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Formulation and evaluation of self nano-emulsifying mouth dissolving film of azathioprine

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Self-nanoemulsifying mouth dissolving films (SNMDFs) integrate the benefits of self-nanoemulsifying drug delivery systems (SNEDDS) with fast-dissolving oral films, offering a potential solution to the instability of liquid SNEDDS. Azathioprine, a poorly water-soluble immunosuppressant, is used to prevent joint damage in autoimmune disorders. Development and evaluation of a solid SNMDF of azathioprine to enhance its stability, oral bioavailability, and patient compliance have been reported in this article. Liquid SNEDDS was converted into a solid SNMDF using Aerosil 200 and pullulan as solid carriers and film-forming polymers. A pseudo-ternary phase diagram was constructed using the water titration method. A 3^2 factorial design was used to optimize film formulation. The optimized SNMDF showed suitable mechanical and physicochemical properties: thickness (0.112 ± 0.061 mm), surface pH (6.2 ± 0.75), folding endurance (>200), and wetting time (~16 ± 3.16 sec). Upon reconstitution in water, a nanoemulsion formed with 208.6 nm particle size and 7.4 mV zeta potential. Drug release followed zero-order kinetics. Solid-state characterization indicated azathioprine reduced crystallinity and partial molecular dispersion of the drug within the polymer matrix. Azathioprine-loaded SNMDF presents a promising approach for improving stability, rapid onset, and oral bioavailability of poorly water-soluble drugs.

Keywords: Azathioprine, Drug delivery, Mouth dissolving film, Self nano-emulsifying drug delivery, Self nano-emulsifying mouth dissolving film

Introduction

Rheumatoid arthritis is a chronic inflammatory disorder that affects joints connective tissues, muscle, tendons, and fibrous tissue. It occurs when immune system mistakenly attacks its own body's tissues. According to world health organization (WHO) report it most commonly affects during the most productive years of adulthood, between the ages of 20 and 40. The frequency is more common in industrialised countries and among women, ranging from 0.3% to 1%. Within 10 years its onset, at least 50% of patients in developed countries are unable to hold down a full-time job. It is estimated that 54.4 million U.S. adults suffer from arthritis, equating to about 25% of the population. By 2040, it is anticipated that 78 million (26%) American individuals aged 18 and over would have arthritis, according to a doctor's diagnosis[1]. Even though a number of new drugs (immune-suppressants) have significantly improved treatment choices, severe rheumatoid arthritis can still result in physical impairments.

Azathioprine belongs to a class of immune-suppressant drugs. It functions by reducing the body's immunological response, and by halting additional joint damage, it aids in the treatment of the illness. And it belongs to the BCS class IV[2]. The buccal cavity (intraoral route) offers a number of benefits over the traditional parenteral and gastrointestinal methods of drug delivery, including the removal of pain and suffering related to injections. The surface of oral cavity was composed of squamous epithelium and submucosa. Drugs can be absorbed straight via the buccal mucosa and subsequently reach the systemic circulation, bypassing the first pass effect, because its permeability is greater than that of the skin but lower than that of the gut[3]. Oral dose forms that dissolve quickly include orally disintegrating tablets, fast disintegrating capsules and oral films can be consumed at anyplace and anytime as per convenience of the individual, and are preferred by patients, especially the elderly and children. Mouth dissolving films (MDF) have some advantages over

other oral formulations including convenience to transport and store, availability of larger surface area leading to rapid disintegrating and dissolution in the oral cavity, easy of swallowing without additional liquid especially for patients with dysphagia, vomit, dyskinesia, and psychonosema. In recent years, increasing attention has been focused on MDF[4].

Self-nanoemulsifying drug delivery (SNEDDS) have demonstrated significant promise for improving the oral bioavailability of medicines with poor water solubility[5]. By combining the benefits of solid dosage forms with those of liquid SNEDDS, solid SNEDDS were created, eliminating the drawbacks of liquid formulations. Solid SNEDDS were created by mixing a liquid self nano-emulsifying formulation into the solid dosage form. In previous investigations, we have developed oral solid SMEDDS, which preserved self-emulsification performances of liquid SMEDDS and exhibited the *in vivo* absorption enhancement as high as liquid SMEDDS[6].

Therefore, the goal of the current study was to create a novel self-nanoemulsifying mouth dissolving film (SNMDF) for azathioprine. Based on MDF and self-nanoemulsifying components, SNMDF would have a significant influence on its *in vitro* and *in vivo* characteristics. Effects might include accelerated medication release and increased oral bioavailability[7]. Therefore, in this article the *in vitro* properties of SNMDF, such as the content uniformity, film thickness, surface pH, *in vitro* wetting time and dissolution were investigated.

Despite advances in lipid-based drug delivery systems, the incorporation of SNEDDS into fast-dissolving oral films remains relatively underexplored for drugs belonging to BCS Class IV, particularly immunosuppressive agents such as Azathioprine. Most previously reported SNEDDS-film systems have focused primarily on BCS Class II drugs, where permeability is not a limiting factor. In contrast, Azathioprine presents a dual biopharmaceutical challenge due to its poor aqueous solubility and low membrane permeability, resulting in variable oral absorption and reduced therapeutic efficiency[8].

Furthermore, conventional liquid SNEDDS formulations are associated with limitations such as leakage, stability concerns, and patient inconvenience, which restrict their clinical applicability. Converting optimized SNEDDS into a solid mouth dissolving film system offers a promising strategy to enhance formulation stability, ensure dose uniformity, improve patient compliance, and enable rapid dispersion in the oral cavity[9].

Additionally, systematic statistical optimization of SNEDDS prior to solidification into a polymeric film matrix has not been extensively investigated for Azathioprine. Therefore, the present study was designed to bridge this gap by developing a statistically optimized SNEDDS and incorporating it into a SNMDF, with the objective of improving dissolution behaviour and exploring the potential for enhanced pre-gastric absorption.

Experimental Section

Materials

Azathioprine was bought from Festiva Pharma in Gujarat, while the commercial (conventional) tablet of azathioprine was obtained from RPG Life Science Ltd. We purchased eucalyptus oil from Burgoyne, Mumbai. Ozone International in Mumbai provided the Cremophore EL AR, PEG-400, Aerosil-200, HPMC, and PEG-8000 that were ordered. The supplier of pullulan was Yarrow Chem Products.From Mumbai's Loba chemicals Pvt. Ltd., methanol, citric acid, and sodium saccharin were acquired.

FTIR spectroscopy

The FTIR is a powerful technique, which is used for determination of drug substance the spectral regions for every substance are characteristics for its structures and functional groups it is associated with. The FTIR spectra selected drugs were recorded using Infrared spectrophotometer (IR Affinity-1S). About 5 mg of drug sample was kept in sample holder & a spectrum was recorded over the wave number 400-4000 cm^{-1} on spectrophotometer[10].

Differential Scanning Calorimetry (DSC)

DSC analysis was carried out by using DSC-60 (Shimadzu) instrument. Accurately weight 1–2 mg of drug sample was placed in aluminum pans and sealed. Sealed empty aluminum pan was used as a reference. Studies were carried out from 30°C to 400°C at a scanning rate of 10°C/min. Headspace was saturated with nitrogen at a flow rate of 20 mL/min[11].

Determination of the solubility of azathioprine in different oils, surfactants and co-surfactants

The solubility of drugs in various oils, surfactants and co-surfactants were determined. 5 mL of selected vehicle were added to cap vial containing excess of drug. The vials were tightly closed and were stirred continuously for 72 h. Using orbital shaker at 25°C.

After equilibrium each vial was centrifuged at 3000 rpm for 10 min. and excess insoluble drug is discarded by filtration using a membrane filter. The samples were taken and diluted with suitable solvent (i.e., Phosphate buffer 7.4) and solubility was quantified with UV-spectroscopy[12].

Pseudo ternary phase diagram

The schematic of a pseudo-ternary system of oil, surfactant: co-surfactant (S_{mix}) and water were constructed using water titration method. The previous solubility research profile was used to choose the oil, surfactant, and co-surfactant. S_{mix} was created by combining co-surfactant and surfactant in a 1:1 ratio. In a volumetric flask, S_{mix} and oil were combined in the following ratios: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 (details are as given in Supplementary Information Table S1). To the resultant mixtures, distilled water was added dropwise till the first sign of turbidity and then a clear solution in order to identify the end point and after equilibrium, if the system became clear then the water addition was stopped. The final emulsion, which is transparent or slightly blush appearance, exhibiting good stability and flow ability as a nanoemulsion[12].

Formulation of SNEDDS

Oil, surfactant and co-surfactant were accurately weighed and mixed by gentle stirring. The formulation quantity of the drug was dissolved in the oil and surfactant mixture based on its solubility. Then, the components were mixed by gentile stirring and vortex mixing at 37°C until drug was completely dissolved. The combination was then placed in a glass vial, sealed, and kept at room temperature until equilibrium[13].

Optimization of SNEDDS

The SNEDDS formulation's composition was optimized using an optimal mixture design. Levels of independent factors were selected from area of self-emulsification found in ternary phase diagram. Oil (Eucalyptus) and S_{mix} (Cremophore EL AR: PEG-400) were selected as independent variables $X_1$and X_2, respectively. Both X_1 and X_2 had concentrations that were set between 30-40-50 and 30-35-40 percent, respectively. Two response factors were chosen as independent variables zeta potential (Y_2) and particle size (Y_1), respectively[14]. Nine experimental runs were determined according to 3^2 Full Factorial Design method given in Supplementary Information (Table S1& S2).

Preparation of Solid SNEDDS

The formulated liquid SNEDDS is converted into S-SMEDDS by simple adsorption method and Aerosil 200 (adsorption carrier) for adsorbing the L-SNEDDS[15].

Formulation of SNMDF

Preliminary trials for screening of component

Development of successful fast dissolving film is based on selection of polymer nature and concentration; several polymers were tried for their film forming property. In the current experiment, Pullulan and HPMC were employed as film formers. Different polymer and plasticizer compositions were dissolved in distilled water to create the blank formulations, as given in Table S3. The end product was cast and let too dry for 24 h in an oven set at 45 °C. Films obtained were assessed for film clarity, surface appearance, stickiness, DT and folding endurance (Table S4)[16].

Preparation of oral mouth dissolving film of SNEDDS by solvent casting method

The water-soluble polymers were dissolved in suitable solvent. The drug (S-SNEDDS) along with other excipients was also dissolved in suitable solvent. Mix and stir both solutions were mixed, stirred, casted into the Petri plate and allowed to dry[17].

Evaluation of SNEDDS

Dilution study by visual observation

Dilution studies were performed by dilution of SNEDDS to 50, 100, 250 and 1000 times with water and buffers. They were retained for 12 h after dilution in order to look for any indications like phase separation, turbidity, or drug precipitation[18].

Self-emulsification time

As formulated SNEDDS are diluted, the moment has come for the accumulation to create a homogeneous mixture. In 300 mL of pure water, 1 mL of the SNEDDS formulation was added. At 37°C, the material was gently blended using a magnetic stirrer. The time needed to emulsify spontaneously and progression of emulsion droplet was observed for triplicate time[19].

Percentage transmission test

The optical clarity of SNEDDS formulations was measured spectroscopically upon dilution. Percentage transmittance was determined using Shimadzu UV spectrophotometer (Shimadzu, Japan). Using 100 mL of distilled water to dilute 1 mL of liquid SNEDDS,

the amount of transmittance was measured at 650 nm and any turbidity was checked. The percentage transmission value near to 100% indicates that the clear and transparent SEDDS(20).

Partial size analysis and PDI

Aliquots of the formulation (1 mL each), serially 100-fold diluted with filtered water, particle size analyzers based on dynamic laser scattering were used to analyse variations in light scattering caused by Brownian motion of the particles to calculate the globule size[21].

Determination of zeta potential

Zeta potential was calculated by photon correlation spectroscopy using Zetasizer (Nano ZS, Malvern Instruments) equipped with, which measures the potential ranged from −120 to +120 V. Zeta potential was assessed after diluting the nanoemulsion formulation 100 times with double-distilled water. All measurements were made at 25°C[22].

Evaluation of SNMDF

Physical parameters

Thickness

Thickness of every oral film was determined at five different places using vernier caliper. Average thickness and standard deviation of each oral film formulation was determined.

Weight Variation

From each film formulation, three films measuring 3×3 cm^2 each were randomly selected. Films were weighed individually on electronic balance and the mean weight for each batch was calculated.

Surface pH

In order to assess how well the surface pH of loaded buccal films matched the pH of the buccal mucosa, this pH was measured. The pH was evaluated after three hours by putting universal pH paper (pH scale from 6.0 to 8.1; Carlo Erba, Milan, Italy) over the films, which were allowed to swell on a sponge saturated in phosphate buffer (pH = 7.4).

Folding endurance

A film with a consistent cross-sectional area and thickness was folded until it broke to ascertain it. Folding endurance is calculated as the number of folds a film can withstand without breaking. This test ensures the tensile strength of the film.

Drug content uniformity

A random sample of every batch was used to assess the drug content. The FDF (3×3 cm^2) was broken down in the phosphate buffer 7.4. This solution was filtered and determined by UV spectrophotometer at 281 nm. A mean of three measurements was used to determine the drug content.

Percentage moisture Loss

A test of % moisture loss was conducted to ascertain the film's integrity and physical stability. A 3×3 cm^2 piece of film was cut and weighed. After that, the film was put for three days in a desiccator with fused anhydrous calcium chloride. The film patch was removed and weighed once more after three days. The formula below was used to compute the % moisture loss of the film:

$$\text{Percentage Moisture Loss} = \frac{(\text{Initial Weight} - \text{final weight})}{\text{Initial Weight}} \times 100$$

In vitro wetting time

The Petri dish contained a circular piece of paper. The Petri plate was filled with 6 mL of a 0.1% w/v amaranth dye solution. On top of the tissue paper, the film strip (3× 3 cm^2) was laid out. Wetting time was defined as the amount of time needed for the dye to show up on the film's surface.

Disintegration time

By putting the film strip (3 x 3 cm^2) in a Petri dish (6 cm in diameter) filled with 6 ml of pH 7.4 phosphate buffer, disintegration time was calculated. The amount of time needed for the film to completely degrade was observed. Three duplicates of each measurement were made, and the average results were reported.

Performance parameters

In vitro release profile

In a beaker with 30 mL of phosphate buffer (pH 7.4) and 1% w/v SLS, the dissolving profile of the films was measured at 37±0.50°C. The entire assembly was kept on a shaker. At various time intervals, the sample aliquot (1.0 ml) was removed and replaced with the same fresh medium. Filtered samples were then diluted with phosphate buffer (pH 7.4) and subjected to UV spectrophotometer analysis. To comprehend the release profile and release mechanism, the acquired in vitro release data were applied to zero order and first order kinetics.

Stability study

The stability study was conducted in accordance with ICH guidelines. All of the formulations were placed in glass vials with tight seals and stored in a humidity room with a temperature control of 40 °C for 3 months. The formulations were exposed to the different SNMDF assessment criteria after being stored for 3 months. The formulations were evaluated against data for stability obtained before[25].

Results and Discussion

FTIR & DSC analysis

Confirmation of pure form of Azathioprine was done by comparing FTIR spectra of Azathioprine samples with standard reference spectrum. The FTIR spectra of obtained Azathioprine show characteristics peaks as shown in Fig. 1 (a & b). Azathioprine showed characteristic peaks corresponding to N–H stretching (~3160 cm^{-1}), aromatic C–H stretching (~3060 cm^{-1}), C=O stretching (~1700 cm^{-1}), and C=N stretching (~1600 cm^{-1}). In the optimized SNMDF formulation, these peaks were retained without significant disappearance, confirming absence of chemical incompatibility. Minor peak broadening observed in the N–H region suggests possible hydrogen bonding interactions. DSC thermogram of Azathioprine shows endothermic peaks at 259.2°C as shown in Fig. 1 (c & d). Combined DSC and FTIR findings indicate reduced crystallinity and partial molecular dispersion of the drug within the polymer matrix.

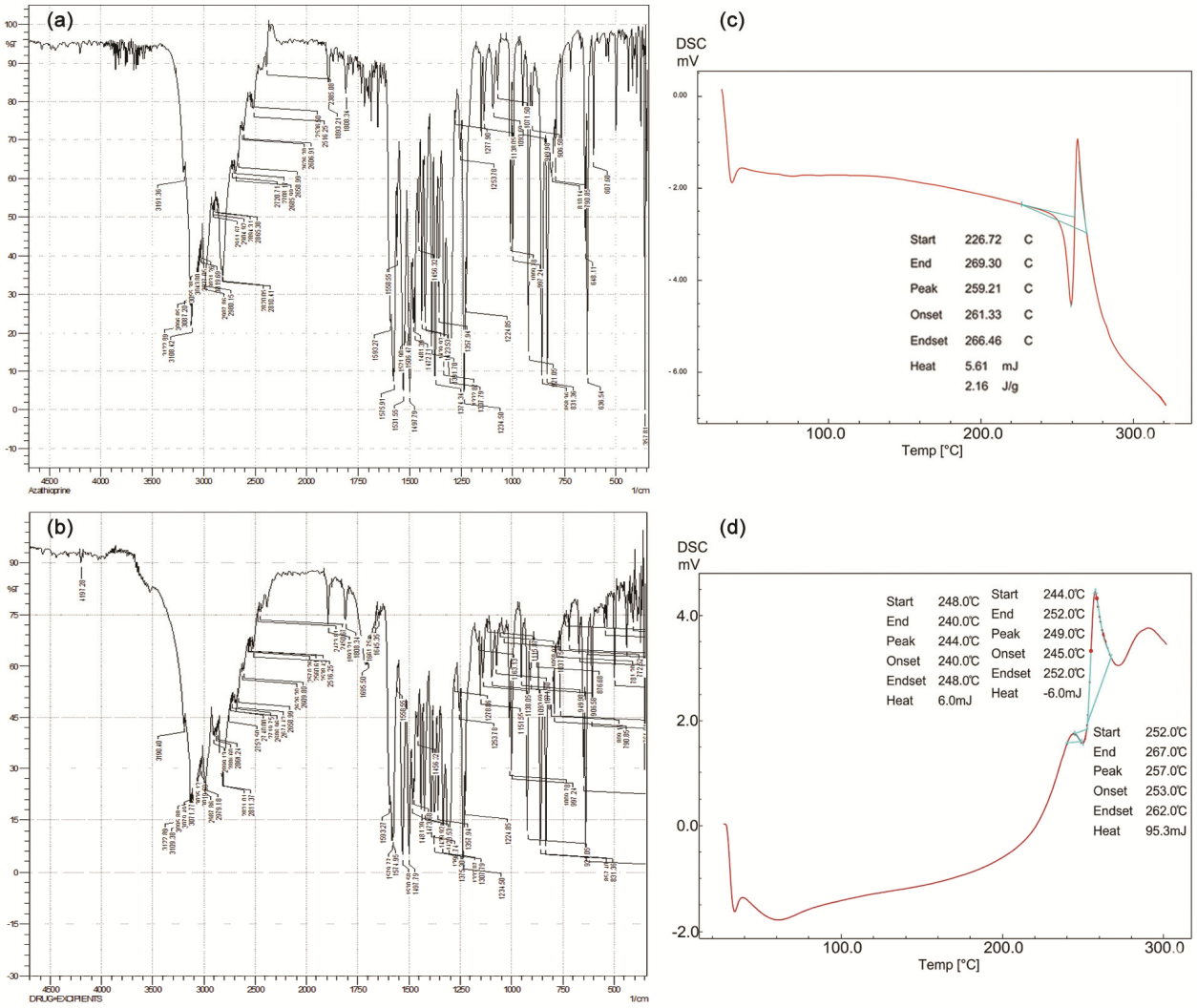

Fig. 1 — (a) FTIR spectrum of Azathioprine, (b) FTIR spectrum of Azathioprine with excipients, (c) DSC of Azathioprine and (d) DSC of Azathioprine with excipients

Table — 1 Solubility of azathioprine in oils, surfactants & co-surfactants

Oils	Solubility (mg/mL)	Surfactant	Solubility (mg/mL)	Co-Surfactant	Solubility (mg/mL)
Arachis oil	1.398	Peacol	15.41	Tween 20	19.44
Castor oil	16.6	Brij 30	1.929	Tween 80	11.42
Cottonseed oil	1.458	Ethyl acetate	8.687	Span 20	15.41
Corn oil	1.618	Ethyl oleate	15.8	Span 80	8.89
Olive oil	1.413	Cremophore EL-AR	28.81	Ethanol	6.02
Sunflower oil	1.423	Transcutol	13.8	PEG-400	21.46
Almond oil	1.58	Campmul MCM EP/NF	10.9		
Eucalyptus oil	18.26	Captex-200P	8.153		
Soyabean oil	1.17	Captex-300 EP/NF	12.63		
Labrafil	12.44	Isopropyl myristate	10.229		

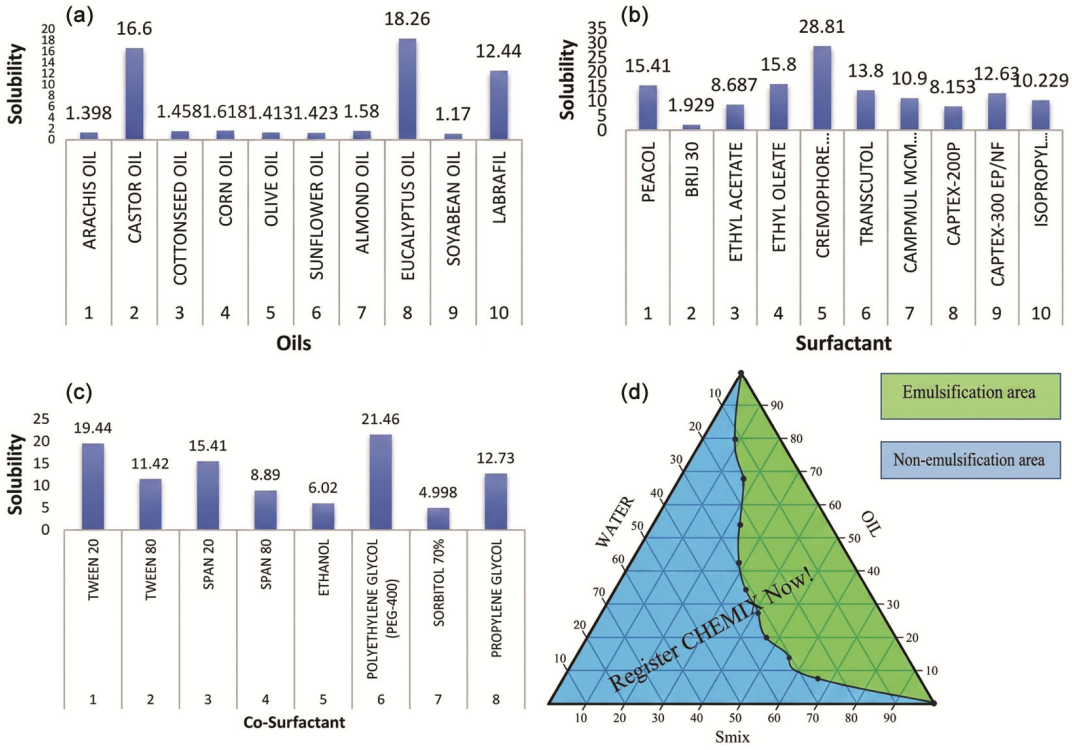

Fig. 2 — Tabular graphical representation of solubility of Azathioprine in (a) oils, (b) surfactants, (c) co-surfactants and (d) pseudo ternary phase diagram of Azathioprine SMEDDS

Solubility study

Identification of oils and surfactants with effective SNEDDS solubilizing capacity is the goal of the solubility investigation. It helps to identify suitable vehicles with maximum potential drug solubilization and having good miscibility with each other. The results of saturation solubility study are shown in Table 1. It is observed that the Eucalyptus as oil phase, Cremophore EL AR as Surfactant, PEG-400 as co-surfactant have high solubility of Azathioprine (Fig. 2) and hence chosen for further formulation.

Construction of pseudo-ternary phase diagram

Based on the results of solubility trials, the oil, surfactant, and co-surfactant for the SNEDDS formulation were selected. In order to examine the phase diagram of SNEDDS, nine distinct possible combinations of surfactant and co-surfactant mixture to oil were employed. Distilled water was gradually added to the resultant mixtures until the first sign of turbidity appeared in order to identify the end point and the system's clarity after equilibrium. After reaching equilibrium, if the system became clear, water addition was stopped, and results were given in

Table S5. The combination was examined visually for phase clarity and flow capability once perfect equilibrium had been established. The colored part of phase diagram shows a nanoemulsion region in Fig. 2 (d). Pseudo-ternary phase diagram was constructed using CHEMIX school software (version-3.51).

Formulation of SNEDDS

Azathioprine SNEDDS was formulated by the Eucalyptus as oil, Cremophore EL AR assurfactant and PEG-400 as co-surfactants. Azathioprine was dissolved into mixture of oil, surfactant and co-surfactants mixed by gentle stirring and vortex mixing at 37°C until azathioprine was completely dissolved in the mixture. The combination was then placed in a glass vial, tightly closed, and kept at room temperature until equilibrium.

Optimization of SNEDDS

A central composite research approach was developed to enhance the developed Azathioprine SNEDDS. In this approach, responses were assumed to dependent over properties of oil and co-surfactant: surfactant (S_{mix}) mixture region of the ternary phase diagram. Oil (Eucalyptus) and S_{mix} (Cremophore EL AR: PEG-400) were picked as independent variables X_1 and X_2, respectively. The proportion of X_1 was set within range of 30-40-50% and the proportion of X_2 was set within range of 30-35-40%, respectively as shown in Fig. 3.

Optimization of SNEDDS formulation was carried out using a 3^2 full factorial design employing Design-Expert® software (Version XX, Stat-Ease Inc., USA). The experimental data were fitted to a quadratic polynomial model. Analysis of variance (ANOVA) was applied to evaluate the statistical significance of the model and individual factors.

For particle size (Y_1), the model was significant with an F-value of 32.45 and a p-value of 0.0008. The coefficient of determination (R^2) was 0.9678, indicating that 96.78% of variability in response could

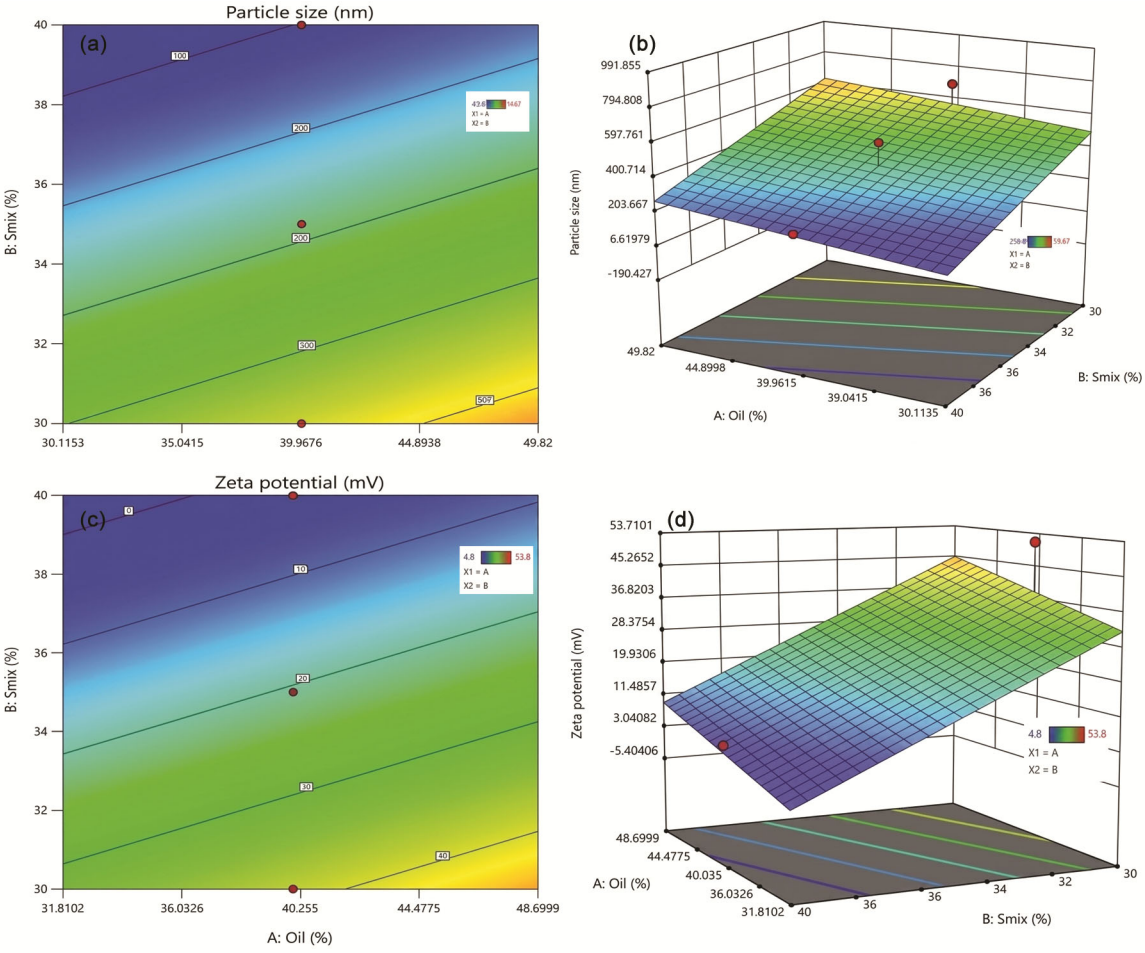

Fig. 3 — (a) Contour plot of particle size, (b) 3D surface plot of particle size, (c) contour plot of zeta potential and (d) surface plot of Zeta potential

be explained by the model. The Adjusted R^2 and Predicted R^2 values were 0.9423 and 0.8834 respectively, demonstrating good model reliability and predictability. Adequate Precision value of 18.67 indicated an adequate signal results were given in Table S6.

Similarly, for zeta potential (Y_2), the model showed an F-value of 21.73 with a p-value of 0.0019. The R^2, Adjusted R^2, and Predicted R^2 values were 0.9542, 0.9178, and 0.8426 respectively. Adequate Precision was 15.24, confirming sufficient model discrimination results were given in Table S7. Two response factors were picked as independent variables viz. zeta potential (Y_2) and particle size (Y_1), respectively. Nine experimental runs were determined according to mixture design method. The outcomes are displayed in Table 2.

Polynomial models for responses

The relationship between independent variables and responses was expressed using polynomial equations in terms of coded factors.

Particle size (Y_1)

$$Y_1 = 472.98 + 168.07A_1 - 34.34A_2 - 118.28B_1 - 6.39B_2$$

The positive coefficient of oil concentration (A_1) indicates a direct relationship with particle size, whereas the negative coefficient of S_{mix} concentration (B_1) demonstrates its reducing effect on droplet size. This suggests that increasing surfactant mixture improves emulsification efficiency, leading to smaller droplet formation. Oil concentration showed comparatively greater influence on particle size.

Zeta potential (Y_2)

$$Y_2 = 20.09 + 7.67A_1 - 1.22A_2 + 20.41B_1 - 6.29B_2$$

The positive coefficient of S_{mix} concentration (B_1) indicates its significant role in enhancing zeta potential and stabilizing the nanoemulsion system. Oil concentration also exhibited a positive effect, although of lower magnitude. The quadratic contrast terms indicate minimal curvature within the studied formulation space.A9 was selected for smaller particle size and stable Zeta potential as shown in Fig. 4.

Table 2 — Three square Full Factorial Design for optimization of Azathioprine SEDDS formulation & self-emulsification time

Mixture number	% Oil (X_1)	% S_{mix} (X_2)	Particle size (nm) (Y_1)	Zeta potential (mv) (Y_2)	PDI	Self-emulsification time (s)
A1	30	30	350.3	21.2	0.344	23 ± 2
A2	30	35	220.6	5.7	0.396	20 ± 2
A3	30	40	240.8	6.7	0.34	19 ± 2
A4	40	30	689.6	51.5	0.376	20 ± 2
A5	40	35	519.7	16.3	0.296	18 ± 2
A6	40	40	215.7	4.8	0.532	16 ± 2
A7	50	30	714.7	53.8	0.378	18 ± 2
A8	50	35	296.8	18.4	0.356	17 ± 2
A9	50	40	208.6	7.4	0.527	15 ± 2

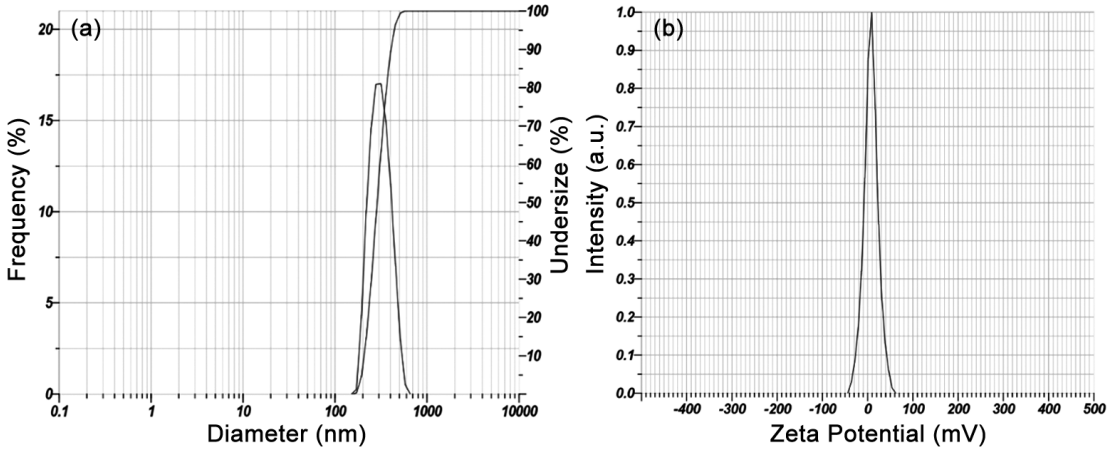

Fig. 4 — (a) Particle size and (b) Zeta potential of Azathioprine SNEDDS [A9]

Dilution study by visual observation

It was concluded from the dilution investigation that there was no evidence of phase separation or drug precipitation.

Self-emulsification time

It is a time for accumulate to form a homogenous mixture upon dilution of formulated SNEDDS. 1 mL of SNEDDS formulation was added to 300 mL purified water and time was recorded in shown in Table 2. Formulation A9 shows least self-emulsification time i.e., 15 ± 2. Based on optimization data i.e., zeta potential and particle size of formulation A9 is 7.4 and 208.6, respectively, with least self-emulsification time. Hence A9 formulation is selected for further film formulation.

Preliminary trail results of blank mouth dissolving film

On the basis of preliminary studies, the BF4, BF5, BF6 were showing significant mouth dissolving film properties. Based on a preliminary finding (Table S4), a variety of polymers and plasticizers were chosen.

SNMDF

SNMDF Formulation is prepared as given in Table 3. Thicknesses of films were determined using a vernier caliper and Table - 4 displays the results. Film thickness was varied with increase in proportion of polymer and thickness values of films were ranged from 0.112 ± 0.06 mm to 0.235 ± 0.06 mm. With the use of a digital balance, the weights of the films were calculated, and Table 4 shows the average weight of all the films. Weights of films were varied with increase in proportion of polymer and values were ranged from 430.94±76.30mg to 580.45±73.20 mg.

Surface pH

The surface pH provides information on the alkalinity and acidity of the films that may cause irritability at the application site. All formulations had a surface pH between6.2±0.75 to 7.4±0.45. All of formulation provides an acceptable pH in between of salivary pH (5.0 to 7.6) hence cannot produce any irritation to buccal mucosa. Table 4 has a listing of the surface pH of each formulation.

Folding endurance

In order to determine the flexibility of films, the folding tolerance study was conducted by constantly folding a tiny strip of the films at the same point until it broke. Folding endurance revealed to be increased with increase in concentration of polymers. Average folding endurance was as given in Table 4 of all films. It was determined that the folding endurance was between 251.33±21.78 to 288.66±15.54. This data revealed that the films had good mechanical strength along with flexibility.

Table — 3 Composition of SNEDDS loaded film

Ingredients	F1	F2	F3	F4	F5	F6
Azathioprine S-SNEDDS (mg)	300	300	300	300	300	300
Pullulan (mg)	200	250	300	350	400	425
HPMC (mg)	250	200	150	100	50	25
PEG-8000 (mg)	150	160	170	180	190	200
Sodium saccharin (mg)	20	20	20	20	20	20
Citric acid (mg)	30	30	30	30	30	30
Tween 80 (mL)	0.06	0.06	0.06	0.06	0.06	0.06
Water (mL)	20	20	20	20	20	20

Table 4 — Evaluation results of films thickness, weight variation, surface pH, folding endurance, % drug content, % moisture loss, in vitro wetting time, % CDR and release kinetics

Evaluation Parameters	F1	F2	F3	F4	F5	F6
Films thickness	0.112±0.061	0.148±0.025	0.213±0.039	0.138±0.035	0.196±0.022	0.235±0.061
Weight variation	430.94±76.30	480.44±26.80	550.02±42.77	460.74±46.50	540.89±33.69	580.45±73.20
Surface pH	6.2±0.75	6.3±0.65	7.4±0.45	7.3±0.35	7.2±0.25	7.3±0.35
Folding endurance	251.33±21.78	275.66±2.54	281.10±7.98	274.66±1.5	288.66±15.54	267.40±5.71
% Drug content	95.48±0.83	96.56±1.91	93.94±0.70	95.88±1.23	93.88±0.76	92.15±2.49
% Moisture loss	15.807±3.12	9.633±3.04	11.61±1.07	12.6±0.08	13.553±0.87	12.89±0.20
In Vitro wetting time	16 ± 3.16	14 ± 5.16	20 ± 0.84	16 ± 3.16	23 ± 3.84	26 ± 6.84
% CDR	97.55	96.44	93.49	89.42	89.98	92.01
Release kinetics	R^2=0.997 (Zero order)	R^2=0.996 (Zero order)	R^2=0.995 (Zero order)	R^2=0.998 (Zero order)	R^2=0.983 (Zero order)	R^2=0.964 (Kosmeyer -Pepas)

Table — 5 Results of in vitro drug release (%) of all film formulations

Time (min)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
5	19.64	18.72	16.68	19.08	13.36	17.24
10	35.52	33.48	33.12	35.33	38.65	19.82
15	52.13	51.21	48.44	51.76	58.04	60.99
20	66.35	63.95	59.88	67.45	68.56	69.12
25	72.25	78.53	76.14	77.05	71.15	76.87
30	84.99	92.19	88.13	79.27	88.13	84.25
45	97.55	96.44	93.49	89.42	89.98	92.01

Drug content uniformity

The percent drug contents of films are found to be in between 92.15±2.49 % to 96.56±1.91 %. This indicates uniform drug content. Results of drug content of all formulations are shown in the Table 4.

Percentage moisture loss

In order to find out the integrity of films at dry conditions, films were assessed for percentage moisture loss. The results obtained are as shown in Table - 4. Among the evaluated formulations, F1 and F2 exhibited highest and little moisture loss i.e., 15.807±3.12 and 9.633±3.04, respectively.

In vitro wetting time

The *in-vitro* wetting time of the film investigation was done and the wetting time ranges between 14 ± 5.16 to 26 ± 6.84 seconds. Table 4 displays the outcomes of the formulation.

In-vitro release of SNMDF

SNMDF in-vitro release data are shown in Table 5 after the experiment was completed.

Stability studies

After 90 days, the formulations were examined to determine their drug content and percent drug release after 45 min. The results have showed negligible variations in the parameters of F1 to F6 after 90 days of storage which is recorded as shown in Table 6.

Table 6 — Results of stability testing of SNMDF at room temperature

Formulation	% Drug content	% CDR
F1	94.98	84.745
F2	95.45	81.689
F3	96.85	87.256
F4	93.74	88.325
F5	93.65	86.698
F6	92.54	83.354

Discussion

Azathioprine exhibits poor aqueous solubility and bioavailability, which significantly limits its therapeutic efficacy. Additionally, the drug suffers from extensive first-pass metabolism and variability in absorption when administered orally in conventional dosage forms[26].

To mitigate the solubility and bioavailability challenges associated with Azathioprine, a lipid-based nanocarrier system SNEDDS was developed employing a systematic approach. Excipients were selected based on equilibrium solubility studies to ensure optimal drug loading and emulsification efficiency. A 3^2 Full Factorial Design was utilized to statistically optimize the formulation variables, focusing on minimizing globule size and achieving a stable zeta potential. The optimized SNEDDS formulation was subsequently incorporated into a fast-dissolving polymeric film matrix to form a SNMDF, enabling rapid dispersion and release in the oral cavity. This dual-delivery approach was aimed at enhancing both dissolution and patient compliance by circumventing hepatic first-pass metabolism and facilitating pre-gastric absorption.

Previous studies on SNEDDS of poorly soluble drugs like Talinolol or similar immune suppressants have demonstrated increased solubility and improved bioavailability through the use of lipid-based systems. For example, the results from the characterization and solubility studies showed that SNEDDS formulations were stable with lower droplet size of 150 nm[18]. In our case, we achieved a particle size of 208.6 nm (Formulation A9), which indicates an enhanced emulsification profile and potential for better absorption.

Although the optimized formulation (A9) exhibited a zeta potential of 7.4 mV, which is lower than the conventional ±30 mV threshold for electrostatic stabilization, the nanoemulsion system is primarily stabilized by non-ionic surfactants. Cremophore EL AR contains polyethylene glycol chains that provide steric stabilization by forming a hydrophilic barrier around oil droplets, thereby preventing aggregation

and coalescence. In such systems, stability is governed more by steric hindrance than by surface charge magnitude[27]. The absence of phase separation during dilution studies further supports the physical stability of the formulation.

Moreover, while previous film-based delivery systems have primarily focused on hydrophilic drugs[28], our SNMDF approach aligns with recent advancements integrating SNEDDS into polymeric films for poorly soluble drugs. This dual-delivery strategy enhances both solubility and patient compliance. Our films (especially F1 and F2) achieved rapid disintegration (wetting time 14 to 16 s) and CDR > 96%, which is either comparable to or better than results reported for fast-dissolving films in other lipid-drug systems.

The superior performance of our formulation can be attributed to the selection of Eucalyptus oil, which showed the highest solubility for Azathioprine among tested oils, and Cremophore EL AR, known for excellent emulsification capacity and biocompatibility. The use of PEG-400 further enhanced drug solubilization and the formation of a robust nanoemulsion. Additionally, using a statistically optimized design ensured precise control over the critical formulation parameters. Variations from other studies could stem from differences in the drug's polymorphic form, or experimental conditions such as mixing time and temperature.

Despite promising results, this study was limited to in vitro evaluations. The lack of in vivo pharmacokinetic or bioavailability studies restricts the direct correlation of enhanced dissolution to therapeutic benefit. Additionally, long-term stability and patient palatability of SNMDFs were not fully assessed. In the future, in vivo bioavailability studies, mucoadhesive property testing, and scale-up feasibility must be conducted to validate the clinical applicability of the developed SNMDF system. Furthermore, exploring alternative biodegradable film-forming polymers may help enhance patient acceptability.

Conclusion

Self-Nanoemulsifying mouth dissolving film of Azathioprine were successfully formulated and evaluated. Screening for the oils, surfactant and co-surfactant was done based on saturation solubility analysis, and SNEDDS made by Eucalyptus as oil, Cremophore El AR as surfactant and PEG 400 as co-surfactant has shown profound increased in solubility and permeability of drug. Every formulation was found within acceptable limits of physical characters. The SNMDF formulation F1 has shown 97.55% drug release after 45 min. Based on the results of the study, it can be said that buccal drug delivery may be a safer method of administration and that SNMDF may be an effective approach for improving the solubility and permeability of Azathioprine.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Information

Supplementary information is available on the website https://nopr.niscpr.res.in/handle/123456789.

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