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Original Article

TERCONAZOLE-LOADED MICRO-SPONGES: PREPARATION, CHARACTERIZATION, AND OPTIMIZATION FOR SOLUBILITY AND DISSOLUTION RATE ENHANCEMENT

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ABSTRACT

Objective: Terconazole is a potent antifungal agent characterized by insufficient aqueous solubility; which is a significant challenge for formulation development and therapeutic efficacy. The current research aimed to develop and optimize an innovative carrier system using micro-sponges to improve the solubility and dissolution rate of Terconazole.

Methods: Fifteen formulations of Terconazole-loaded micro-sponges were prepared by quasi-emulsion solvent diffusion technique, with various parameters such as polymer type and concentration, emulsifying agent concentration, plasticizer percentage, and pore inducer amount being systematically investigated. The impact of these parameters on particle size, production yield, loading efficiency, saturation solubility, and *In vitro* dissolution profiles was thoroughly assessed.

Results: The results indicated that formula F15, comprising 0.1g Eudragit L100, 25 mg Poly Vinyl Alcohol (PVA), 0.1 ml glycerol, and 1.5g Pre-Gelatinized Starch (PGS), exhibited the smallest particle size, higher production yield and loading efficiency, achieving a remarkable nine-fold enhancement in saturation solubility (752.26±19.84µg/ml) compared to pure Terconazole of 83.42±3.39µg/ml. furthermore, the percentage of Terconazole released after one hour from F15 was 92.85%, significantly higher than the 33.54% from its pure powder. Scanning Electron Microscope **(**SEM) analysis revealed highly porous structures of the micro-sponges, while Fourier Transform Infra-Red (FTIR) studies showed no evidence of chemical interaction, and Differential Scanning Calorimetry (DSC) indicated no change in Terconazole's nature during micro-sponges production.

Conclusion: Overall, the findings suggest that micro-sponges represent a promising system for enhancing the saturation solubility and dissolution rate of poorly water-soluble Terconazole, potentially improving its bioavailability and therapeutic outcomes in clinical settings, especially ocular medications. The implications of this study extend beyond Terconazole, offering valuable insights and methodologies that can be applied to improve the solubility and bioavailability of a wide range of pharmaceutical compounds.

Keywords: Terconazole, Solubility, Dissolution rate, Micro-sponges, Eudragit

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INTRODUCTION

Solubility is the most critical pre-formulation property, which significantly influences formulation development and therapeutic performance [1].

The main challenge associated with drug delivery systems is that most medications are poorly aqueous soluble, which creates many difficulties during formulating them in traditional dosage forms [2].

Micro-sponges technology was developed by Won in 1987, which is currently considered a revolutionary manner for delivering drugs in a unique, versatile structured form [3].

Micro-sponges are actually polymeric porous microspheres, with a large porous sponge-like structure and very tiny spherical shape particles. The size of micro-sponges is varied, usually from 5 to 300µm in diameter. A typical 25µm sphere can have up to 25×10^4 pores, with internal structure equivalence to 10 feet per pore in length, providing a total pore volume of about 1 ml/g for extensive retention of pharmaceutical active ingredients [4].

Micro-sponges are designed to deliver a drug efficiently at a minimum dose, which improves the drug stability and reduces the adverse effects. In addition, they can enhance the solubilization and dissolution rate of poorly aqueous soluble drugs and also modify the drug release profile in a controlled manner [5].

Previously, the micro-sponges drug delivery system was mostly used for topical administration, while nowadays as per current information according to many researchers; it is used for drug delivery via oral, parental, and ophthalmic routes.

Micro-sponges are predominantly composed of polymers dispersed in aqueous media, along with an appropriate emulsifying agent. For the enhancement of drug dissolution and controlling of drug release in Micro-sponges, Eudragit are frequently reported as a polymer of choice [6].

The quasi-emulsion solvent diffusion technique is a prevalent method for micro-sponge engineering, involving the dissolution of a water-insoluble polymer in a water-immiscible solvent, which is then emulsified with a hydrophilic plasticizer aqueous phase. The organic solvent gradually diffuses out while stirring, forming spherical scaffold structures. Key factors influencing the efficacy of these micro-carriers include the active drug-to-polymer ratio, surfactant, and plasticizer. Eudragit polymers are extensively utilized in the fabrication of micro-sponges [7].

Eudragit polymers are biologically inert, non-toxic, and non-biodegradable polymers. They encompass various types of pH-dependent and pH-independent coating polymers with a wide range of apparent viscosity; this versatility in properties confers a wide scope in the preparation of micro-sponges and enhancement of desired attributes such as particle size, loading efficiency, and saturation solubility [8].

Corneal pathologies significantly contribute to visual impairment, with various ocular regions vulnerable to fungal infections. Fungal keratitis is a notable condition caused by various fungal species, predominantly Candida. Despite being the leading cause of ocular fungal infections, Candida's resistance to common antifungal agents poses challenges. Historically, amphotericin B was the primary treatment for fungal keratitis, but it often led to diminished visual acuity. In complex cases, corneal grafts became necessary. Alternative methods for applying topical amphotericin B have been suggested, particularly the use of topical azoles. Fluconazole, voriconazole, and terconazole proved to have less toxicity to corneal epithelia, demonstrating better ocular tolerability than amphotericin B [9].

Terconazole is a potent antifungal agent over a wide range of yeasts and mycelium-forming fungi. It inhibits the fungal cytochrome P-450 dependent ergosterol synthesis, which is a vital component of a fungal cell membrane, leading to disruption of the fungal structure, function, and growth [10]. Terconazole was the first triazole marketed for the topical management of vaginal candidiasis (0.4% and 0.8% creams and 80 mg suppositories).

Chemically Terconazole ($C_{26}H_{31}Cl_2N_5O_3$) is 1-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3dioxolan-4-yl]methoxy]phenyl]-4-1-methylethyl)piperazine [11]. It is a weak base with a molecular weight of 532.47 Daltons. It has pka and log P of 8.41 and 5.37, respectively [12].

Terconazole, which belongs to Biopharmaceutical Classification System (BSC) Class-II, has insufficient aqueous solubility. Unfortunately, Terconazole suffers from a limit dissolution rate, poor ocular absorption, and rapid drainage from ocular tissues, which collectively restrict its ocular bioavailability despite its good efficacy [13]. Consequently, an effective delivery system is essential to overcome these drawbacks and improve its therapeutic activities. Thus, micro-sponges may provide significant improvements in the *in vitro* and *in vivo* performance of poorly water-soluble drugs and, therefore, may serve as effective vehicles for ocular drug delivery [14].

Moreover, it is noteworthy that micro-sponge carriers have not been utilized in previous literature for Terconazole loading despite being regarded as a promising method for enhancing drug solubility and bioavailability.

Various studies have explored different carrier systems to improve the bioavailability and therapeutic efficacy of Terconazole, for ocular applications. Terconazole was loaded onto mesoporous silica microparticles, specifically Syloid® 244 FP, modified with Poly(Lactic-co-Glycolic Acid) to enhance its solubility and dissolution rate. The optimal formulation showed a significant increase in mean residence time, Cmax, and AUC0–24-values in rabbit eyes, indicating improved ocular bioavailability [12]. Silica/Chitosan Nanoparticles (SCNs) were developed using tetraethyl ortho silicate and chitosan HCl, with cyclodextrins as cryoprotectants. The optimized SCNs demonstrated excellent mucoadhesive properties and increased Cmax and AUC0–24 values, suggesting enhanced ocular bioavailability compared to Terconazole suspension [9]. While these innovative carriers show promise in enhancing the solubility and dissolution rate of Terconazole for ocular delivery, challenges such as formulation stability, scalability, and long-term safety need to be addressed.

The objective of this study was to develop an optimized formulation of Terconazole-loaded micro-sponges for improving Terconazole solubility and dissolution rate, which is the limitation of ocular delivery system developments.

MATERIALS AND METHODS

Materials

Terconazole powder was provided from Nosch Labs Private Limited, India. Eudragit polymer S100 powder was supplied from Röhm GMBH Weiterstdt, Germany. Eudragit polymers (L100 powder and E100 granules) and PGS powder were obtained as a gift sample from Samara Drug Industry, Iraq. Eudragit polymers (RS100 and RL100) powders were supplied from Vikram Thermo Limited, India. PVA powder was received from Panreac, Espana. Glycerol was supplied from BDH Chemicals Limited, England. All other ingredients used in this research were of analytical grade.

Preparation of terconazole-loaded micro-sponges

Terconazole-loaded micro-sponges were prepared via quasi-emulsion solvent diffusion technique, using different polymers, as shown in table 1.

Firstly, the organic (inner) phase was prepared by dissolving one type of Eudragit polymers (S100, L100, RS100, RL100, or E100) and glycerol as plasticizer, in 2.5 ml of ethanol, which can dissolve all used polymers as well as Triconazole. On the other hand, 100 ml of the aqueous (outer) phase was prepared by dissolving PVA, as surfactant, in distilled water at 70 °C, until it dissolved completely and then allowed to cool down to room temperature.

1g of Terconazole was added to the organic phase and dissolved under ultrasonication (ultrasonic power 100W and frequency 40KHZ) at 35 °C for 15 min, until a clear solution was obtained. The resulting solution was then poured, drop by drop, into the aqueous phase. The mixture was stirred at 500rpm for 1h at room temperature. Hence, micro-sponges were created by expelling ethanol from the solution through evaporation. The prepared mixture was left in a refrigerator for one day in order to complete precipitation of the micro-sponges. Subsequently, the performed mixture was filtered to separate the micro-sponges, and then washed several times with distilled water. The formed Terconazole-loaded micro-sponges were dried in an oven at 40 °C for 12h and stored for subsequent investigations [15, 16].

Table 1: The composition of different micro-sponges formulas prepared by quasi-emulsion solvent diffusion technique

Formula code	*Terconazole (g)	Inner phase				Outer phase		PGS (mg)
		Eudragit type	Polymer (mg)	Glycerol (µl)	*Ethanol (ml)	PVA (mg)	*Water (ml)	
F1	1	S100	125	250	2.5	25	100	-
F2	1	L100	125	250	2.5	25	100	-
F3	1	RS 100	125	250	2.5	25	100	-
F4	1	RL 100	125	250	2.5	25	100	-
F5	1	E100	125	250	2.5	25	100	-
F6	1	L100	75	250	2.5	25	100	-
F7	1	L100	100	250	2.5	25	100	-
F8	1	L100	150	250	2.5	25	100	-
F9	1	L100	100	125	2.5	25	100	-
F10	1	L100	100	500	2.5	25	100	-
F11	1	L100	100	500	2.5	5	100	-
F12	1	L100	100	500	2.5	50	100	-
F13	1	L100	100	500	2.5	25	100	0.5
F14	1	L100	100	500	2.5	25	100	1
F15	1	L100	100	500	2.5	25	100	1.5

*Value were kept constant, PVA is Poly Vinyl Alcohol and PGS is Pre-Gelatinized Starch, The specific ranges for polymer, plasticizer, emulsifying agent, and pore inducer concentrations were chosen based on preliminary experiments, in addition to using what was mention in previous literature.

Evaluation of terconazole-loaded micro-sponges

Particle size analysis

The particle size of the prepared micro-sponges was measured with an Olympus BX51 optical microscope, by using a calibrated ocular and stage micrometer under a regular polarized light. A tiny amount of sample was spread on a clean glass slide and observed under 100X lens magnification. The average particle size was calculated using the following equation [16]:

$$D_{av} = \frac{\sum nd}{\sum n}$$

Where:

D_{av} is the average diameter of at least 100 particles (μm).

n is the number of particles per group.

d is the middle value of particles diameter (μm).

Determination of the micro-sponges production yield

The production yield of micro-sponges was obtained by comparing the accurate weight of the final formed micro-sponges with the initial weight of the combined raw components. The percentage yield was calculated by the following equation [17]:

$$Y_{\%} = \frac{M_m}{M_0} \times 100\%$$

Where:

$Y_{\%}$ is the percentage of Micro-sponges production yield.

M_0 is the theoretical total mass of Terconazole and polymer used.

M_m is the mass of the formed micro-sponges.

Determination of terconazole loading efficiency

The amount of Terconazole loaded into the micro-sponges pores was quantified spectrophotometrically.

Initially, an accurate weight (10 mg) of Terconazole-loaded micro-sponges sample was crushed in a mortar; and then kept in 100 ml of Phosphate Buffer Solution (PBS) pH7.4 for 24h, to extract the Terconazole.

Subsequently, the solution was filtered through a 0.45μm cellulose membrane filter. After that, the filtrate was appropriately diluted with PBS (pH7.4), and spectrophotometric absorbance was taken at the maximum wave length of Terconazole.

The Terconazole content was determined from the standard calibration curve. The loading efficiency of Terconazole was estimated using the following equation [8, 18]:

$$LE_{\%} = \frac{D_m}{D_0} \times 100\%$$

Where:

$LE_{\%}$ is the percentage of Terconazole loading efficiency.

D_0 is the Theoretical Terconazole amount that was fed initially to prepare a formula.

D_m is the amount of free Terconazole that leaks from the micro-sponges formula.

Saturation solubility study

The saturated solubility determination was achieved by using the shake flask method. This study was performed, at room temperature, for the unprocessed pure Terconazole and all the prepared micro-sponges formulas.

To gain maximum solubility, an excess amount of the sample was dispersed in 10 ml of PBS (pH7.4). The suspension was sonicated for 15 min and then stirred in a water bath shaker at a constant temperature of 24±0.5 °C. After 72h, which was sufficient to achieve an equilibrium state, the suspension was filtered through a 0.45μm cellulose membrane filter. The filtrate was collected and suitably diluted with the same buffer solvent.

The absorbance of Terconazole was analyzed by a UV-Visible Spectrophotometer, at the previously scanned λ_{max} in a particular BPS (pH7.4) of 245 nm. Finally, the concentration of the dissolved Terconazole was determined using a standard calibration curve [19].

The saturation solubility of pure Terconazole was also determined at 37±0.5 °C for sink condition.

***In vitro* dissolution study**

The *in vitro* dissolution rate of Terconazole from the optimized micro-sponges formula was compared with pure Terconazole powder, using United States Pharmacopeia (USP) apparatus-II (paddle assembly). 900 ml of PBS (pH7.4) was used as a dissolution medium close to lachrymal fluid. Keeping temperature at 37±0.5 °C and stirring rate at 50rpm. An accurately measured amount of micro-sponges equivalent to 10 mg of Terconazole was used. An aliquot of 10 ml of dissolution fluid was collected at regular predicated intervals (10, 20, 30 45, 60, 120, and 180 min) and immediately replaced with 10 ml of the fresh dissolution medium to maintain a constant volume. The samples were filtered through a 0.45μm filter, suitably diluted, and assayed at λ_{max} of Terconazole using a UV-Visible spectrophotometer [8, 20].

Kinetic modeling of drug release from micro-sponge

In order to elucidate the mechanism underlying the release of Terconazole from the formulations, the *in vitro* release data were subjected to fitting with various kinetic models of release. The models employed include: zero order, first order, Higuchi model, Hixon-Crowell model, and Korsmeyer-Peppas. The model exhibiting the highest correlation coefficient was designated as the most appropriately fitting model [21].

SEM study

SEM technique was employed to survey the morphology and surface topography of the optimized micro-sponges formula. The sample was coated with gold-palladium, and then scanned using SEM (Inspect S50-FEI Company, Netherlands) under an argon atmosphere at room temperature [22].

Compatibility studies

Compatibility studies were carried out using thermal powerful analytical techniques, to determine the possibility of physicochemical interaction between Terconazole and a polymer used in the preparation of the optimized micro-sponges formula.

FTIR analysis

FTIR spectra of pure Terconazole, Eudragit-L100, a physical mixture of Terconazole and Eudragit-L100 at a ratio 1:1, and an optimized micro-sponges formula were recorded to achieve compatibility. An accurately weighed sample of 1 mg was grounded finely, mixed thoroughly with 100 mg of potassium bromide, and then scanned in the range from 400 cm^{-1} to 4000 cm^{-1} of 2 cm^{-1} resolution, using the FTIR spectroscopy-8300 Shimadzu model [23].

DSC analysis

DSC thermogram can be used to confirm the crystallinity of Terconazole, especially in the optimized formula, and also to ascertain compatibility. It was performed for the same FTIR samples by using a DSC analyzer-60plus Shimadzu model. An accurately weighed sample of 5 mg was placed in an aluminum pan, sealed hermetically, and scanned at a heating rate of 10 °C/min covering a temperature range of 0-350 °C, under a nitrogen atmosphere with a flow rate of 20 ml/min [24].

Statistical analysis

All the experimental studies were repeated in triplicate. All factors and evaluation parameters were statistically calculated individually. Results were presented as a means of three samples±standard deviation and were analyzed according to the One-Way Analysis of Variance (ANOVA) test using Microsoft Excel Program 2013. Differences were considered to be statistically significant at the level of $p<0.05$.

RESULTS AND DISCUSSION

Preparation of Terconazole-loaded micro-sponges

Quasi-emulsion solvent diffusion technique is a simple, safe, cost-effective, and reproducible procedure, involved in the preparation of Terconazole-loaded micro-sponges [25].

Micro-sponges were formed by rapid diffusion of ethanol into the inner phase, resulting in reduced solubility of hydrophobic Eudragit polymer in the droplets. Immediate mixing of ethanol and water at the droplet interface resulted in the precipitation of Eudragit, thus

forming a shell surrounding the dissolved Terconazole with ethanol. Ethanol diffusion resulted in the solidification of finely dispersed droplets in the aqueous solution [8].

Different variables were studied for their effect on the prepared Terconazole-loaded micro-sponges, to select the optimum formula that achieves the aim of this study.

The saturation solubility of pure Terconazole powder in PBS (pH7.4) at 24±0.5 °C was 83.42±3.59µg/ml, which is similar to the result reported previously [26].

Effect of polymer type of inner phase

Initially, five formulations were prepared using different Eudragit polymers to study their effect on the Terconazole-loaded micro-sponges, as shown in fig. 1.

The type of Eudragit has a considerable impact on the nature of micro-sponges formulations [27]. It was observed that the average particle size of micro-sponges was inversely proportional to the apparent viscosity of Eudragit polymers, as shown in the following order:

Eudragit polymers S100<L100<RS100<RL100<E100 as found in the micro-sponges formulas (F1-F5) respectively.

The apparent viscosities of Eudragit polymers are 50-200mpa. s for Eudragit-S100, 60-120mpa. s for Eudragit-L100, 1-15mpa. s for Eudragit-RS100 and-RL100, and 3-6mpa. s for Eudragit-E100 [28].

This result was attributed to the viscosity difference between the inner and the outer phases. Since, when the difference between the viscosity of the Eudragit dispersed phase and the aqueous dispersion medium was decreased, the emulsion was barely broken down into small droplets, and micro-sponges with larger particle sizes were formed [29].

It was shown that as the particle size decreased, the loading efficiency of Terconazole increased significantly ($p<0.05$). Further reduction in particle size increases their surface area with more active sites available for drug uptake, leading to improved loading efficiency [30].

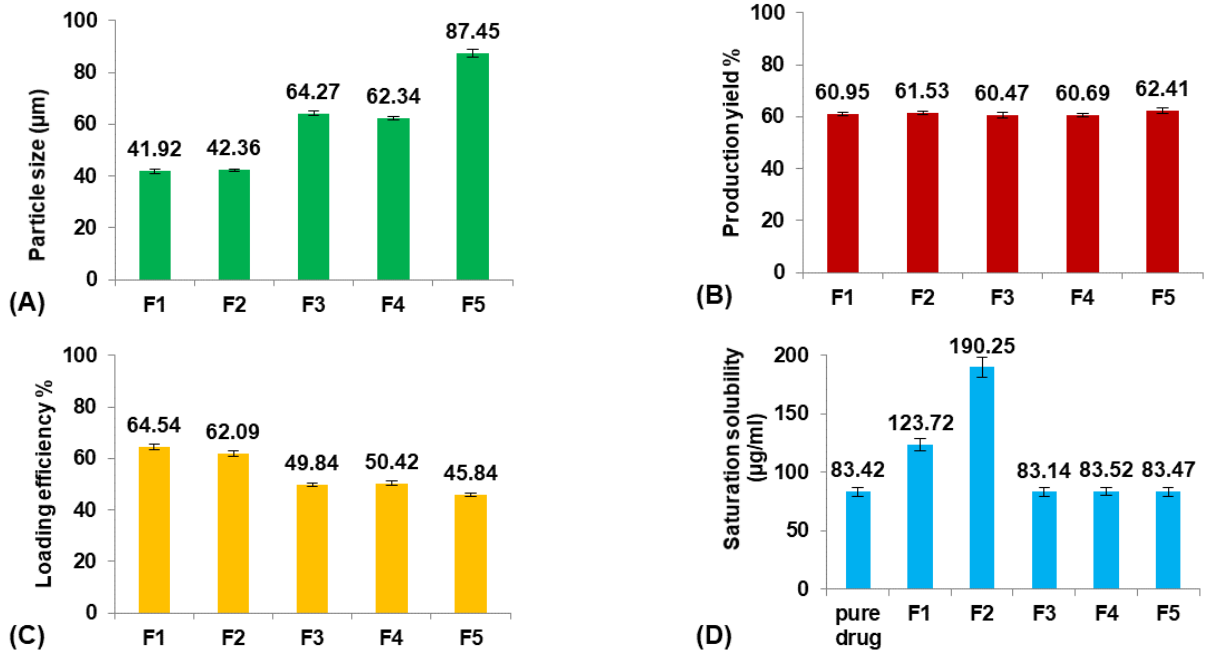


Fig. 1: Effect of polymer type of inner phase on: A) particle size (µm), B) production yield %, C) loading efficiency %, and D) saturation solubility (µg/ml) of Terconazole-loaded micro-sponges formulas F1-F5, which prepared by using Eudragit polymers-S100,-L100,-S100,-RL100, and-S100 respectively (all data expressed as mean±SD, n=3)

Moreover, the apparent viscosity of Eudragit polymers also has a noticeable effect on the loading efficiency. As the viscosity of the inner phase increased, the movement of Terconazole out of the dispersed droplets reduced, resulting in a greater amount of Terconazole entrapment [8].

The saturation solubility of Terconazole in PBS (pH7.4) was significantly improved ($p<0.05$) in micro-sponges formulas F1 and F2, prepared from Eudragit-S100 and-L100, respectively. Formulas F3-F5, prepared from Eudragit-RS100,-RL100, and-E100 respectively, gave no significant difference ($p>0.05$) in the saturation solubility of Terconazole.

The reason behind these results is that the Terconazole release occurs after complete swelling and degradation of the Eudragit matrix. Eudragit-S100,-L100, and-E100 are pH-dependent coating polymers that dissolve in definite pH media with the formation of their polymeric salt as follows:

Eudragit-S100 dissolves above pH7, Eudragit-L100 dissolves above pH6 and Eudragit-E100 dissolves below pH5, while Eudragit-RS100 and-RL100 are pH-independent polymers [31].

Formula F2 of Eudragit-L100 gave the best improvement in the solubility of Terconazole, in comparison with Formula F1 of Eudragit-S100. This result is because increasing Eudragit viscosity increases the adhesion of Terconazole molecules with polymeric molecules and reduces their mobility outside of the formed micro-sponges [17].

The production yield of micro-sponges was not significantly affected ($p>0.05$) by using different types of Eudragit polymers.

Formula F2, with acceptable particle size, production yield, and loading efficiency, and best solubility improvement, was selected for further optimization studies.

Effect of polymer concentration

Different concentrations of Eudragit-L100 (30, 40, 50, and 60 mg/ml ethanol) were used to prepare formulas F6, F7, F2, and F8, respectively, to study their effect on the Terconazole-loaded micro-sponges, as illustrated in fig. 2.

At a decreased concentration of Eudragit-L100, below 30 mg/ml, the finely dispersed spherical quasi-emulsion droplets were observed in

a solvent under agitation. Still, as the agitation was discontinued, emulsion droplets adhered together and aggregated. The result suggests that the Eudragit-L100 concentration of the inner phase needs to be controlled within an appropriate range to maintain the optimal viscosity that affects the formation of quasi-emulsion droplets at the initial stage and the solidification of Terconazole and Eudragit-L100 in the droplets. The good micro-sponges were produced only when 30 mg/ml of Eudragit-L100 was used.

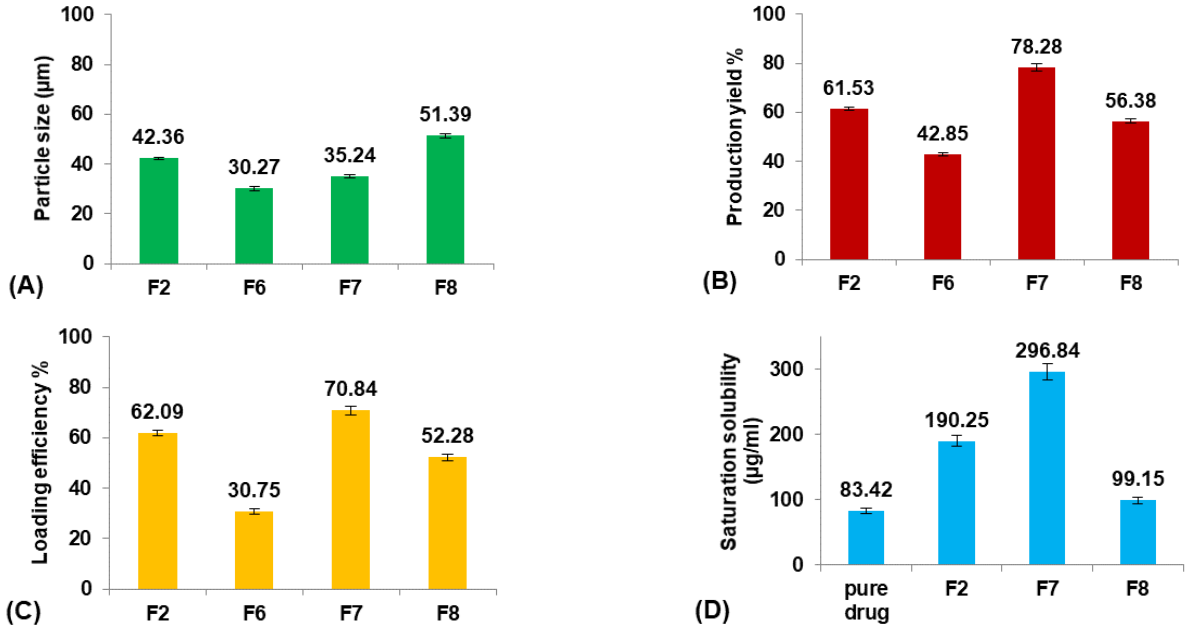


Fig. 2: Effect of polymer concentration on: A) particle size (µm), B) production yield %, C) loading efficiency %, and D) saturation solubility (µg/ml) of Terconazole-loaded micro-sponges formulas F2 and F6-F8, which prepared by using 50, 30, 40, and 60 mg/ml of Eudragit-L100, respectively, (all data expressed as mean±SD, n=3)

It was observed that decreasing the concentration of Eudragit-L100 led to a significant decrease ($p<0.05$) in the average particle size of micro-sponges. This occurred because the proportion of polymer available per micro-sponges was decreased, and thus a smaller particle size was obtained. This provides an extensive surface area for high entrapment [32].

Production yield and loading efficiency increased significantly ($p<0.05$) with decreasing particle size, as shown in formulas F2, F7, and F8. At the lowest concentration of Eudragit-L100 (Formula F6), production yield and loading efficiency decreased significantly ($P<0.05$) despite the decrease in particle size due to the reduction in the Eudragit-L100 fraction available for Terconazole encapsulation [8]. Therefore, formula F6 was rejected from the study.

The saturation solubility of Terconazole in PBS (pH7.4) was significantly improved ($p<0.05$) by reducing the concentration of Eudragit-L100. This result was due to an increased ratio of Terconazole to Eudragit-L100, which resulted in a decreased matrix wall thickness of the micro-sponges pores, and thus a more extensive diffusion pathway and ultimately more Terconazole release [20].

Formula F7, with lower particle size, higher production yield and loading efficiency, and best solubility improvement, was selected for further optimization studies.

Effect of plasticizer percentage

Glycerol was used as a plasticizer to overcome the problem of coalescence during solvent evaporation [33].

Different glycerol percentages of (5, 10, and 20%v/v ethanol) were used to prepare formulas F9, F7, and F10, respectively, to study their effect on the Terconazole-loaded micro-sponges, as shown in fig. 3.

The results indicated that increasing the percentage of glycerol led to a significant decrease ($P<0.05$) in the average particle size with a significant increase ($P<0.05$) in production yield and loading efficiency, in addition to a significant improvement ($P<0.05$) in the saturation solubility of Terconazole in PBS (pH7.4).

These results were attributed to a higher stabilizing effect of glycerol on small emulsion droplets, as it reduces the phase tension between the droplets and the dispersion medium, preventing them from aggregating into larger particles. Therefore, micro-sponges with smaller particles and larger surface areas were formed [34].

Formula F10, with lower particle size, higher production yield and loading efficiency, and best solubility improvement, was selected for further optimization studies.

Effect of emulsifying agent concentration

PVA was employed as a non-ionic emulsifying agent in the preparation of micro-sponges to maintain the viscosity of the aqueous outer phase [35].

Different concentrations of PVA (5, 25, and 50 mg/100 ml water) were used to prepare formulas F11, F10, and F12, respectively, to study their effect on the Terconazole-loaded micro-sponges, as illustrated in fig. 4.

It was observed that increasing the PVA concentration (from 5 to 25 mg/100 ml) gave better stability against the coalescence of the emulsion and led to a significant decrease ($p<0.05$) in the average particle size.

On the other hand, increasing the PVA concentration (from 25 to 50 mg/100 ml) also resulted in a significant increase ($p<0.05$) in the average particle size, which was attributed to the lower viscosity difference between the inner and outer phases. This would result in larger emulsion droplets and consequently larger micro-sponges with a lower active surface area [16].

Production yield, loading efficiency, and saturation solubility of Terconazole in PBS (pH7.4) decreased significantly ($p<0.05$) with increasing particle size.

Formula F10, with lower particle size, higher production yield and loading efficiency, and best solubility improvement, was selected for further optimization studies.

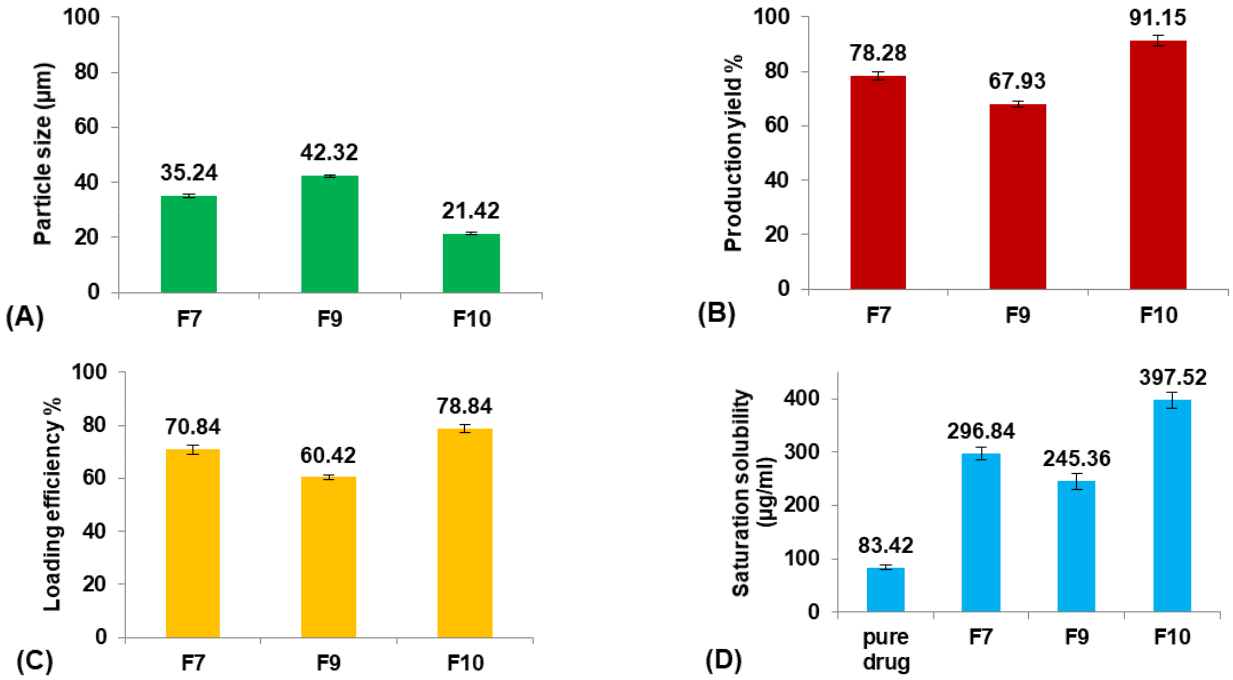


Fig. 3: Effect of plasticizer percentage on: A) particle size (µm), B) production yield %, C) loading efficiency %, and D) saturation solubility (µg/ml) of Terconazole-loaded micro-sponges formulas F7, F9, and F10, which contained of 10, 5, and 20% glycerol, respectively, (all data expressed as mean±SD, n=3)

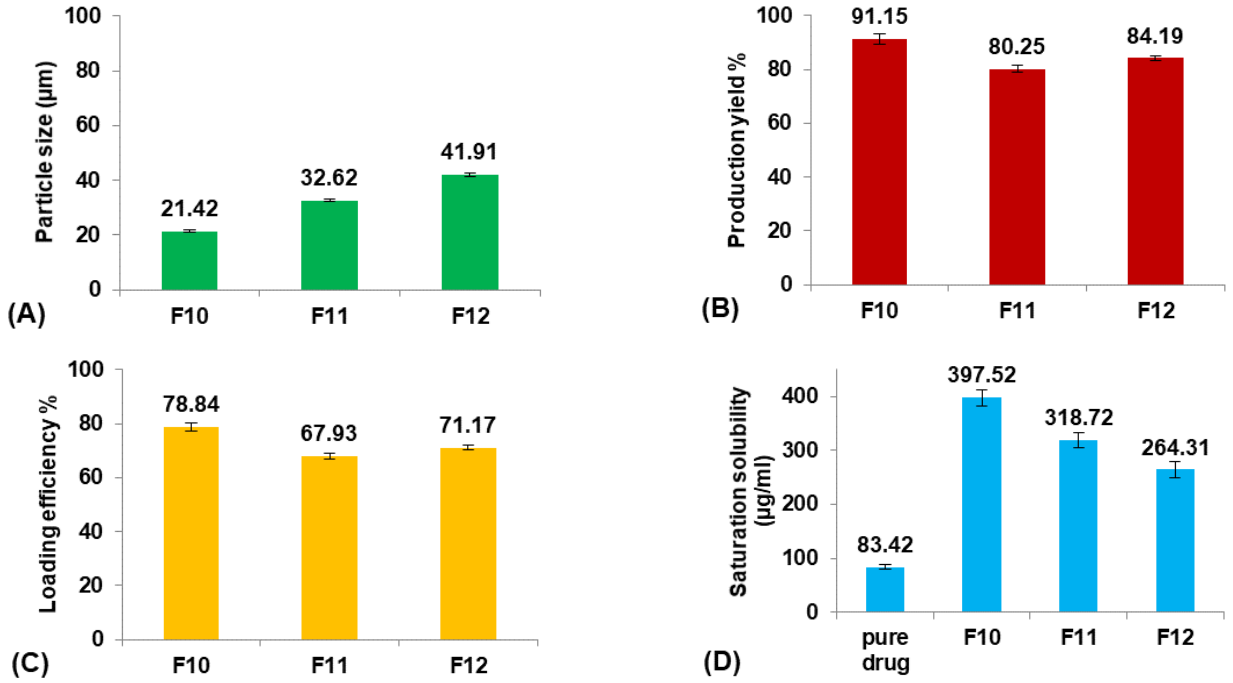


Fig. 4: Effect of emulsifying agent concentration on: A) particle size (µm), B) production yield %, C) loading efficiency %, and D) saturation solubility (µg/ml) of Terconazole-loaded micro-sponges formulas F10-F12, which contained of 25, 5, and 50 mg PVA/100 ml water, respectively (all data expressed as mean±SD, n=3)

Effect of pore inducer amount

PGS was introduced as a pore inducer in the preparation of micro-sponges to study its effect on Terconazole solubility. First, Terconazole was triturated with PGS and dispersed in 3 ml of ethanol. This dispersion was allowed to dry completely and then incorporated into the organic phase, after which the general preparation method was carried out [31].

Formulas F13-F15 (containing 0.5, 1, and 1.5 gm of PGS, respectively) were prepared for comparison with the selected formula F10, as shown in fig. 5.

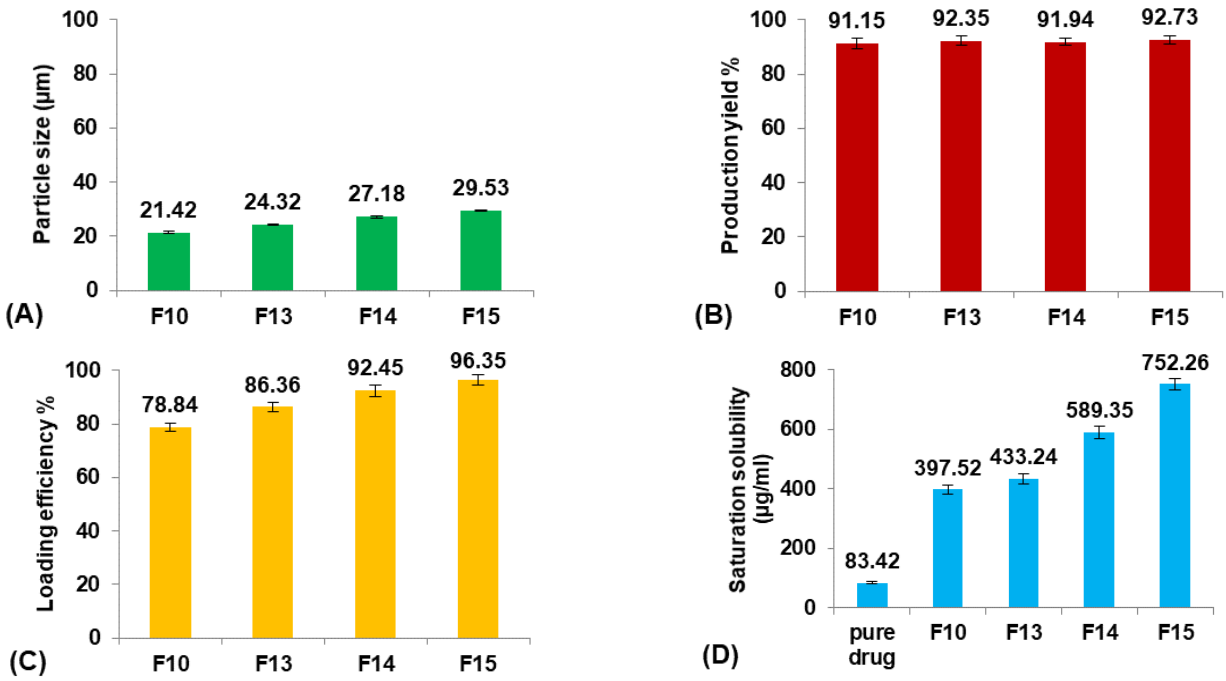


Fig. 5: Effect of pore inducer amount concentration on: A) particle size (µm), B) production yield %, C) loading efficiency %, and D) saturation solubility (µg/ml) of Terconazole-loaded micro-sponges formulas F10 and F13-F15, which contained of 0, 0.5, 1, and 1.5 mg PGS, respectively, (all data expressed as mean±SD, n=3)

It was observed that increasing the amount of PGS significantly increased ($p<0.05$) the particle size, entrapment efficiency, and saturation solubility, with no significant difference ($p>0.05$) in the production yield.

These results can be attributed to increased pores and channels within the particles, which provided a large surface area for Terconazole to be evenly absorbed into the micro-sponges, resulting in increased loading efficiency. On the other hand, the PBS (pH7.4) solution can pass through the pores of the micro-sponges, releasing the drug carried onto their surface by osmotic effect, resulting in enhanced solubility of Terconazole molecules [15].

Formula F15 possessed a higher percentage of uniform spherical particles during optical microscopy analysis.

Table 2 summarizes the statistical significance (p-value) of the different effects on each evaluation parameter of Terconazole-loaded micro-sponges formulas.

From all these studies, F15 was considered the optimized Terconazole-loaded micro-sponges formula.

***In vitro* dissolution study**

The *in vitro* dissolution profile (fig. 6) detects that formula F15 gave a better release of Terconazole than its pure powder because of the high thermodynamic activity of Terconazole molecules. The percentage of Terconazole release from micro-sponges formula F15 reached 92.85% within one hour, while Terconazole powder had only 33.54% content dissolved at that time.

In comparison, 70% of Terconazole was released after 3 h from the optimized formula prepared as Cyclodextrin Stabilized Freeze-Dried Silica/Chitosan Nanoparticles as reported previously [10].

Micro-sponges enhance the solubility of terconazole by trapping its molecules within their pores. Terconazole is effectively reduced to microscopic particles and increased surface area results in improved dissolution rate.

These results are in good accordance with Noyes–Whitney equation which states that the decrease in drug particle size caused an increase in the surface area and consequently enhanced the contact between particles and the dissolution medium, leading to an increased dissolution rate [8, 36].

On the other hand, the porous structure of micro-sponges allows easy penetration and access of dissolution medium to the entrapped drug molecules, as the pores provide channels for drug release. The results indicated that most of the Terconazole molecules are adsorbed on the surface of the micro-sponges and thus have the potential to undergo rapid solubilization and then quick drug release [20, 32].

Table 2: The statistical significance (p-value) of the different effects on each evaluation parameter of terconazole-loaded micro-sponges formulas

Effects	Evaluation parameters			
	Particle size	Production yield	Loading efficiency	Saturation solubility
Eudragit type (Increase the apparent viscosity)	Significant decrease ($p<0.05$)	No significant difference ($p>0.05$)	Significant increase ($p<0.05$)	Significant increase ($p<0.05$) for Eudragit-S100 and L100 no significant difference ($p>0.05$) for Eudragit-E100, RS100 and RL100
Decrease Eudragit-L100 concentration	Significant decrease ($p<0.05$)	Significant increase ($p<0.05$)	Significant increase ($p<0.05$)	Significant increase ($p<0.05$)
Increasing glycerol percentage	Significant decrease ($p<0.05$)	Significant increase ($p<0.05$)	Significant increase ($p<0.05$)	Significant increase ($p<0.05$)
Increasing PVA concentration	Significant increase ($p<0.05$)	Significant decrease ($p<0.05$)	Significant decrease ($p<0.05$)	Significant decrease ($p<0.05$)
Increasing PGS amount	Significant increase ($p<0.05$)	No significant difference ($p>0.05$)	Significant increase ($p<0.05$)	Significant increase ($p<0.05$)

PVA is poly vinyl alcohol and PGS is pre-gelatinized starch

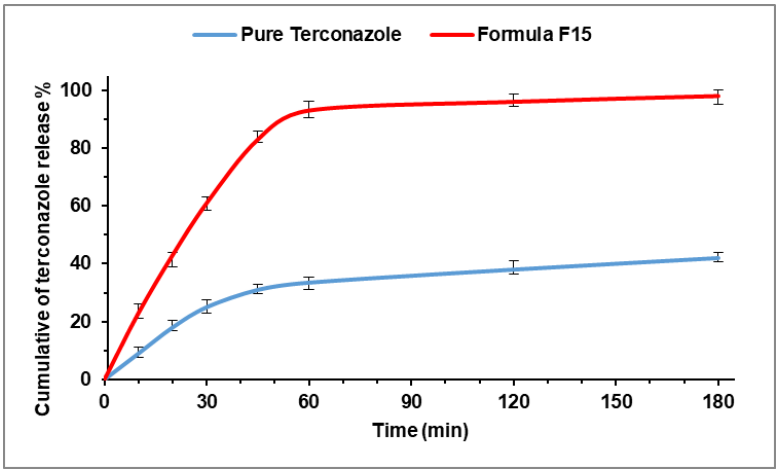


Fig. 6: Dissolution profile of Terconazole from micro-sponges formula F15 and pure Terconazole in PBS (pH 7.4) at 37±0.5 °C (all data expressed as mean±SD, n=3)

Kinetic modeling of drug release from micro-sponge

The release kinetics of Terconazole from micro sponge formulation F15 predominantly conform to the Hixon-Crowell release model, as indicated by the superior (R^2) values obtained. The findings demonstrated that the release exponent "n" value for F15 micro-sponges is greater than 0.5 and less than 1, signifying a non-Fickian (anomalous) release profile. Consequently, it is posited that this formulation delivers its active ingredient through a mechanism involving a combination of diffusion and erosion [21, 32].

SEM study

The SEM micrograph of the optimized micro-sponges formula F15 is presented in fig. 7. The image shows that the particles formed were mostly spherical with a finely porous sponge-like structure. A rough texture topography containing fine particles of Terconazole crystals adhered to the porous surface is also visible [31].

Compatibility studies

FTIR analysis

FTIR spectra are presented in fig. 8: A-D. Terconazole spectrum shows characteristic absorption bands at 1514 cm^{-1} (C=C stretching of aromatic ring), 1273 cm^{-1} (aromatic ether stretching), 1215 cm^{-1} (C-O stretching), 1149 cm^{-1} (C-O-C stretching) and 650 cm^{-1}(C-Cl bond) [38].

FTIR spectra of the physical mixture and formula F15 revealed no change in functional group peaks of Terconazole, confirming that there is no significant change in the chemical integrity of Terconazole as well as no chemical interaction or complexation between Terconazole and Eudragit-L100. The lack of interaction signals in FTIR spectra implies that the molecular structures of the drug and polymer remain intact, supporting the formulation's stability [20, 39].

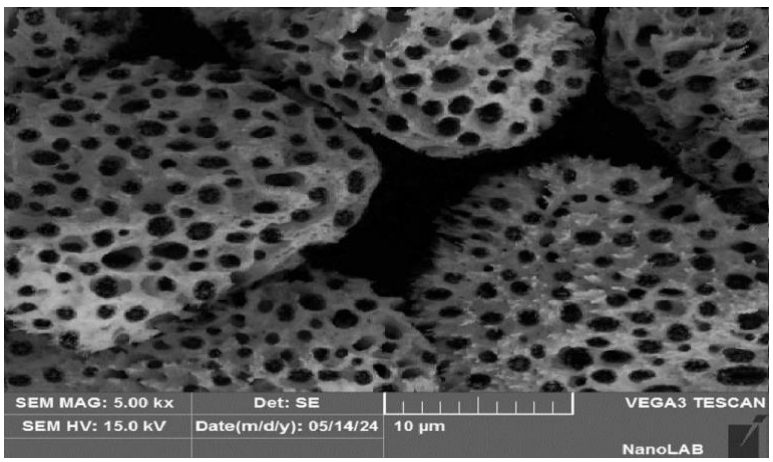


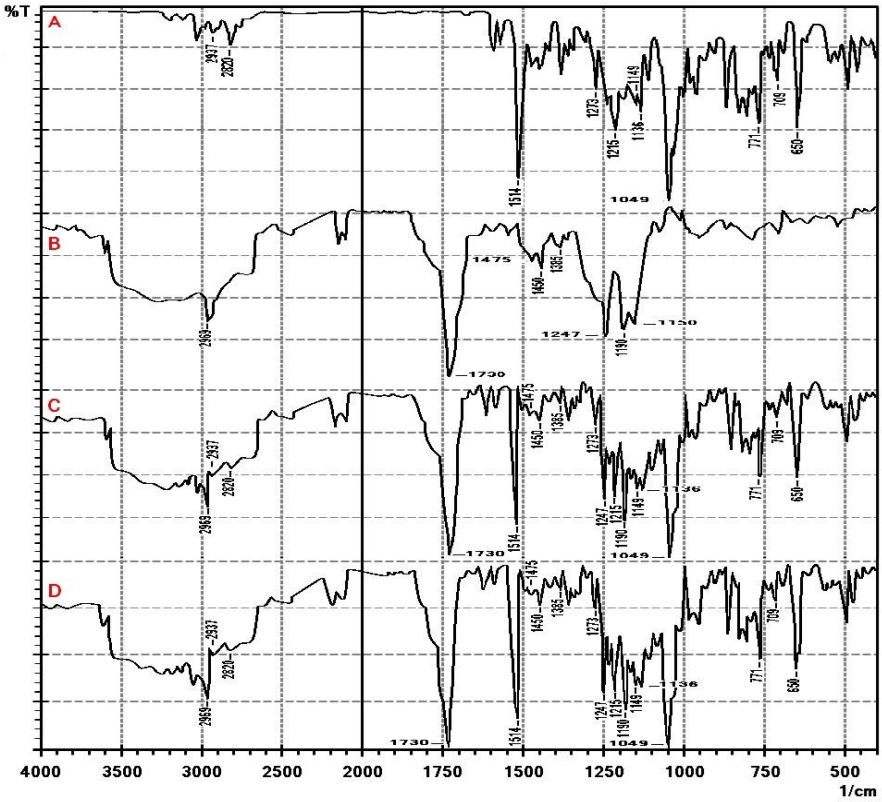
Fig. 7: SEM image of micro-sponges formula F15

DSC analysis

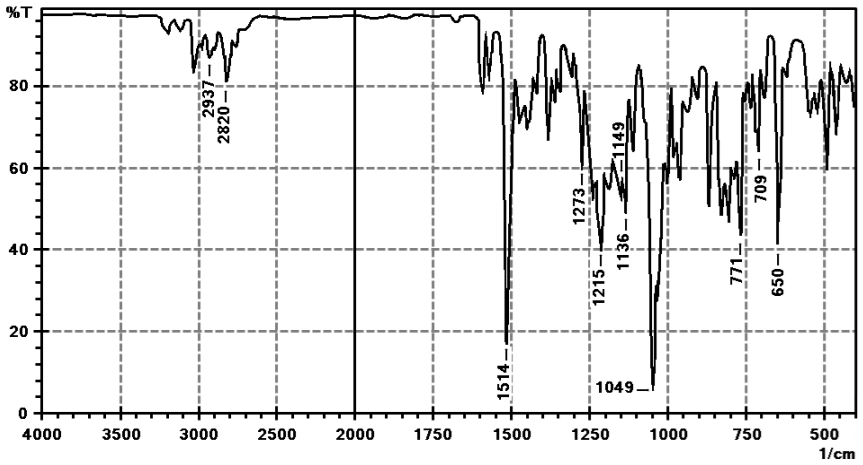
The DSC thermograms are presented in fig. 9: A-D. The terconazole curve shows a sharp characteristic endothermic peak at 126 °C, corresponding to its melting point, which indicates that Terconazole is used in a pure crystalline state [9].

DSC curves of the physical mixture and formula F15 show the typical peak of Terconazole crystals. The disappearance of Eudragit-L100 signal in the formula F15 is primarily due to the lower amount of Eudragit-L100 used compared to Terconazole, as well as the amorphous nature of Eudragit-L100.

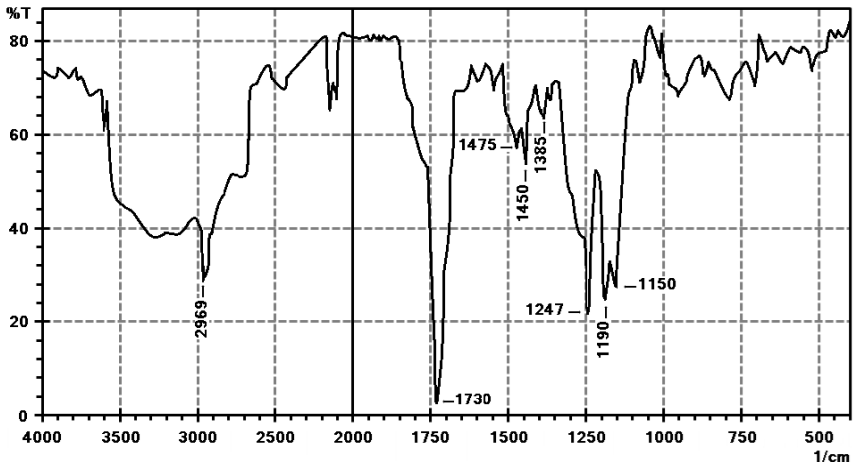
These results confirmed the compatibility between Terconazole and Eudragit-L100, which indicates that the micro-sponges production process did not change the nature of Terconazole in micro-sponges, as well as it suggests that the active ingredient remains unaltered and effective over time [8].



A) Pure Terconazole



B) Eudragit L100



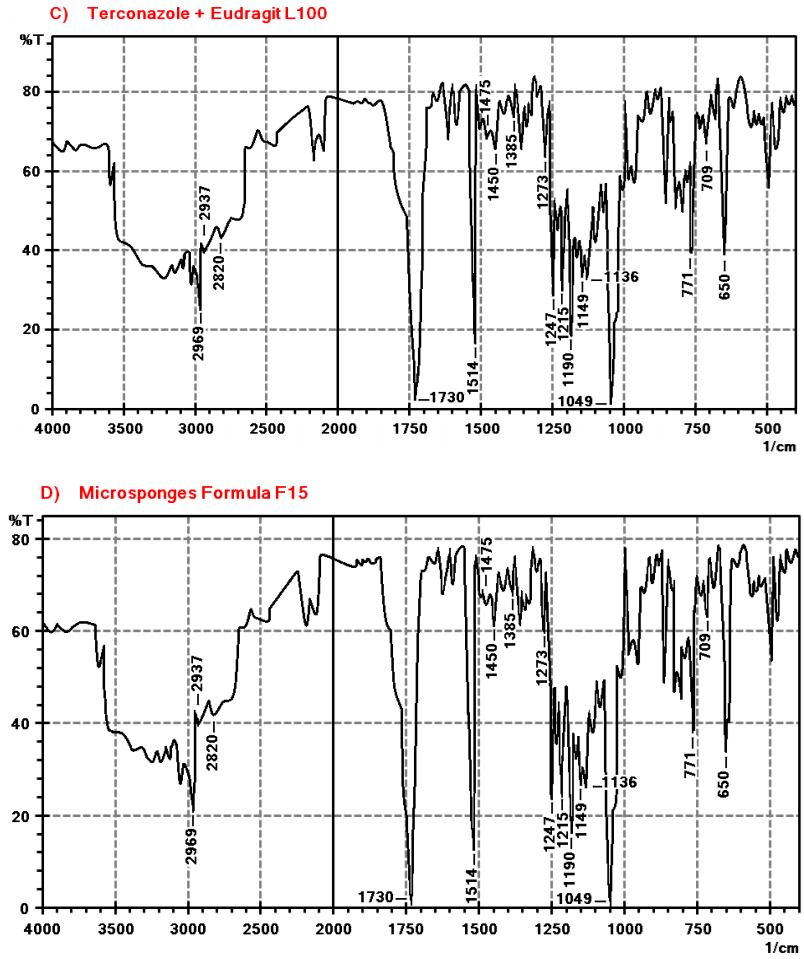
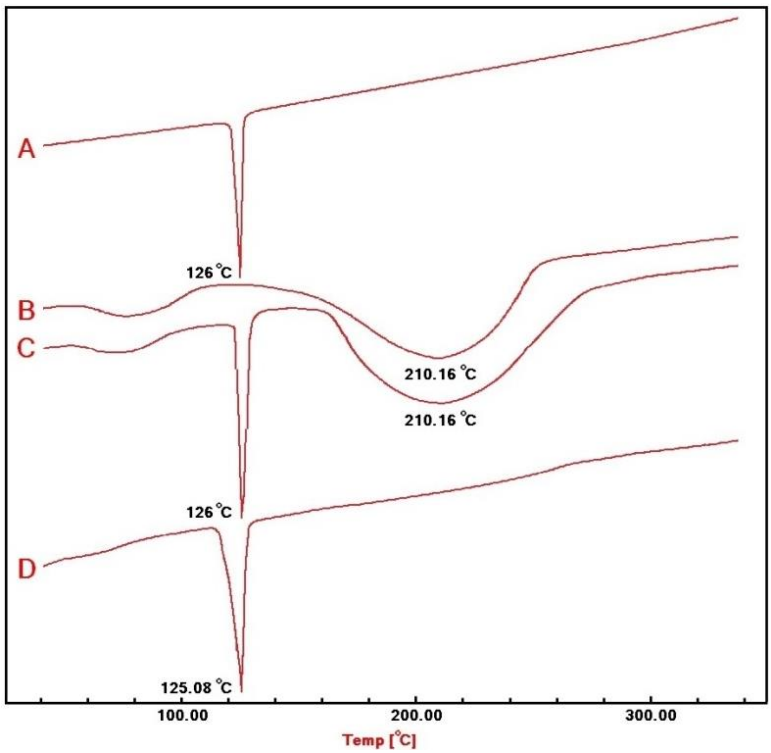


Fig. 8: FTIR spectra of A) Terconazole, B) Eudragit-L100, C) physical mixture, and D) formula F15



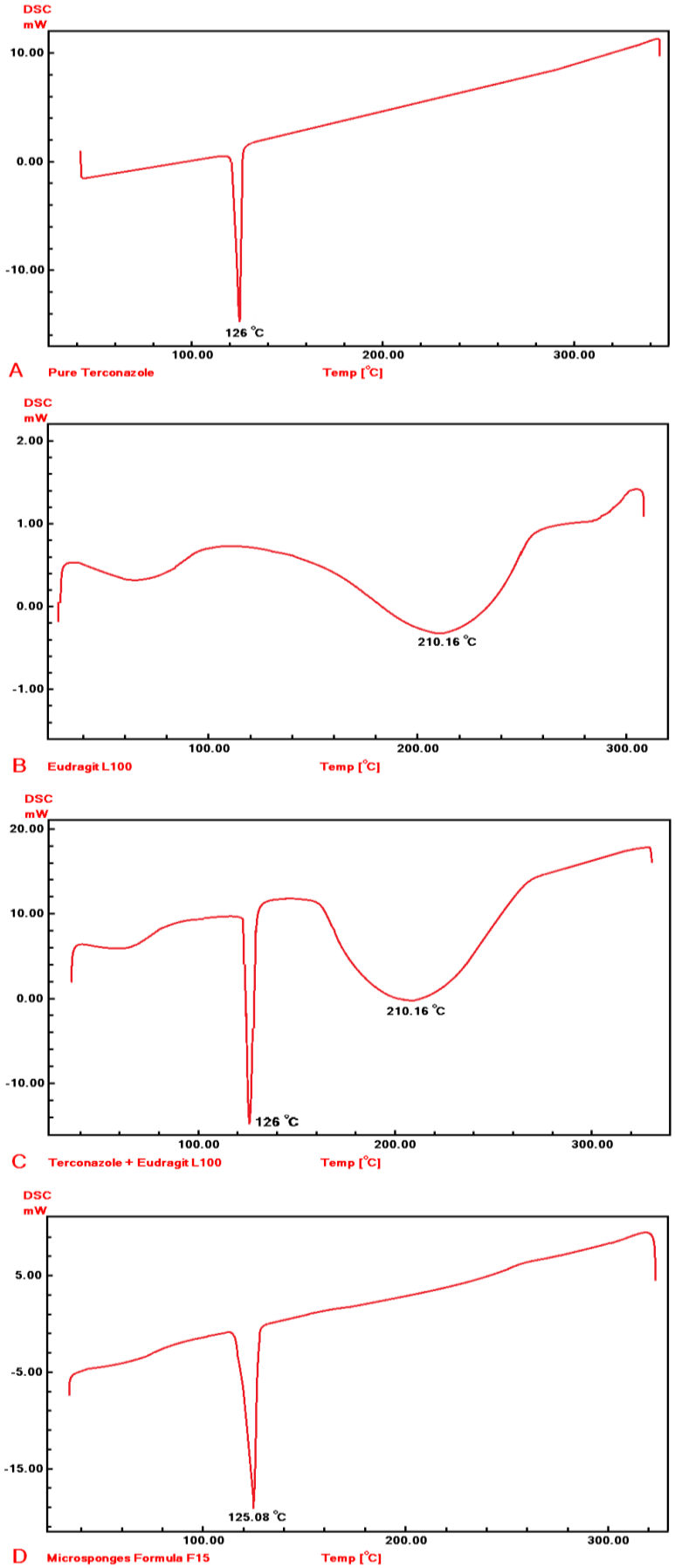


Fig. 9: DSC thermograms of A) Terconazole, B) Eudragit-L100, C) physical mixture, and D) formula F15

CONCLUSION

The investigation into Terconazole-loaded micro-sponges signifies a noteworthy progression in the formulation of drugs with poor aqueous solubility. The study validates the quasi-emulsion solvent diffusion technique as an effective method for micro-sponge preparation, with Eudragit-L100 identified as the most proficient polymer for enhancing Terconazole's solubility and dissolution rate. Optimization of formulation parameters notably improved micro-sponge characteristics, achieving a nine-fold increase in saturation solubility and superior dissolution profiles. The research establishes a foundational understanding for advancing ocular drug delivery systems and emphasizes the necessity of *in vivo* studies to assess the therapeutic efficacy of the developed micro-sponges.

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Nil

AUTHORS CONTRIBUTIONS

Maha Mahdi Ali designed the research work, performed the experiment, and analyzed the results. Manar Adnan Tamer contributed to the preparation and revision of the manuscript and provided guidance. Saba Abdulhadi Jaber monitored the research outcomes and finalized the paper for submission.

CONFLICT OF INTERESTS

Declared none

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