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Pharmaceutical Sciences **RESEARCH ARTICLE ……!!!**

DESIGN AND DEVELOPMENT OF MICROEMULSION OF AZATHIOPRINE

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KEYWORDS:

Microemulsion, azathioprine, oleic acid, propylene glycol.

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ABSTRACT

The aim of the present study was to design microemulsion of azathioprine and study its characteristics. Oil and surfactants were selected on the basis of drug solubilizing capacity. Pseudoternary phase diagrams were developed separately for surfactant /cosurfactant ratio. Azathioprine loaded microemulsion using oleic acid, tween 80 and propylene glycol as oil, surfactant and cosurfactant respectively was prepared and characterized. Drug solubilisation capacity of microemulsion was determined. The prepared microemulsion was evaluated regarding their particle size, solubility, scanning electron microscopy and release .The result showed that the solubility of the azathioprine was maximum in oleic acid as compared to soybean oil, isopropyl myristate and isopropyl maleate.

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INTRODUCTION:

An ideal liquid vehicle for a drug delivery should have certain requirements like thermodynamic stability, solubilisation capacity, small droplet size and low viscosity. A microemulsion possesses all these including ease of formulation. Due to small droplet size and large inner phase in microemulsion, the density of droplets and their surface area are assumed to be high. Hence, the droplets settle down close in contact with the skin which provides high concentration gradient and improved drug permeation. Microemulsions have a low surface tension which ensures good contact to the skin, the dispersed phase can act as a reservoir making it possible to maintain an almost constant concentration gradient over the skin for a long time. Microemulsions are clear transparent, thermodynamically stable dispersion of water and oil stabilized by inter facial film of surfactant in combination with a cosurfactant. Microemulsions are dynamic systems, structurally they are divided into oil in water (o/w), water in oil (w/o) and bicontinuous microemulsions. In all these three types, the interface is stabilized by an appropriate combination of surfactants and Cosurfactants. Microemulsions offer several significant advantages including low skin irritation, powerful permeation ability and high drug loading capacity as compared to other carriers like liposomes or solid lipid nanoparticles [1] .A topical drug delivery is preferable if we need the sustained release of drug into the deep skin layers. Microemulsions can be considered ideal vehicle for drug delivery as they have most of the requirements for this including thermodynamic stability, ease of formulation, low viscosity and small droplet size. These characteristics provide better chance for adherence to biological membranes transporting drugs in a controlled manner. Microemulsions have been reported to enhance the thermodermal penetration of drugs compared to conventional formulations such as solutions, gels or creams. A polar organic solvent like soybean oil can be used to form a microemulsion which can be characterized by high viscosity and optical transparency.

In the present study various polymers like isopropyl palmitate, isopropyl myristate etc. have been employed. Optimum drug delivery through topical route can be achieved if the active drug is formulated in the shape of microemulsion because of its various characteristics like

- Thermodynamic stability
- Ease of formulation
- Low viscosity and small droplet size

These characteristics promote the ease and adherence for transportation of drugs through biological membranes in controlled manner. Azathioprine is a purine antimetabolite which has marked immunosuppressant than antitumor action.The basis for this difference is not clear , but may be due

to its selective uptake into immune cells and intracellular conversion to the active metabolite 6-mercaptopurine ,which then undergoes further transformation to inhibit de novo purine synthesis and damage to DNA.The purine antimetabolite acts after getting converted to 6-mercaptopurine by the enzyme thiopurine methyl transferase (TMPT). It is a potent suppressant of cell mediated immunity, appears to selectively affect differentiation and function of T cells and natural killer cells. It also suppresses inflammation. However, remission is induced in smaller percentage of RA patients and it is less commonly used, especially in cases with systemic manifestations.

Azathioprine also suppresses inflammation. It is also used in arthritis and deep vein thrombosis.

The components of microemulsion have been shown to interact with lipid layer of the stratum corneum and change the structural integrity leading to the enhanced permeability of drug. This is the basic principle for this study i.e. the study of azathioprine in microemulsion form to investigate various aspects.

- Therapeutic efficacy in dermatology
- Steroid sparing effect
- Effective in controlling ocular and extra ocular manifestations

  Thus in this research we tried to form a novel microemulsion which improves the topical permeation of the drug and hence enhancing the absorption.

Material and Method

Solubility Studies

To find out the suitable oil which can be used as the oil phase in microemulsion preparation and provide excellent skin permeation , the solubility of the drug in various oils like oleic acid, isopropyl palmitate, isopropyl myristate and ethyl oleate was determined using shake flask method. An excess amount of azathioprine was added to each vial containing 5ml of the selected vehicle. The mixtures were sealed and vortexed using a vortex mixerfor10minutes.The mixture was then kept at 37±1ºC in an isothermal shaker for 72 hrs.to achieve equilibrium. The filtrates were analysed spectrophotometrically at 250nm after appropriate dilution with methanol. Similarly solubility of azathioprine was determined in various surfactants like Tween 80, Tween 20 and propylene glycol using shake flask method.

Pseudoternary Phase Diagram

Pseudoternary Phase Diagram was constructed using water titration method [2] at ambience temperature(25ºC) in order to find the concentration range of components for the existence range of microemulsion. Three phase diagrams were prepared with the 1:1, 2:1and 3:1volume ratios of Tween 80 and Propylene Glycol. For each phase diagram,oil and specific surfactant/cosurfactant

(S_{mix}) ratio were mixed in different combinations of oil and S_{mix} (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 & 9:1) to cover maximum ratios for the study. Slow titration with distilled water was carried out for each ratio under moderate stirring. After being equilibrated, the mixtures were assessed visually and designated as microemulsions, crude emulsions or gels.

Preparation of Microemulsions

The microemulsions was prepared using spontaneous emulsification (water titration) method with a few modifications as described by [3]. In brief, propylene glycol was dissolved in oleic acid to form a clear oily phase. Tween 80 was solubilized and added to clear oily phase drop by drop under continuous magnetic stirrer at a temperature of 42 ºC.

Characterization of microemulsion

Centrifugation studies

The physical stability of the selected microemulsions was determined by centrifugation studies [4]. Microemulsion systems were subjected to centrifugation at 3000 rpm for 30 minutes and then examined for any phase separation**.** This technique helps to determine behaviour of small particles in gravitational field i.e. their separation rate and is a quite simple and inexpensive method providing a rapid full-proof identification of the system as microemulsion

Refractive index, pH and conductivity measurement

Refractive index provides the information about the clarity and isotropy of the system [5]. The refractive index of blank and drug loaded formulation was determined by using Abbe's Refractometer by placing one drop of formulation on the slide of Refractometer at 25ºC. All the experiments were performed in triplicate. The pH was determined for the optimized microemulsion by using digital pH meter Standardized using pH 4.0 and 7.0 standard buffers before use. The effect of theamount of water phase of microemulsion was monitored by measuring the electrical Conductivity. The conductivity of the blank and drug loaded formulation was measured by using thermo conductivity meter at 25ºC±1ºC.

In Vitro drug release studies

The in vitro release studies were carried out using Franz diffusion cell. Activated dialysis membrane was used in this study. The membrane was saturated with acetate buffer (pH5.5) prior to study and was clamped between donor and receptor compartments [6].The receptor compartment was filled with acetate buffer (pH 5.5).The assembly was thermostated at 37±1ºC and receptor phase was stirred at 100rpm by means of Teflon coated magnetic bead using a magnetic stirrer. 1ml of formulation was applied to the upper side of membrane in donor compartment. Aliquots of 2ml were withdrawn through the sampling port at predetermined time intervals over 24 hrs and replaced

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with an equal volume of fresh acetate buffer (pH5.5)after each sampling. The samples were analysed for drug content by UV spectrophotometrical analysis at 250nm and cumulative amount of drug released in the specific time was determined.

Result and Discussion

Solubility Studies

The maximum solubility of azathioprine was found in oleic acid as compared to soybean oil, isopropyl myristate and isopropyl palmitate. The solubility of azathioprine in oleic acid was 11.507±0.848mg/ml which was highest amongst the oils investigated. Among the surfactants, highestdrug solubility was found in Tween 80 i.e.8.885±0.598mg/ml. The solubility of drug in propylene glycol was found to be 6.235±0.80mg/ml. It was concluded on the basis of solubility studies that oleic acid, Tween 80 and propylene glycol were the most appropriate components for the preparation of microemulsions.Fig.1 & 2 shows the solubility result.

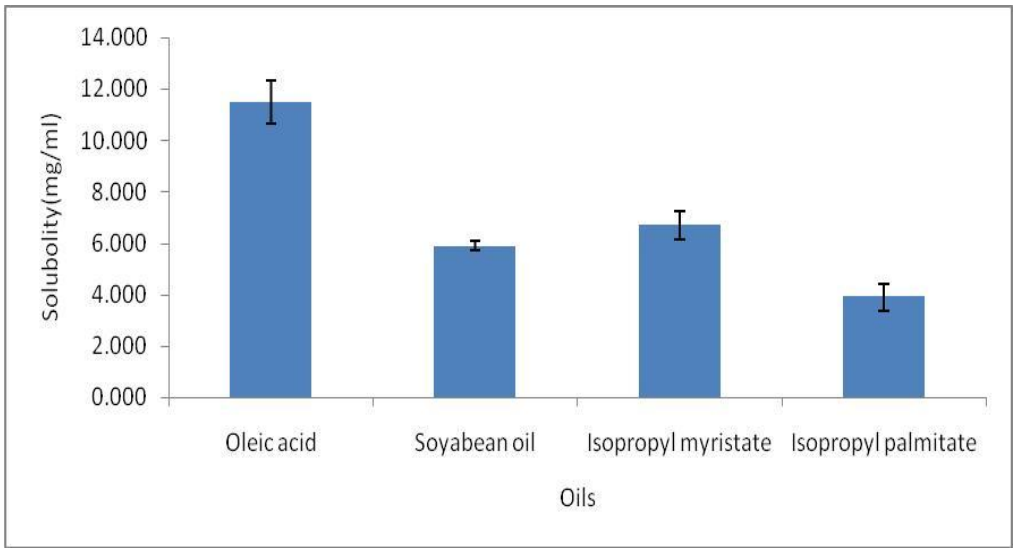

Fig.1 Solubility of Azathioprine in various oils

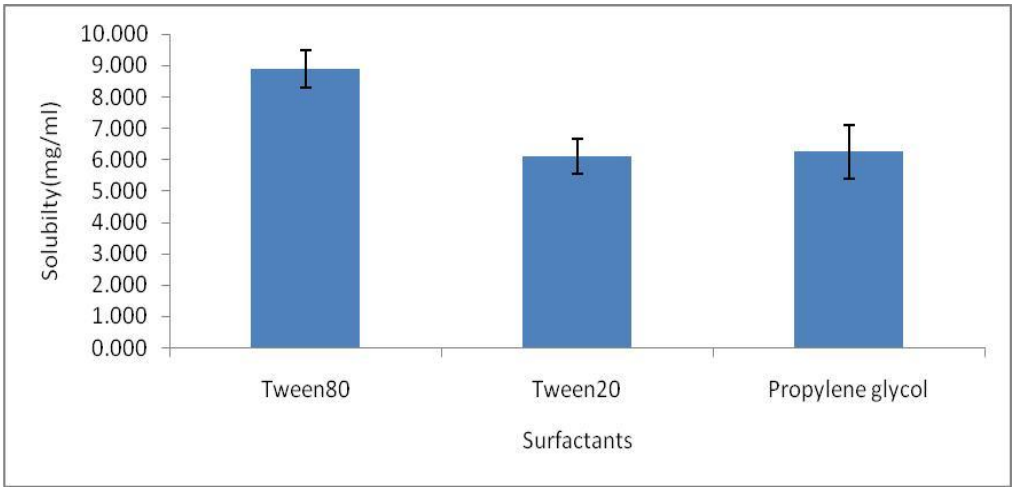

Fig.2 Solubility of Azathioprine in various surfactants

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Pseudoternary Phase Diagram

Pseudoternary phase diagram constructed with three different surfactant/cosurfactant ratios are given in fig.3. From fig.3a it is observed that maximum water solubilization capacity of microemulsion system obtained with S_{mix} ratio of 1:1 was around 50% which was found in the system containing 5% oil and 45% S_{mix}. When the conc. of oil present in the system was minimum, the maximum amount of solubilized and amount of water incorporated was reduced with increasing oil concentrations. The fluidity of microemulsion was reduced with increasing water content.The gel systems were observed when the surfactant concentration was greater than 60% and the water content in the system was in the range of 25-60%. The gel structure was broken down upon further dilution with water before transformation into coarse emulsion. This behaviour could be attributed to the fact that the content below 25% is insufficient to hydrate the polyoxyethylene groups which are critical for the swelling of surfactant chains to demonstrate the gel structure. Accordingly water content more than 60% will increase the distance between the polyethylene groups and destabilize the gel structure leading to breaking of the swollen gel [7].As the surfactant ratio was increased in the S_{mix} ratio 2:1, a higher microemulsion region was observed, perhaps due to further reduction of the interfacial tension, increasing the fluidity of the interface, thereby increasing entropy of the system. At this S_{mix} ratio, the maximum concentration of water incorporated was 44% v/v which was found in system comprising 5.5% of oil and 50% of S_{mix}. When the S_{mix} ratio of 3:1 was studied, it was found that microemulsion region decreased slightly, which may have been due to decreased concentration of cosurfactant. The maximum concentration of water that could be incorporated with this ratio was 39% which was found in the system comprising 6% of oil and 54.5% of S_{mix}. From the phase studies it can be observed that the oleic acid-Tween80-propylene glycol-water system at S_{mix} ratio of 2:1 formed a larger single phase region as compared other ratios and the same ratio was selected for the development of microemulsions.

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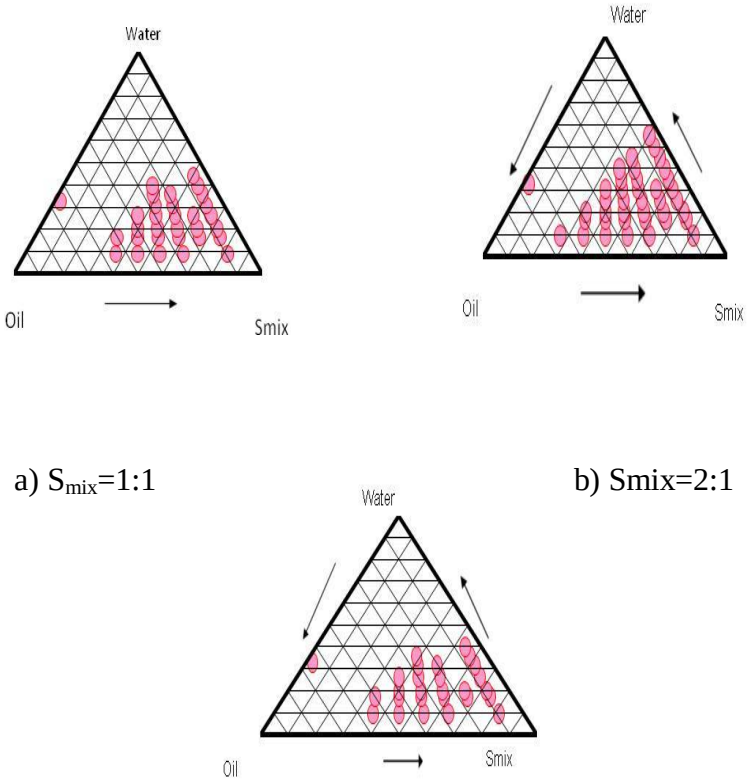

a) S_{mix}=1:1 b) Smix=2:1

c) S_{mix}=3:1

Fig.3 .Pseudoternary phase diagrams using the aqueous titration method, indicating microemulsion region of oleic acid (oil), Tween 80 (surfactant), Propylene glycol (cosurfactant) at different S_{mix} ratios.

Preparation of microemulsion

From the phase diagram selected, microemulsion with suitable composition containing maximum amount of water and minimum surfactant concentration was selected.

Formulation code	Oleic acid %v/v	Tween 80%v/v	Propylene glycol%v/v	Water %v/v
F1	6.46	38.71	19.34	35.49
F2	6.07	36.35	18.18	39.39
F3	5.57	33.32	16.65	44.45
F4	5	30	15	50

Table 1 Composition of selected microemulsion

Selection of Formulation

Optimized formulation was selected on the basis of droplet size, polydispersity index, and drug loading capacity from the tested formulations. Drug loading capacity was found to increase with the increase in concentration of oil and $S_{mix.}$ The formulation F1 showed highest drug loading capacity which can be attributed to enhanced solubilisation capacity of microemulsion component for drug as investigated in solubility studies.The average droplet size was varied from 2361 nm to 711 nm when the content of S_{mix} concentration was varied from 45 to 54%. The result showed that the smaller droplet size of microemulsion F2 was obtained due to presence of higher concentration of S_{mix} in the microemulsion. The decrease in droplet size with increase in S_{mix} concentration can be attributed to solubilisation of the internal phase within a larger number of surfactant micelles, which are consequently swollen to a lesser extent. When the oil content was at 6.06% and S_{mix} content was 54.54%, the formulation was found to exhibit smallest globule size. However in case of formulation F1, containing of 6.45% oil and 58.05% of S_{mix}, the average droplet was found to be increased significantly up to 1120 nm with PDI value of 0.519 , which can be attributed to the expansion of oil droplets of microemulsion by increased amount of oil. Based on the results obtained, the formulation F2 showing minimum droplet size and PDI and a satisfactory drug loading capacity was selected.

Formulation Code	Oleic Acid (%v/v)	Tween 80 (%v/v)	Propylene glycol (%v/v)	Total Smix Conc. (%v/v)	Water (%v/v)	Average droplet size (nm)	Polydis-persity index	Drug loading capacity (mg/ml)
F1	6.46	38.71	19.34	58.05	35.49	1120 nm	0.519	17.21
F2	6.07	36.35	18.18	54.54	39.39	711.0 nm	0.504	13.60
F3	5.57	33.32	16.65	49.99	44.45	1246nm	0.801	9.982
F4	5	30	15	45	50	2361nm	1.085	6.93

Table.2.Physiochemical characterization of the prepared micro emulsion formulations

Refractive index, pH and conductivity measurement

The mean values of the refractive index of drug-loaded and blank formulation are given in Table 6.5. When the refractive index values for drug loaded formulation were compared with those of the blank formulation, it was found that there was no significant difference between the values. Therefore, it can be concluded that the formulation was isotropic in nature and there were no interactions between microemulsion components and drug.

The conductivity measurement can be used to characterize the microemulsion system. It has been previously reported that the conductivity of w/o microemulsion is generally lower than 10µs/cm and the o/w microemulsions have relatively high conductivity as compared to w/o systems (about 10-100µs/cm). The electric conductivity of the blank and drug loaded formulations revealed that both the formulations were o/w type. Also, the conductivity of the microemulsion formulation was found to be constant before and after loading which showed that drug loading into microemulsions did not change the electric conductivity of the system. pH of the blank and drug loaded formulations were found to be within the required physiologic pH range accepted for dermal preparations that is 4.0–7.0 pH units.

Parameters	**Blank formulation**	**Drug loaded formulation**
Refractive index	1.421±0.006	1.421±0.004
Conductivity (µS)	31.7±0.1	31.8±0.2
pH	5.937±0.005	5.254±0.006

Table.3.Physiochemical characterization of azathioprine loaded microemulsion

In Vitro Drug release Studies

Drug release studies were conducted through dialysis membrane in a Franz diffusion cell utilizing acetate buffer (pH 5.5). The drug release from drug loaded microemulsion (F2) and drug solution was studied over a period of 24 hours [8]. Percentage cumulative amount of azathioprine released from the microemulsions and drug solutions at various time points are presented in Table .4.

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Time (h)	% Cumulative Release from drug sol ± S.D.	% Cumulative Release from Microemulsion F2 ± S.D.
0.5	0.791±1.913	2.279±0.931
0.1	17.7641±2.335	5.153±1.015
1.5	24.339±1.379	8.326±2.013
2.0	33.829±2.379	13.059±1.002
2.5	36.167±1.190	13.280±2.531
3.0	41.017±2.302	22.075±3.038
3.5	45.815±2.114	24.324±1.523
4.0	48.764±3.023	28.109±2.592
5.0	55.874±0.525	36.245±4.064
6.0	62.288±1.435	40.315±1.096
7.0	65.640±2.452	48.335±1.150
8.0	68.994±1.025	51.369±3.107
9.0	72.055±0.070	62.205±1.059
10	75.789±1.381	76.223±3.714
12	80.017±2.550	72.279±2.617
24	94.178±3.430	78.056±2.507

Table 4.Cumulative percent release of azathioprine from microemulsion F2 and drug solution.

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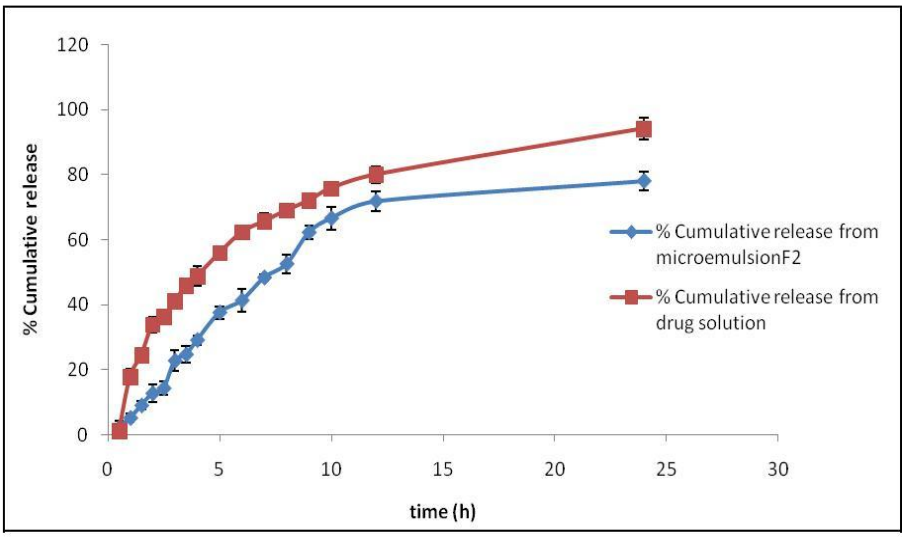

Fig.4 In vitro release of drug from microemulsion F2 and drug solution.

From the drug release profile of microemulsions, it was found that maximum amount of drug was released within 12hours with percentage cumulative release of 72.279 ± 2.617µg/ml. A steady increase in amount of azathioprine was observed with time up to 12 hours which indicated controlled and sustained drug release from the formulation.

CONCLUSION

The aim of the present study was to develop and evaluate microemulsion of Azathioprine for topical delivery.

For the microemulsion preparation, oleic acid was selected as oil phase due to its good solubilizing capacity. Tween 80 and propylene glycol were selected as surfactant and cosurfactant, respectively on the basis of their solubility with the drug and their ability to solubilize oil to form microemulsion.Various formulations were selected from the phase diagram showing maximum microemulsion area and were assessed for their drug loading capacity and particle size distribution. Among all the formulations selected formulation F2 comprising 6.06 % of oleic acid, 36.36 % of Tween 80, 18.18 % of propylene glycol and 39.39 % of water showed minimum droplet size (711 nm) and PDI (0.504) and was selected.

Refractive index of the formulation was found to be 1.421 indicating isotropy and clarity of the system. The conductivity studies revealed that the formulation was an o/w type microemulsion.

Drug release studies were carried out through dialysis membrane. The microemulsion was found to decrease the drug release as compared to control solution which verified that the drug was present in the internal phase of microemulsion, hence retarding drug release.

The results indicated that the formulated microemulsion may be promising vehicle for topical delivery of azathioprine.

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