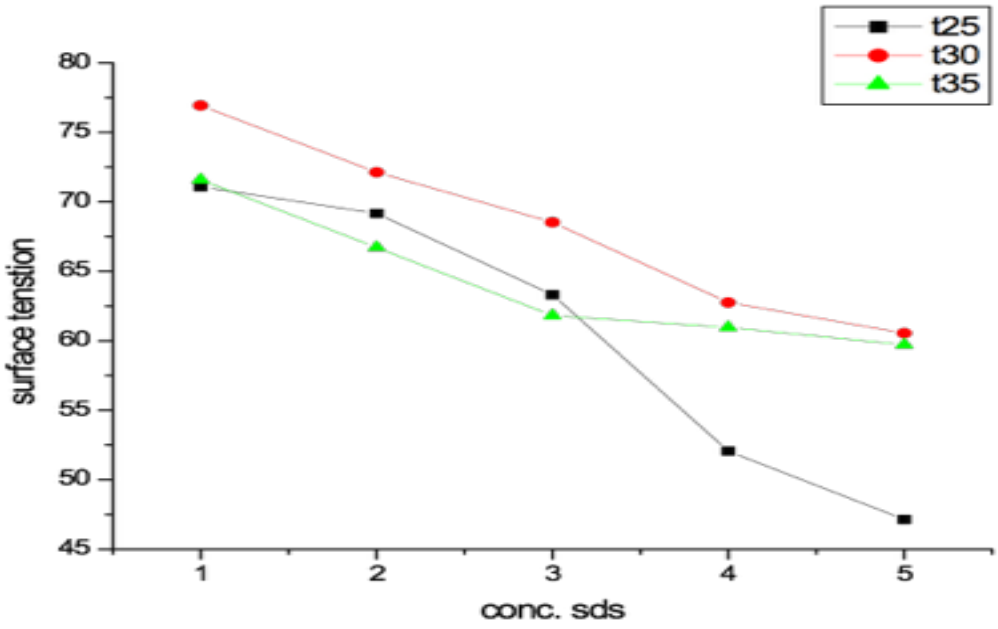

Fig. 4.6. The plot of surface tension versus SDS concentration in solution of Itraconazole at temperatures T=25, 30, 35°C.

4.3 VISCOSITY STUDIES:

Viscosity is calculated using time of flow at each temperature at different concentrations.

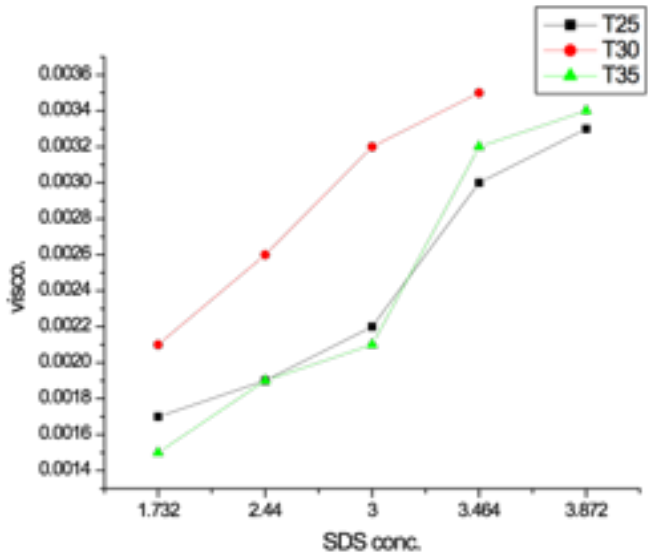

Fig. 4.7. The plot of viscosity versus SDS concentration in solution of itraconazole at temperatures T=25, 30, 35°C.

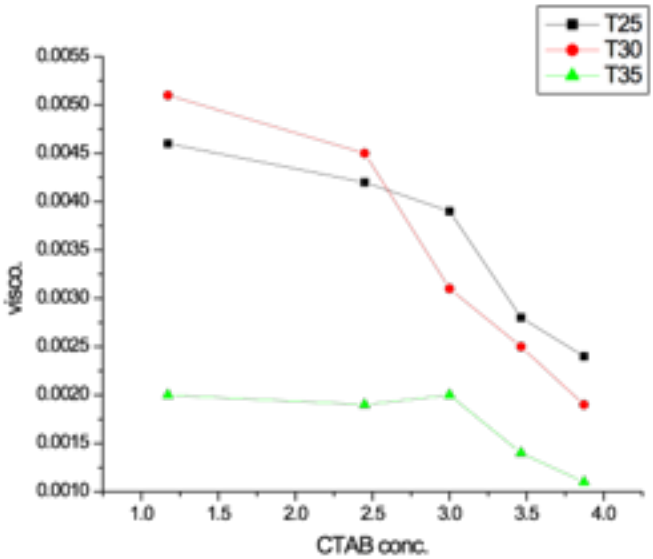

Fig. 4.8. The plot of viscosity versus CTAB concentration in solution of itraconazole at temperatures T=25, 30, 35,°C.

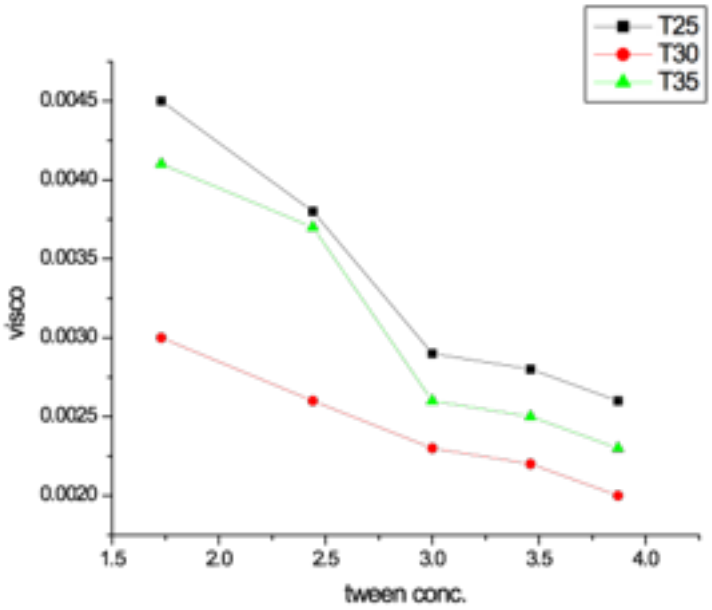

Fig. 4.9. The plot of viscosity versus Tween 20 concentration in solution of itraconazole at temperatures T=25, 30, 35°C.

From above equation ψ was calculated by dividing n by n not at each temperature for different concentration.

Relation between ψ and half of conc. is plotted(conc. versus ψ)

nr = n/n0 =1+ AC1/2 + BC or

(nr - 1)/C1/2 = ψ = A + BC1/2

where nr = (n/n0), and n and n0 are viscosities of solution and solvent system,C is the molar concentration ..

Viscosity is calculated using time of flow at each temperature at different concentrations.

Viscosity tends to increase with increase in concentration but decreases with increase in temperature.

Table 4.3 Representing coefficients of viscosity i.e. A & B with SDS

	At 25°C	At 30°C	At 35°C
A	0.018	0.016	0.013
B	0.05	0.01	0.03

Table 4.4 Representing coefficients of viscosity i.e. A & B with CTAB

	At 25°C	At 30°C	At 35°C
A	0.0408	0.055	0.014
B	0.1	0.3	0.4

Table 4.5 Representing coefficients of viscosity i.e. A & B with Tween 20

	At 25°C	At 30°C	At 35°C
A	0.019	0.018	0.002
B	0.2	0.5	0.2

where

A accounts solute-solute interaction.

B accounts for contribution arising from the size of solute, molar volume of the solvent and contribution due to solute-solvent interactions and estimates the order or disorder as a result for addition of solute into solvent.

Hence from above table we can conclude that solute- solvent interactions dominates as value of B is greater in each case. B coefficient illustrates the solvent –solute interaction and directly depend upon size, shape and charge of solvent molecules , hence this shows that here is strong solute-solvent interaction and temperature dependent changes.

4.4 Preparation of Standard Plot

The standard plot of itraconazole were prepared in phosphate buffer (pH 7.4) : methanol [1:1]. 100 mg/ 100ml stock solution was prepared and further, dilutions were done to obtained concentration range 10-50 ug/ml. A straight line was obtained indicating the drug follows Beer's law within the specified concentration range. Thereafter, it was further analysed by UV spectrometer at 260 nm.

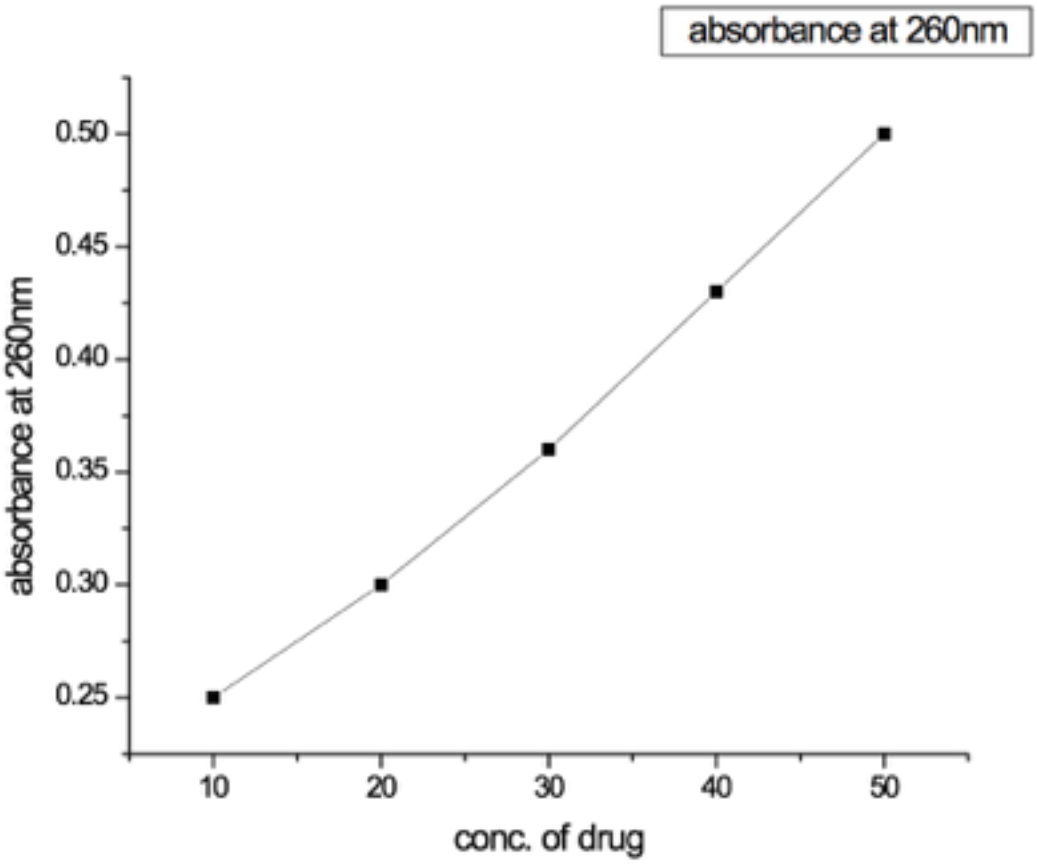

Fig. 4.10. Standard plot of itraconazole

4.5 Analysis of Formulations

Fig 4.11. Formulation of gel with different surfactants

Formulation of gel with SDS, CTAB and Tween-20 were made and has been found that sodium dodecyl sulphate (SDS) is thermodynamically stable and has good spreadability as compared to CTAB and Tween-20.

The prepared gels were evaluated for the pH, spreadability. The pH of all gels were lying in range 5.6 to 7.2 as shown in Table 4.6, 4.7 and 4.8) which favoursours formulations the pH of the skin varies in between 4.5-7.4. These resultts indicate that gels were suitable for topical delivery. Spreadability was also evaluated. The values of spreadability for SDS aided and itraconazole loaded gels were found lie in between 15.5 to 14.8 and 14.8 respectively as shown in **Table 4.6**, indicating that the gels were easy spearable by small amount of shear. Whereas the results were not satisfied with CTAB and Tween-20.

Table 4.6 Rheological characterisation of SDS aided gel

Formulation Code (Gel)	Surface pH	Spreadability
F1	7.1	15.5
F2	6.9	14.8
F3	7.2	14.8

Table 4.7 Rheological characterisation of CTAB aided gel

Formulation Code (Gel)	Surface pH	Spreadability
F4	5.9	22.5
F5	5.9	15
F6	5.6	15.5

Table 4.8 Rheological characterisationof Tween 20 aided gel

Formulation Code (Gel)	Surface pH	Spreadability
F7	6.2	7.5
F8	6.7	7.5
F9	6.4	7.5

Conclusion

The current study demonstrates the development of both thermodynamically stable sodium dodecyl sulphate (SDS) aided formulation for the topical delivery of itraconazole. Series of experiments had done for the development and the results showed that the formulations could be utilised to delivery itraconazole topically.

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