

Formulation and *In-vitro* Evaluation of Itraconazole Floating Microparticles

Taha A. Basher^{*,1} and Entidhar J. Muhammad^{*}

^{*}Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract

Itraconazole (ITZ) is an antifungal drug (BCSII) used for the treatment of local and systemic fungal infections. Furthermore, ITZ used as an antifungal prophylaxis for immunocompromised patients.

The objective of the study is to overcome the two problems of low and pH dependent solubility of ITZ by its preparation as floating microparticles.

Firstly, pH-dependent floating microparticles were prepared using oil in water solvent evaporation method, from which the best one (F7) selected as a best pH-dependent formula with composition of ITZ (200mg), EC (800mg), HPMC 15cps (200mg) and safflower oil (2ml). Then, F7 was compared with the selected Relatively pH-independent ITZ floating microparticles formula with composition of ITZ(200mg), a first coat of HPMC15cps (200mg), a second coat of EC (800mg), HPMC 15cps (200mg) and safflower oil (2ml) which prepared by a dual coating solvent evaporation method using a first coat which provides relatively pH-independent solubility, while the second coat applied as a bouncy producing agents. Polyvinyl alcohol (PVA) was used as a surfactant in both cases.

The prepared floating microparticles were subjected to various evaluation parameters such as yield percent, drug loading and drug entrapment efficiency (EE), particle size analysis, *in-vitro* bouncy, drug release, Fourier Transforms Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and X-ray Diffractometry (XRD) studies.

The selected relatively pH-independent formula (F16) has a particle size of 11.29 μm with a polydispersity index of 0.651, and the best pH-dependent formula (F7) has a particle size of 2.56 μm with PDI of 0.37. *In vitro* drug release per cent from the selected formula (F16) was 100 % at 10 hour (pH 1.2) while was 84% at 12 hour (pH 1.2) for F7 ($p > 0.05$), 62 % at 12 hour (pH 3.7) for formula (F16) while was 22% at 12 hour (pH 3.7) for F7 ($p < 0.05$), was 35 % at 12 hour (pH 6.8) from F16 while, 7% at 12 hour (pH 6.8) from F7 ($p < 0.05$). Therefore ITZ release from the selected formula (F16) is significantly better ($p < 0.05$) and the bouncy per cent of selected formula (F16) was 95% that was significantly better than bouncy percent of F7 that was 86 % ($p < 0.05$) in formula 16. In F7, %DL and %EE were 97% and 89%, respectively, while in F16 both %DL and %EE were 96%.

FTIR results showed no change in the peaks of the ITZ microparticles functional group while XRD and DSC show change the physical state of ITZ which indicate conversion from crystalline to amorphous, which had better stability (F16).

Thus, dual-coated floating microparticles (F16) appeared to be a promising approach to improve solubility at different pH values and prolong the release of the ITZ within the stomach that may increase its oral bioavailability.

Keywords: Itraconazole, Hydroxypropylmethylcellulose 15cps, Eudragits S100, solvent evaporation method, microparticles

تصنيع وتقييم خارج الجسم لجسيمات الايتروكانازول العائمة

طه عبد القادر بشير^{*}، و انتظار جاسم محمد^{*}

^{*} فرع الصيدلانيات، كلية الصيدلة، جامعة بغداد، بغداد، العراق .

الخلاصة

ايتراكونازول هو دواء مضاد للفطريات يستخدم لعلاج الالتهابات الفطرية الموضعية والجهازية. علاوة على ذلك، يستخدم ITZ كوقاية مضادة للفطريات للمرضى الذين يعانون من نقص المناعة.

انخفاض القابلية للذوبان في الماء والذوبان في درجة الحموضة المعتمدة هما العاملين الرئيسيان المسؤولين عن التوافر البيولوجي المنخفض عن طريق الفم. الهدف من الدراسة هو التغلب على هاتين المشكلتين من خلال صياغة الايتراكونازول كجسيمات مجهرية عائمة مستقلة نسبياً عن الأس الهيدروجيني لزيادة قابلية الذوبان وإطالة أمد إطلاق الايتراكونازول والذي من الممكن ان يؤدي إلى زيادة التوافر الحيوي.

¹Corresponding author E-mail: babantaha497@yahoo.com

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أولاً ، تم إعداد الجسيمات الدقيقة العائمة المعتمدة على الرقم الهيدروجيني ، وتم اختبار الصيغة (٧) كأفضل صيغة لأنه نتج عنه نسبة إطلاق دواء مقبول (٨٤%) خلال ١٢ ساعة ونسبة الجسيمات العائمة خلال ١٢ ساعة مقبول (٨٦%) ، تحضير جسيمات الاتروكانازول العائمة المستقلة نسبياً عن الأس الهيدروجيني بطريقة تبخير مذيب الطلاء المزوج باستخدام HPMC15 cps كطبقة أولى توفر قابلية ذوبان مستقلة عن درجة الحموضة (pH) نسبياً و EC و HPMC15cps وزيت القرطم كعوامل توفر العوم واستخدم PVA كمثبت مساعد. الجسيمات الدقيقة العائمة المحضرة التي تخضع لمعايير تقييم مختلفة ، مثل كمية الجسيمات المايكروية المنتجة ، كمية العلاج المحمل على الجسيمات المايكروية ، وكفاءة انحسار الدواء (DEE) ، وتحليل حجم الجسيمات ، وتحرر الدواء ، وتحويل فورييه للطيف بالأشعة تحت الحمراء (FTIR) ، مسعر المسح التبايني (DSC) و حيود الأشعة السينية (XRD).

تحتوي الصيغة المحددة المستقل نسبياً عن الأس الهيدروجيني (F16) على حجم جسيم يبلغ ١١,٢٩ ميكرون مع مؤشر متعدد الاختلافات يبلغ ٠,٦٥١ ، وأفضل صيغة تعتمد على الأس الهيدروجيني (F7) لها حجم جسيم ٢,٥٦ مع PDI 0.37. نسبة تحرر الدواء من الصيغة المحددة (F16) كان ١٠٠٪ خلال ١٠ ساعات (درجة الحموضة ١,٢) بينما كان ٨٤٪ خلال ١٢ ساعات (pH 1.2) من (P > 0.05) F7 ، ٦٢٪ خلال ١٢ ساعات (درجة الحموضة ٣,٧) من الصيغة المحددة (F16) بينما كان ٢٢٪ في ١٢ ساعات (pH 3.7) من (p < 0.05) F7 ، وكان ٣٥٪ خلال ١٢ ساعات (pH 6.8) ، بينما ٧٪ في ١٢ ساعة (p < 0.05) F7 (pH 6.8) ، وبالتالي فإن تحرر ITZ من الصيغة المحددة أفضل بكثير (p < 0.05). بلغت نسبة الكريات المايكروية الطافية ٧٨٦٪ وفي F16 كانت ٩٥٪.

لم تظهر نتائج FTIR أي تغيير في موقف المجموعة الوظيفية للجزيئات الدقيقة من ITZ ، بينما أظهرت XRD و DSC تغيير الحالة الفيزيائية لـ ITZ من البلورية إلى غير المتبلورة والتي كانت تتمتع بثبات أفضل

وهكذا ، يبدو أن الجسيمات الدقيقة العائمة المزوجة المغلفة هي طريقة واحدة لزيادة القابلية للذوبان وإطالة أمد إطلاق ITZ مما قد يزيد من التوافر البيولوجي عن طريق الفم.

الكلمات المفتاحية : ايتراكونازول ، هايدروكسي بروبيل ميثيل سيليلوز ، ايثيل سيليلوز ، طريقة تبخير المذيبات ، الجسيمات الدقيقة.

Introduction

Oral drug administration is the most common route to get a systemic effect due to its numerous advantages like safety, noninvasive, painless, does not require assistance, more convenient for chronic drugs administration and the medicament not need to be sterile.⁽¹⁾ However, several factors can affect the oral bioavailability, including water solubility and pH-dependent solubility. Hence, several techniques have been used to overcome these challenges include a reduction of the particle size that solves just water solubility problem (not the pH-dependent problem) such as micronisation or nanonisation, use of surfactants and conversion of crystalline to amorphous forms⁽²⁾.

Oil in water solvent evaporation method is most straight forward method for microencapsulation, widely used in pharmaceutical industries and cost-effective. This directly modified technique can be used to overcome many drugs physicochemical problems including both water solubility and pH-dependent solubility using specific coating materials⁽³⁾. Microparticles defined as solid particles with range of 1 and 1000 µm in diameter consisting of a core (drug) and coat layer prepared using one of the microencapsulation methods to overcome core (drug) related undesired physicochemical properties to obtain a more therapeutically effective drug⁽⁴⁾.

Itraconazole is an active triazole, antifungal agent (C₃₅H₃₈Cl₂N₈O₄), which is active against a broad spectrum of fungal species including *Cryptococcus*, *Candida*, *Aspergillus*, *Blastomyces* and *Histoplasma capsulatum*.^(5, 6)

Itraconazole is (BCS II) and has a pKa of 3.7⁽⁷⁾. ITZ has the characteristic of pH-dependent solubility having the highest solubility at the acidic media (4 µg/ml) compared to basic pH (1 µg/ml). It is slightly soluble in alcohols and freely soluble in dichloromethane and practically insoluble in water⁽⁸⁾, indicating that low aqueous solubility and pH-dependent solubility are the main reasons for low oral bioavailability. Itraconazole is a weak basic drug with Pka of (3.7), therefore at a low pH, of gastric juice, ITZ will be ionised therefore, the

gastric acidity is essential for adequate dissolution, and its oral bioavailability increased when taken with food since gastric pH decrease after food intake⁽⁹⁾. However, Firstly the pH of the stomach is variable and impossible to remain below 1.2 value, secondly there are cases that raise stomach pH value thus lead to decrease of ITZ solubility, and therefore bioavailability include fasting state, patients taking gastric acid-reducing agents such as protein pump inhibitors (PPI) and H₂-blockers and Furthermore patients with AIDS who complain from Hypochlorhydria.⁽¹⁰⁾

The objective of this study is to overcome both low water solubility and pH-dependent solubility of Itraconazole by formulating it as floating microparticles and one with a relatively pH-independent floating microparticles and compared between them thus to improve the solubility at variable pH values and prolong the release of the ITZ in stomach, which may lead to increase in ITZ bioavailability.

Materials and Methods

Materials

Itraconazole (ITZ) powder (Baji Gaokang Bio-Technology Co., Ltd., China), Hydroxy propylmethylcellulose (HPMC15cps) (Shanghai Ruizheng chemical Tech Co., Ltd., China), Ethylcellulose (EC) (BDH Chemical Ltd., England), Extra virgin olive oil (EVOO), Rice bran oil (RBO), Palm oil (PO) and safflower oil (SFO) (Furkan Dogalurunler.TIC.SAN.LTD, Turkey). Dichloromethane (DCM) (Thomas Baker Chemicals PVT.Ltd., India), Ethanol, methanol and n-hexane (Chem-Lab NV., Belgium). Hydrochloric acid (HCL) (Central Drug House (P) Ltd., India). Eudragit S100 (ED S100) (ATHOS CHEMICALS, Co., Ltd., India).

Methods

Saturation solubility determination

Solubility studies for the pure drug achieved in 0.1N HCl (pH 1.2), HCl buffer (pH 3.7) and phosphate buffer (pH 6.8) alone and with SLS at a concentration of 2, 3 and 6% (w/v), respectively.

Excess amount of pure ITZ was added to 10 ml of each solution in a test tube and agitated at $25 \pm 0.5^\circ\text{C}$ in a rotary test tube shaker for 72hr. Then, samples were filtered using $0.45\mu\text{m}$ Millipore filters. Each filtrate suitably was diluted with the respective solvent and analyzed by measuring the absorbance at the determined maximum wavelength (λ_{max}) in each solvent using UV-1800 UV-Vis Spectrophotometer (Shimadzu Scientific Instruments Inc., Japan). The solubility (mg/ml) of ITZ was calculated⁽¹¹⁾.

Preparation of ITZ floating microparticles (pH-dependent)

Oil in water solvent evaporation method was used to prepare floating microparticles (pH-dependent) of ITZ. Fifteen formulas prepared using different concentrations of polymers including; EC, HPMC 15cps and ED S100. Different oils were

also used such as; Extravirgin olive oil (EVOO), Rice bran oil (RBO), palm oil (PO) and safflower oil (SFO). Polyvinyl alcohol (PVA) was used as a surfactant, as shown in table (1).

The vegetable oils were not used in formulas F1-F3. The used oils dissolved in a vehicle composed of (DCM) and ethanol at 1:1 ratio forming the oil phase to which the drug added. While the aqueous phase containing 0.75% PVA as a surfactant. The oil phase was added to the aqueous phase drop wise using a bared syringe, under high-speed mechanical agitation of 1000rpm by utilizing magnetic stirrer for one hour at $25 \pm 1^\circ\text{C}$ to allow the organic solvent to evaporate and get the microparticles that filtered and dried at room temp overnight. Each formula was washed with 5 ml DCM to remove the excess oil if needed and dried again⁽¹²⁾.

Table 1. Composition of ITZ floating microparticles (pH-Dependent)

| Ingredient | Formula no. | | | | | | | | | | | | | | |
|-------------------|-------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 | F13 | F14 | F15 |
| ITZ(mg) | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 300 | 200 | 200 | 200 | 200 | 200 |
| EC(mg) | 900 | 800 | 700 | 800 | 800 | 800 | 800 | 700 | 900 | 800 | 800 | 800 | - | 800 | 800 |
| HPMC15 CPS(mg) | 100 | 200 | 300 | 200 | 200 | 200 | 200 | 300 | 100 | 200 | 200 | 200 | 200 | 200 | 200 |
| EDS100 (mg) | | | | | | | | | | | | | 800 | | |
| EVOO (ml) | | | | 2 | | | | | | | | | | | |
| RBO (ml) | | | | | 2 | | | | | | | | | | |
| PO(ml) | | | | | | 2 | | | | | | | | | |
| SFO(ml) | | | | | | | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 |
| Ethanol (ml) | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | |
| Methanol (ml) | | | | | | | | | | | | | | | 20 |
| DCM (ml) | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| DW(ml) | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 200 | 400 | 400 | 400 |
| PVA(%) | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Speed(rpm) | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 500 | 1000 |

Preparation of relatively pH-independent ITZ floating microparticles

Dual coating solvent evaporation method used for the development of the relatively pH-independent ITZ floating microparticles. The first coat composed of HPMC 15 cps. It was applied by using oil in water solvent evaporation method then without filtration the second coat was an organic solution of HPMC15, EC and SFO in 1:1 of DCM: ethanol as a solvent⁽¹³⁾ as represented in table 2.

Table2. Composition of ITZ relatively pH-independent floating microparticles

| Ingredient | F 16 | |
|-----------------|----------------------|----------------------|
| ITZ (mg) | 200 | |
| | 1 st coat | 2 nd coat |
| HPMC15 CPS (mg) | 200 | |
| DCM (ml) | 10 | |
| Ethanol (ml) | 10 | |
| EC (mg) | | 800 |
| HPMC15 CPS (mg) | | 200 |
| SFO (ml) | | 2 |
| Ethanol (ml) | | 20 |
| DCM (ml) | | 20 |
| DW (ml) | 400 | |
| Speed (rpm) | 1000 | 1000 |

Characterization of the prepared floating microparticles

Percentage yield determination

Yield of the prepared microparticles calculated by dividing the dry weight of the prepared microparticles (practical weight) by the total weight of the drug and excipients used (theoretical weight) as stated in the following equation:

$$\text{Yield (\%)} =$$

$$(\text{Actual weight/theoretical weight}) \times 100 \quad (1)$$

Particle size and polydispersity index (PDI) determination

All of the prepared formulations were in the micro size that is below 212 μ m because all are sieved using sieve number 70. In addition, particle size and PDI of the prepared ITZ floating microparticles F7, F12, F14, F15 and F16 measured by dynamic light scattering (DLS) using Nano Brook 90Plus particle size analyzer (Brookhaven instruments. the USA). Measurements were performed in triplicate by measuring the intensity of light scattered by the

molecules in the sample as a function of time at 90° scattering angle and constant temperature 25 °C. The PDI was determined, which measured the width of the size distribution of each formula; it is an index of spread or variation within the particle size⁽¹⁴⁾.

Drug loading and entrapment efficiency (EE) determination

25 mg of each formula dissolved in 50 ml methanol. The solution filtered by 0.45 μ m Millipore filters and 1 ml was taken and diluted with methanol if required. The samples were analyzed for drug content spectrophotometrically at 262 nm⁽¹⁴⁾.

$$\text{EE (\%)} = (\text{Actual drug loading} / \text{theoretical drug loading}) \times 100 \quad (2)$$

$$\text{Drug loading (\%)} = (\text{Actual drug content} / \text{Weight of powdered microsphere}) \times 100 \quad (3)$$

Buoyancy per cent determination

Weight of the formula equivalent to 100mg ITZ was spread over the test media using dissolution apparatus (type II) that filled with 900 ml of 0.1N HCl (pH 1.2, pH 3.7) and phosphate buffer (pH 6.8) separately in each case. The medium was agitated with a paddle rotating at 50 rpm for 12 h. both floating time (FT) and floating lag time were calculated. The floating and the settled portions of microspheres recovered separately were dried and weighed. All the bouncy studies were performed in duplicate⁽¹⁴⁾

$$\text{Bouncy \%} = (W_f / W_f + W_s) \times 100 \quad (3)$$

In- vitro release studies

All formulations were subjected to *in-vitro* release studies. The studies were carried out by USP apparatus II (paddle method) at 50 rpm using 900 ml of 0.1N HCL (pH 1.2, pH 3.7) and phosphate buffer (pH 6.8). The temperature maintained at 37 \pm 0.5 °C. At specific time intervals (every two hours), five ml aliquots were withdrawn and analyzed by UV spectrophotometer at the respective λ_{max} after suitable dilution against suitable blank. The removed volume replaced with an equal volume of fresh solvent⁽⁹⁾. All release studies performed in duplicate.⁽¹⁴⁾

Differential scanning calorimetry (DSC)

The DSC of pure ITZ powder and selected formula (F16) was taken on (Shimadzu DSC-60 plus, Japan). Ten milligrams sealed in the flat-bottomed aluminum pan of the differential scanning calorimeter. Data collection was achieved at a temperature range of 25–200°C and the heating rate was 5°C/min under nitrogen gas at a flow rate of 25 ml/min⁽¹⁵⁾.

Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR spectra of pure ITZ and selected formula were recorded using FTIR -7600,

Australia spectrophotometer. Powders were mixed with potassium bromide and compressed into disks using hydraulic press before scanning from 4000 to 400 cm^{-1} ⁽¹⁶⁾.

X-ray powder diffractometry study

The XRD patterns for pure ITZ and selected formula (F16) were analyzed using an XRD-6000, Shimadzu-Japan. The sample chosen scanning was conducted over powder X-ray diffractometer at the axis of 2 thetas, with the continuous scan range of 5-80 degree. The operating voltage was 40 kV and current 30mA and scans step size of 0.050° (2 θ) and scan step time of 60 seconds ⁽¹⁷⁾.

Statistical analysis

To investigate the significance of the difference between the results of studied formulations, T-test used. The level of significance set at a α 0.05, and ($P < 0.05$) was considered to be statistically significant.

Results and Discussion

Saturation solubility of pure ITZ

The saturated solubility of ITZ were 0.1, 0.01 and 0.0044 in 0.1N HCl (pH 1.2), HCL buffer (pH3.7) and phosphate buffer for (pH 6.8), respectively as shown in Fig (1). Since Sodium lauryl sulfate (SLS) is a surfactant, so it used to increase the solubility of ITZ to fulfill sink condition. The saturated solubility of ITZ were 0.42, 0.37 and 0.36 at 0.1N HCl (pH1.2with2% SLS), (pH3.7with3% SLS) and phosphate buffer of pH 6.8 with 6% SLS.

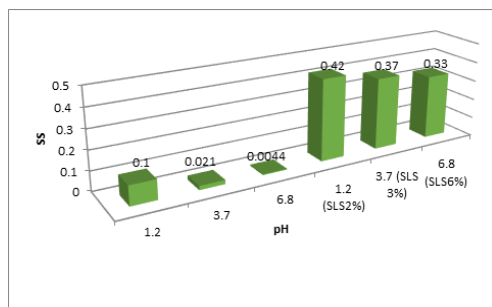


Figure 1. Saturated solubility of pure ITZ at different pH.

Percentage yield

The yield per cent of various floating microparticles formulas prepared depicted in figure (2). Per cent yield does not reach 100% usually due to mechanical variables during preparation method and precipitation of the formula components on the tools used in the preparation. The percent yield was 58% in F2 were no vegetable oils used. However, the use of vegetable oils results in significant increase in yield per cent, F4 98%, F5 95%, and F6 91%, F7 86% were different vegetable oils used ($p < .05$). Also, the higher SFO volume (F7 two ml

versus one ml in F11) result in the higher per cent yield (F7 92% and F11 88%) may be due to the higher oleic acid content when higher volume used. In F16, the lower yield was due to the longer stirring time that is three hours compared to one hour in F7 (different method was used). ⁽¹⁸⁾

Similarly, the lower HPMC15 cps concentration results in a higher yield per cent, in F8 were 300mg of HPMC and 700 mg of EC the yield was 83% while in F9 were 100mg of HPMC and 900 mg of EC used a yield of 98%. This result may be due to increasing HPMC 15cps concentration result in a higher viscosity of the oil phase which results in flocculation and agglomeration of the prepared microparticles thus lower yield per cent. Nepal et al. had found that the higher polymer concentration, the higher yield percent until an certain value after which the yield decreased ⁽¹²⁾.

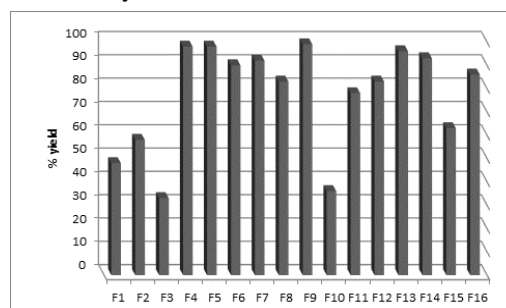


Figure 2. Percentage yield of ITZ floating microparticles.

Particle size analysis

All the prepared formulations were in the micro size below 212 μm because all are sieved using sieve number 70. However, F7, F12, F14, F15 and F16 particle size determined using, dynamic light scattering (DLS) by Nano Brook 90Plus particle size analyzer, and their particle size varied from 2.56 μm to 6.086 μm as shown in figure 3. the higher stirring rate; the smaller microparticles size due to less tendency of the formed microparticles to coalesce and aggregate ^(18,19). Regarding the (F16), the particle size is bigger due to the higher drug-to-polymer ratio and the longer stirring time ⁽²⁰⁾.

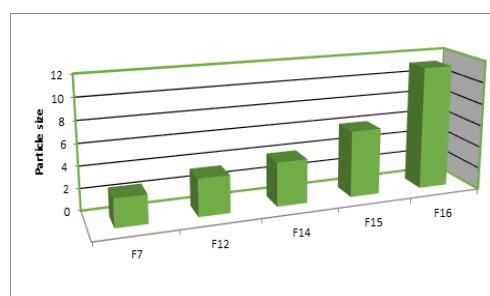


Figure 3. Particle size (μm) of ITZ floating microparticles

Drug loading and entrapment efficiency (EE) determination

The results of drug loading (DL) and drug entrapment efficiency (EE) have been shown in figure (4) and figure (5), respectively. In F4, F5, F6, F7, the %DL was 98, 98, 92 and 97%, respectively, and %EE was 98, 97, 84 and 89%, respectively while in F1, F2 and F3 the %DL was range from 50-79 and %EE range from 20-46. Using vegetable oils results in a significant increase in both drug loading and entrapment +efficiency ($p < 0.05$). Vegetable oil with higher oleic acid content results in higher DL and EE (F4). Similarly, these parameters increase upon using of lower concentration of HPMC15cps; in F8 were 300 mg of HPMC15cps used, %DL was 86, and EE was 71 while in F9 were 100mg of HPMC15cps used %DL was 99 and %EE was 98. The preparation technique dual coating solvent evaporation method also results in higher DL and EE ⁽²¹⁾.

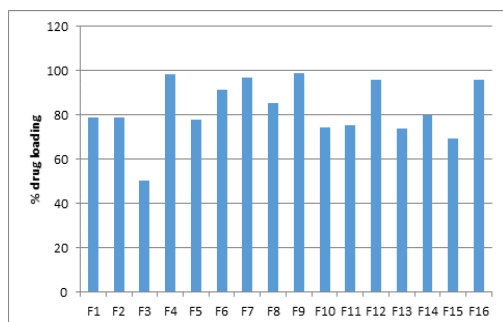


Figure 4. Drug loading of ITZ floating microparticles

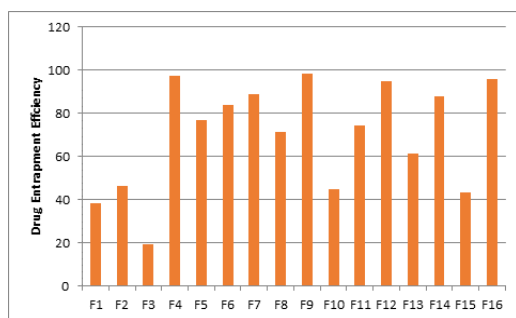


Figure 5. Entrapment efficiency of ITZ floating microparticles.

Buoyancy percent

The floating lag time (FLT) was zero for all formulas since floating has occurred immediately after the formula spread over the test media ⁽²³⁾. The floating time (FT) was range from 3 % (F3) to 98 % (F4) as shown in Fig (6). Vegetable oils used were the key factor that provides buoyancy; with higher oleic acid content of the vegetable oil; higher buoyancy per cent obtained so in (F4) EVOO used that had the highest oleic acid content among the used vegetable oil resulting highest buoyancy per cent

(94%). Also, the concentration of HPMC15cps played an essential role in the determination of buoyancy per cent with the lower the HPMC14cps concentration, the higher buoyancy per cent (95% in F9). Floating ability result from the low density of the prepared microparticles. Similar results observed with floating microparticles of resperidone ⁽¹²⁾.

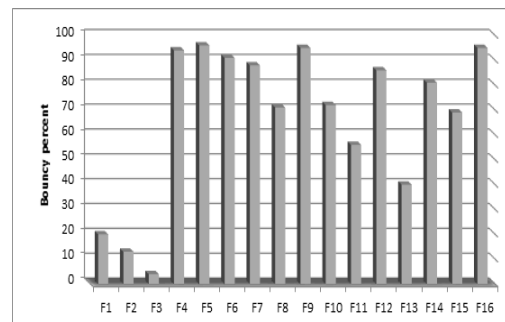


Figure 6. Bouncy percent of ITZ floating microparticles

In vitro drug release

In vitro cumulative release per cent from the selected formula (F16) was 100 % at 10 hour (pH 1.2) while was 84% at 12 hour (pH1.2) from F7 ($p > 0.05$), 62 % at 12 hour (pH 3.7) from selected formula(F16) while was 22% at 12 hour (pH 3.7) from F7 ($p < 0.05$), was 35 %at 12 hour (pH 6.8) while, 7% at 12 hour (pH 6.8) from F7($p < 0.05$), therefore ITZ release from the selected formula is significantly better($p < 0.05$)as shown in figures (7) and (8). These results indicated the efficiency of the dual coating solvent evaporation method and the pH-independent polymers used in solving the problem of the pH-dependent release of Itraconazole. Furthermore, since, Itraconazole is BSCII drug thus decreasing the particle size to micro-scale resulted in higher solubility this coordinated with Noyes–Whitney equation, in which the dissolution rate enhanced as the saturation solubility increased and the particle size decreased. Also, relatively pH-independent solubility advantage resulted from the first coat, namely HPMC 15cps^(13,22,23).

Another factor that may contribute to the fast release was the entrapment efficiency which had a direct effect on the drug release profile. As it increased, the release rate also increased ⁽²⁴⁾.

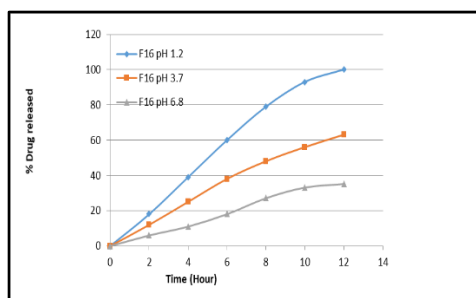


Figure 7. Release profile for pH-dependent ITZ floating microparticles at various pH.

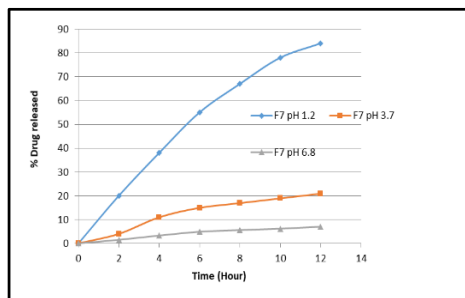


Figure 8. Release profile for relatively pH-independent ITZ floating microparticles at various pH.

Differential scanning calorimetry (DSC)

The DSC was used to estimate the effect of excipients and conditions on the physical properties of the drug. Pure ITZ exhibited a sharp endothermic melting peak at 170° (Figure 9) indicating no change in its melting temperature and the drug had a crystalline nature with high purity. The DSC of the selected formula (F16) in figure 10 showed the complete disappearance of the melting peak of ITZ gave clear evidence of the transformation of the drug from crystalline to the amorphous state (25, 26).

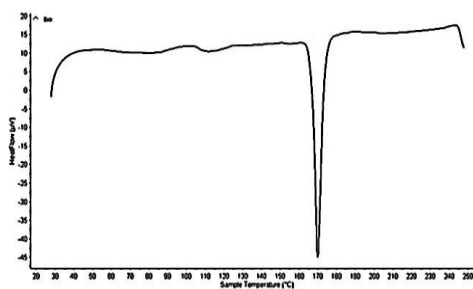


Figure 9. DSC thermogram of pure ITZ at temperature range (20-250 °C)

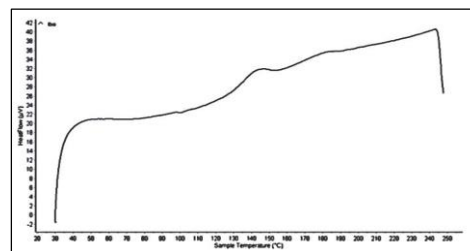


Figure 10. DSC thermogram of selected formula (16) at temperature range (20-250 °C)

Fourier transforms infrared spectroscopy (FTIR)

The FTIR spectrum of pure ITZ shown in figure 11. The main peaks of pure ITZ observed at wavenumbers, 3466, 3130, 2965, 3067, 1698, 2822, 1611, 1512 and 1453 cm⁻¹. It should be noted that all the characteristic bands of ITZ were detected in the spectrum of the physical mixture of drug, EC, HPMC15cps, as shown in figure 12. Therefore, there is no interaction between the drug and polymers used. A sharp band at 1698 cm⁻¹ in the pure drug spectrum was due to C=O, in the FTIR spectrum of the selected formula (F16) all of the ITZ characteristic peaks are present as shown in figure 13 excepted that, the peak 1698 that shifted to 1745 this is because the fatty acids content of safflower oil has a characteristic peak at 1745 that shown in figure 14 (27) thus, in conclusion, the drug, polymers and safflower oil used in the formulation were compatible with each other. (28,29).

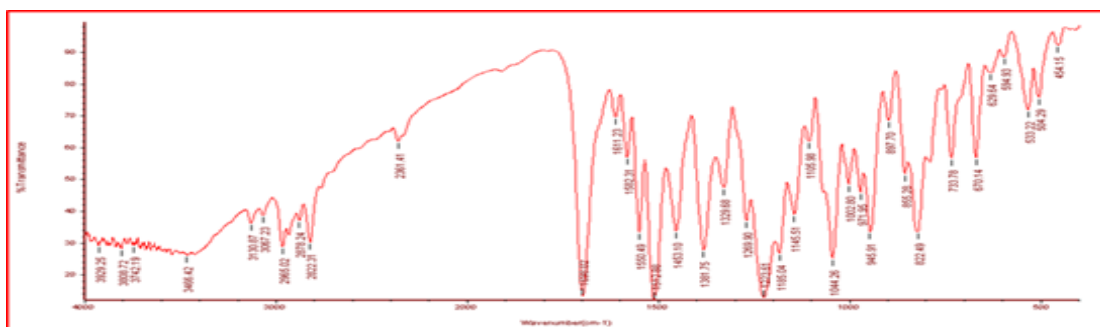


Figure 11. FTIR spectrum of pure ITZ.

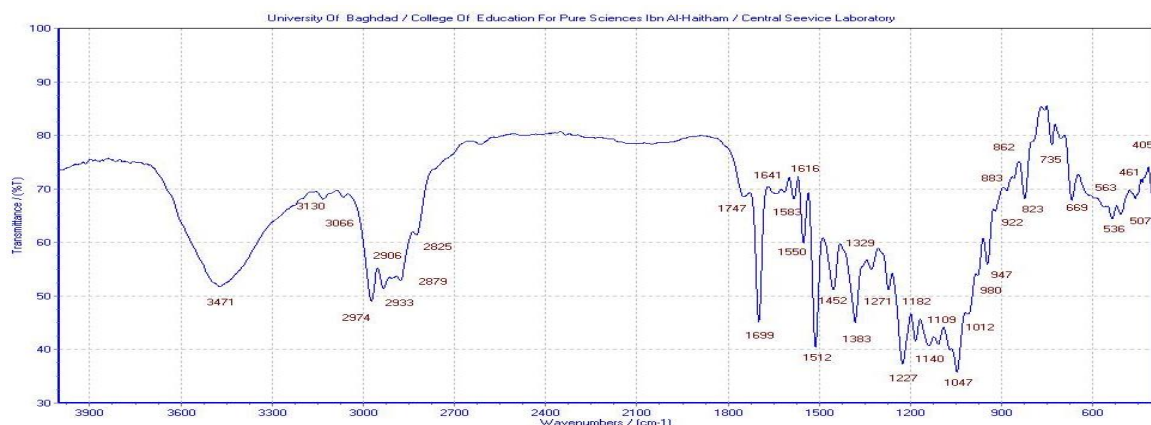


Figure 12. FTIR spectrum of the physical mixture of drug, EC and HPMC 15cps.

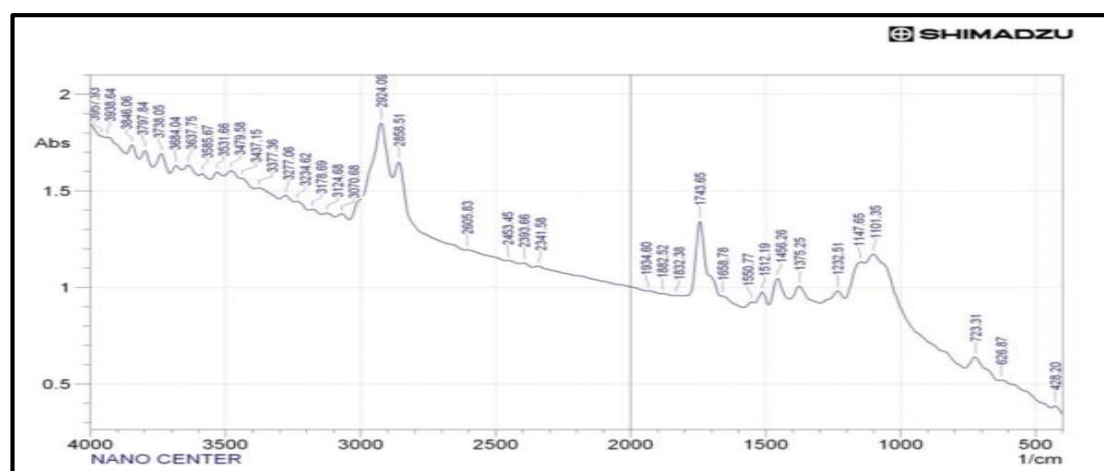


Figure13.FTIR spectrum of floating microparticles (selected formula F16)

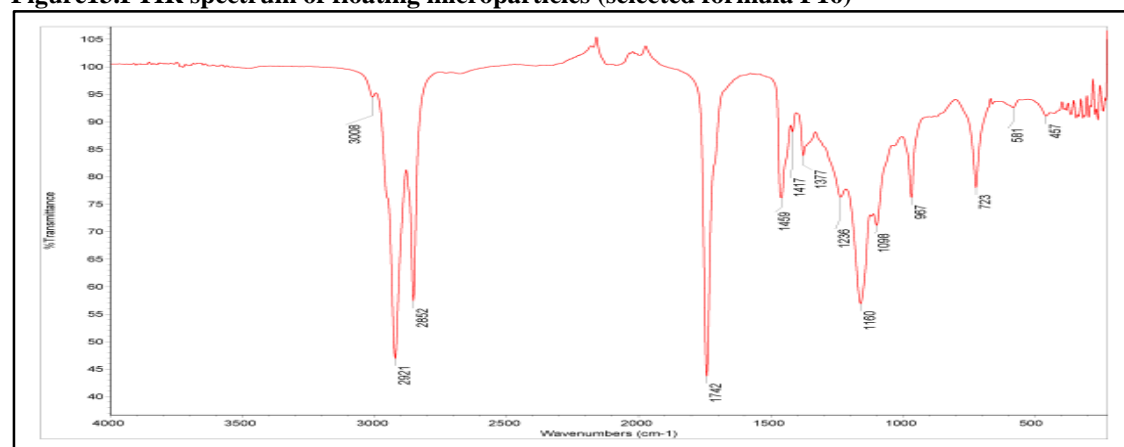


Figure 14. FTIR spectrum of safflower oil. (27)

Powder X-ray diffraction (PXRD)

XRD test of the pure drug and the selected formula (F16) was done to detect the polymorphic structure of the drug before and after formulation. Generally, the amorphous mater sate has higher physical stability. Crystalline nature of pure ITZ easily detected

through the numerous distinctive peaks as shown in figure 15 (27). The XRD of F7 and F16 showed disappearance of the characteristic peaks of pure ITZ as shown in figure 16 and 17 indicated that ITZ incorporated into the polymer matrix that was in the amorphous state (30).

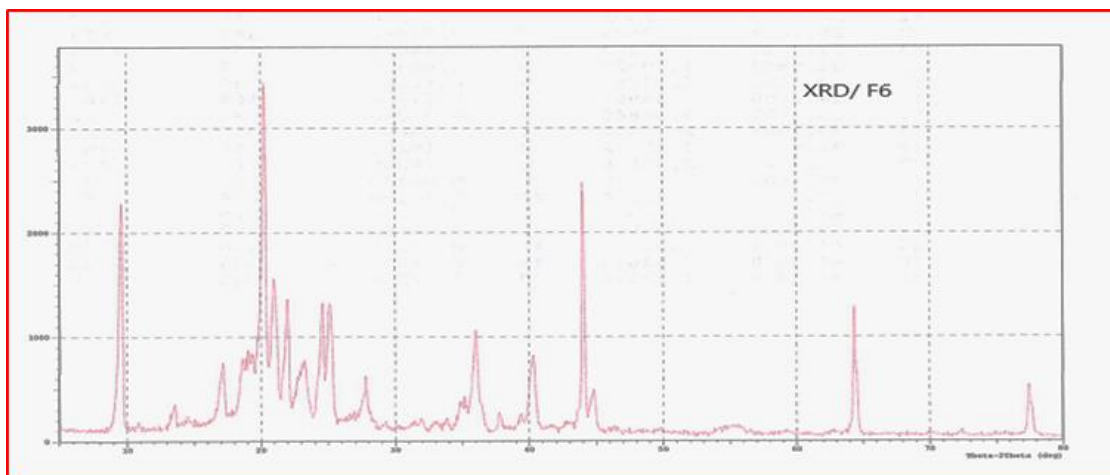


Figure 15. XRD of pure ITZ.

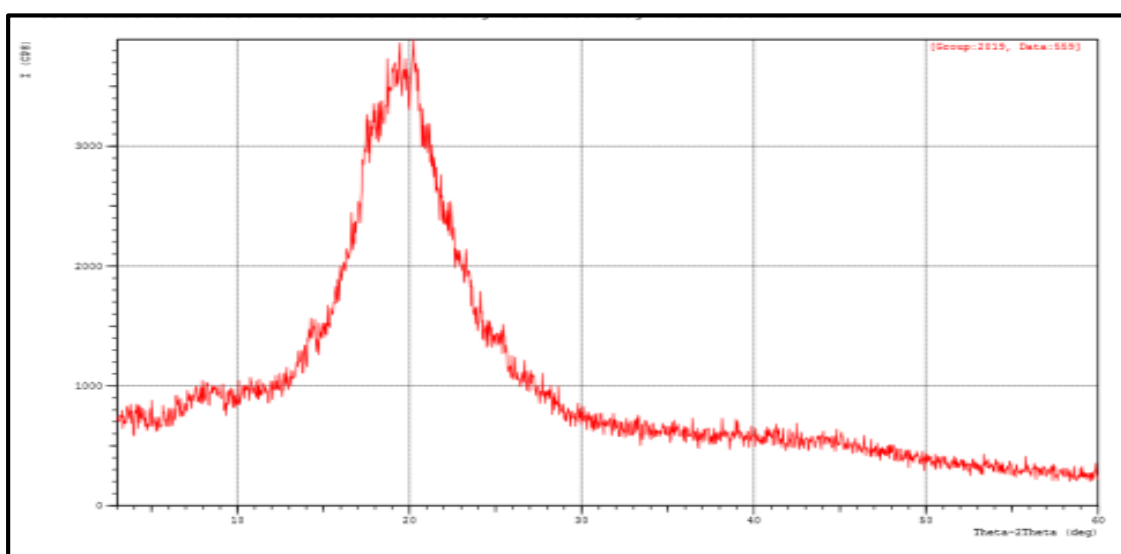


Figure 16.XRD of ITZ selected formula (F16)

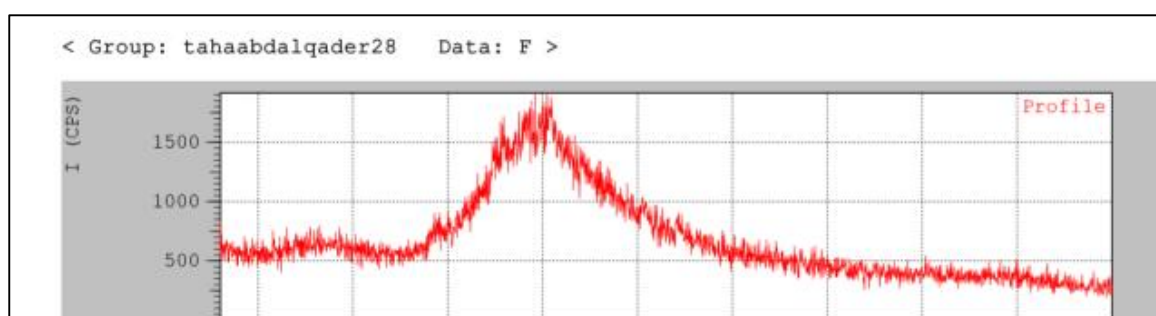


Figure 17. XRD of ITZ best pH-dependent formula (F7)

Conclusion

Dual coating solvent evaporation method was successfully used to produce relatively pH-independent ITZ floating microparticles by using the pH-independent water-soluble polymer (HPMC15 cps) that provided relatively pH-independent solubility of ITZ with a hydrophobic polymer (EC) and safflower oil that provided buoyancy.

Different parameters, such as HPMC 15cps versus EC concentration, various vegetable oil including, safflower oil volume, stirring speed, oil to water ratio, co solvent type, drug- to - polymer ratio were investigated and optimized to produce a formula that provided both excellent buoyancy for 12 hours with maximum drug release. PH-independent ITZ floating

microparticles (F16) was significantly better than

PH-dependent ITZ floating microparticles (F7) in both bouncy per cent at 12 hours and dissolution profile at all test media of different pH values ($p < .05$), therefore, Dual coating solvent evaporation method is a promising method to improve pH-dependent and solubility problems of ITZ.

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