Preparation and Evaluation of Oral Microsponge Drug Delivery System of Ketoconazole

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Abstract:

In oral applications, the microsponge system has been shown to increase the rate of solubilization of poorly water-soluble drugs by entrapping such drugs in the microsponge system's pores.Because these pores are very small, the drug is in effect reduced to microscopic particles and increased surface area thus greatly increases the rate of solubilization.

This investigation was done to increase solubility and then bioavialibility of poorly soluble ketoconazole (BCS class II drug) by preparing it as microsponges by quasi emulsion solvent technique using different types of Eudragits as Eudragit E 100, Eudragit RS or Eudragi tRL. Physicochemical interaction between drug and excipients as individual one, physical mixture and prepared microsponge has been evaluated using Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) which indicates the absence of interaction between them. The1: 0.1 ratio drug: Eudragit E 100 at stirring rate of 2000 rpm (F1b) was found to give the best production yield of 80.46±2.41, entrapment efficiency of 70.84%±1.71, and smallest particle size of 57.42±2.26 µm. Scanning electron microscopy of F1b at magnification of 1000x and 50000x shows the presence of drug as nanocrystals inside microsponge. All prepared formulas shows good flow properties indicated the possibility of capsule formulation which show significant ($P<0.05$) high drug release percent in 0.1 HCl compared with traditional Nizoral® oral tablet (83.15%±1.10) but Eudragit E (F1b) shows the best drug release percent of 100±0.91 at 20 minutes. Finally, the overall obtained data revealed the feasibility of preparing fast release ketaconazole as microsponges in oral capsules dosage form.

Key words: *micosponag, ketocanazol, and quasi emulsion solvent technique.*

ألخلاصة :

في التطبيقات عن طريق الفم، تبين ان نظام الاسفنجات الصغيرة (microsponges) تؤدي الى زيادة ذوبان المواد قليلة الذوبان بواسطة تغلغلها في مسامتها الصغيرة جدا لذلك يخفض العقار الى الجزيئات المجهرية وبالتالي زيادة المساحة السطحية. ان الكتوكانازول هو من المواد ضعيفة الذوبان (BCS class II) لذلك تم تحضيرها على شكل اسفنجات صغيرة بواسطة تقنية شبه المستحلب باستخدام انواع مختلفة من الايدروجيت مثلا الايدروجيت E100 والايدروجيت RS والايدروجيت RL يؤدي الى زيادة ذوبان الكتوكانازول ومن ثم زيادة التوافر الحيوي، وقد تم تقييم التفاعل الفيزياوي بين الكتوكانازول والمواد المضافة مرة بصورة مفردة واخرى كمزيج فيزيائي بالاضافة الى الاسفنجات الصغيرة المحضرة باستخدام التحليل الطيفي بالاشعة تحت الحمراء (FTIR) وفرق المسح الكلوري (DSC) الذي يشير الى غياب التفاعل. ان نسبة 10:1 (الكتوكانازول : لايدروجيتE100) بسرعة تحضر 2000 دورة بالدقيقة (F1B) اعطت افضل محصول انتاج وبمعدل 80,46%±2,41 وانحباس كفاءة بمعدل 70.84 ±1.71 وحجم الجزيئات الصغيرة هو 57.42 ± 2.26 ميكرون. فضلاً عن ان المجهر الإلكتروني (F1B) في التكبير 1000 و 50000 مرة بين ان وجود الكتوكانازول بشكل بلورات نانوية داخل microsponges. كل الصيغ المعدة تظهر أشارت خصائص تدفق جيد في إمكانية صياغة كبسولة التي تظهرنسبة ارتفاع كبيرة ($P < 0.05$) لتحرير الكتوكانازول في حمض الهيدروكلوريك 0.1 مقارنة مع قرص نيزورال عن طريق الفم ® التقليدية (83.15 ٪ ± 1.10) ولكن F1B (Eudragit E100) يبين أفضل نسبة تحرير عن الكتوكانازول 100% ± 0.91 في 20 دقيقة. وأخيرا، كشفت البيانات التي تم الحصول عليها عموما جدوى إعداد الكتوكانازول بشكل كبسولات تحوي اسفنجات صغيرة محملة بالكتوكانازول عن طريق الفم سريعة التحلل.

Introduction:

Microsponges are porous, polymeric microspheres that designed to deliver a pharmaceutically active ingredient efficiently at minimum dose and also to enhance stability, reduce side effects, and modify drug release profiles[1]. This technique is being used for topical formulation and also for oral administration[2].

Microsponge systems can enhance the rate of dissolution of water-insoluble drugs. In oral applications, the microsponge system has been shown to increase the rate of solubilization of poorly water-soluble drugs by entrapping such drugs in the microsponge system's pores. Because these pores are very small, the drug is in effect reduced to microscopic particles and the significantly increased surface area thus greatly increases the rate of solubilization. An added benefit is that the time it takes the microsponge system to traverse the small and large intestine is significantly increased thus maximizing the amount of drug that is absorbed. Microsponges has capacity to adsorb or load high degree of active ingredients into the particles or onto the surface[3].

Microsponges were prepared by liquid-liquid suspension polymerization or Quasi-emulsion solvent diffusion method. Application of microsponge involve: solubility enhancement, site specific action, increase stability of drug, controlled release drug delivery, topical drug delivery, oral drug delivery, and bone tissue enginereering[4,5].

Ketoconazole (KTZ) is an imidazole agent which interference with the synthesis of ergosterol and therefore alters the permeability of the cell membrane of sensitive fungi [6]. It is a broad-spectrum antimycotic, which is administered topically or by mouth; it is practically insoluble in water[7].

Ketoconazole is classified in the Biopharmaceutics Classification Scheme as a class II drug, since it has a high permeability, but a solubility in aqueous media which is insufficient for the whole dose to be dissolved in the gastro-intestinal fluids under normal conditions [8].

Materials and Methods:

Ketocanazole, Eudragit E 100, Eudragit RS and Eudragit LS (Röhm, GmbH,Weiterstdt, Germany), PVA and Mg stearate (Sammara Drug Industries , Iraq), Hydrochloric acid (Gainland Chemical Company UK), dichlormethane (Sharlau, Barcelona- Spain).

Experimental Design Preparation of Microsponges:

The microsponges containing KTZ were prepared by quasi emulsion solvent diffusion method [9]. using different polymers and different drug-polymer ratio as shown in Table-1. The inner phase, EudragitE 100, Eudragit RS or EudragitRL was dissolved in 5 ml of dichloromethane under ultrasonication until a clear solution was obtained. A predermined weight of KTZ was added to the inner phase under sonication at 37^0C for 5 min. A 50 mg of PVA was added of distilled water and heated to 90^0C to get clear solution and then cooled to room temperature. One ml of glycerin was added to PVA. The KTZ - EudragitE 100, Eudragit RS or Eudragit RL was added stepwise to PVA solution. The resultant mixture was stirred at different speed for 60 min, and filtered to separate the microsponges. The microsponges were dried in an air heated oven at 40^oC for 12 hr. and stored for subsequent investigation.

Effect of drug: polymer ratio:

The ratio of ketoconazole to Eudragit E100 at 1: 0.1, 1: 0.125, and 1: 0.2 as denoted by F1-F3 were prepared to study the effect of drug: polymer ratio.

Effect of Eudragits type:

Eudragit E100, Eudragit RS 100, and Eudragit RL for F1, F4, and F5 were used to study the effect of polymer types on microsponage formulation.

Effect of stirring rate:

Formula F1 was selected to study the effect of stirring rate on the size of microsponges at 1000 rpm, 2000 rpm, and 3000 rpm for F 1a, F1b, and F1c respectively.

Production yield and entrapment efficiency:

Percentage yield was calculated using the following equation[10].

Percentage yield = (Final obtained mass of microsponges / initial mass of polymer and drug) × 100

The entrapment efficiency [9] of the microsponges was computed using the equation:

Entrapment Efficiency (%) = (Actual drug content / Theoretical drug content) × 100.

Flowbility measurement of microsponge formulas:

The angle of repose was measured for estimating the flow properties of microsponge formulas. It was determined by calculating tan θ from the height and the radius of the cone formed by the microsponge particles as they flowed out of an orifice of a funnel and subsequently obtaining the inverse of tan θ.

In Vitro Drug Release Studies of Microsponge Formulations:

In vitro drug release studies were carried out according to United States Pharmacopeia (USP) apparatus (basket) keeping stirring rate at 50 rpm and temperature at 37 ± 0.5°C. Initially drug release from micorsponge formulations and pure drug filled in soft gelatin capsules were carried out in 900 mL of 0.1N HCl (pH 1.2) for 1 hr. Samples were withdrawn after regular predicated intervals of time, and drug amount was determined using UV spectrophotometry at 270 nm. Dissolution tests were performed in triplicate for each sample.

Statistical analysis:

The results of the experiments are given as a mean of triplicate samples ± standard deviation and were analyzed according to T-test at the level of ($P< 0.05$).

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Physicochemical characterization Scanning electron microscopy:

The surface topography of microsponge was examined using scanning electron microscopy (Hitachi S-4500 SEM, Japan). The prepared microsponges were coated with platinum prior to examination and scanned under vacuum at room temperature [5].

Thermoanalytical methods:

Thermal analysis using differential scanning calorimetry[11] (*Shimadzu-DSC* – TA 60) was carried out for the pure drug, excipients, drug-excipients physical mixture in ratio of ketoconazole: Eudragit E100:PVA : to confirm compatibility. DSC was also performed for the best microsponge formula to ensure that the formulation process does not effect on crystallinity of the drug. Samples (approximately 2 mg) were placed in aluminum pans, sealed and scaned at a heating rate of 10°C/min over a temperature range 40 to 300°C.

Fourier transform infrared spectroscopy (FTIR):

To indicate that no chemical interaction[5] or changes took place during the preparation of the formulations. Fourier transform infrared spectroscopy (Shimadzu, Japan) was carried out for the pure drug, excipients and the drug-excipient physical mixture and the best microsponge formula. The samples were incorporated in potassium bromide discs and evaluated using FTIR spectrometer.

Particle size analysis:

Particle size and size distribution studies of microsponge particles[5] were done by using particle size analyzer (Mastersizer 2000, Version 2.0, Malvern Instruments Ltd, UK). The results are the average of three experiments.

Result and Discussion:

As the drug: polymer ratio decreased as demonstrated in table-2, the particle size decreased. This may be explained by fact that as drug-polymer ratio decreased, the amount of polymer available for microsponges becomes more, thus increase thickness of polymer wall and hence larger microsponges[12].

This results fit with the result obtained by Vikas Jain et al, for preparation and characterization of dicyclomine-loaded Eudragit®-based microsponge with potential for colonic delivery[5]. Figure-1, in which Eudragit E100 was used, indicated higher production yield (80.46±2.41) than formulas in which Eudragit RS and Eudragit RL were used (77.86±1.89 and 78.52±2.51 for F4 & F5 respectively), the same thing with entrapment efficiency but decrease in particle size as demonstrated in Table-3. In addition to that, as stirring rate increase, particle size decreases demonstrated by table-4. The smaller microsponges size obtained at higher stirring rate and this may be attributed to better dispersion at higher stirring rates[4]. This result is similar to that obtained by Orlu for design and evaluation of colon specific drug delivery system containing flubiprofen microsponges [4].

As stirring rate increase, production yield decrease due to turbulence created within external phase to which polymer adhered to the paddle [12]. The flowability of a powder is of critical importance in the production of pharmaceutical dosage forms in order to get a uniform feed as well as reproducible filling of capsules, otherwise, high dose variations will occur [13].

The angle of repose (Θ) is a characteristic of the internal friction or cohesion of the particles, the value of the angle of repose will be high if the powder is cohesive and low if the powder is non-cohesive [14]. Values less than 35 indicate good flow whereas greater than 35 indicate poor flow[15]. So, the formulated microsponges had good fowability, value of angle of repose at 27-28 (Figure-1). DSC is used to detect possible interactions between a drug entity and the excipients in its formulation to ensure the success of the subsequent stability studies. ketocanazole shows sharp endothermic peak at around 152^{o}C. Figure-2 corresponding to its melting point as prescribes in references. This peak sill present in its microsponage formulation Figure-3, which certified that drug maintains its crystal form; in addition to that, this conclusion was support by scanning electron microscopy analysis that indicated presence of ketocanazole in nanocrystals inside microsponge figures-5.

This results is agreement to that obtained by Mahajan et al. in production of indomethacin microsponges[16].

Figure-4 demonstrated that ketocanazol melting point present in both physical mixtures as well as in microsponage formulation which indicated absence of interaction between drug and used excipients. FTIR also excluded possible interaction between drug and the used additives since the characteristic peak of ketocanazol at 1,643, 813 and 1,285 cm^{-1} due to C=O, -Cl and aromatic groups respectively [17-18] still present in all samples analysis as indicated in Figures (6-8).

Conclusion:

The present study demonstrates that the KTZ microsponges capsulea confirm as a promising fast release oral dosage form since it significantly ($P<0.05$) increased drug release as well as providing excellent evaluations.

In release study, percent of all prepared ketocanazole microsonges release were significantly ($P<0.05$) increase in compares with Nizoral ® tablet figure-9. since drug present in nanoscalein pores and the significantly increased surface area thus greatly increases the rate of solubilization[3]. Also, as stirring rate of ketocanazole microsonges preparation increase, time to reach complete drug release would be decreased as result of particle size reduction [2] (Figure-1a and Figure-1b).

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Table-1: Composition of Microsponage Loaded with Ketocanazol.

Constituents	F1*	F2	F3	F4	F5
Ketoconazole (gm)	1	1	1	1	1
Eudragit E 100 (gm)	0.1	0.125	0.2		
Eudragit RS 100 (gm)				0.1	
Eudragit RL 100(gm)					0.1
Dichloromethane (ml)	5	5	5	5	5
Glycerin (ml)	1	1	1	1	1
PVA (gm)	50	50	50	50	50
Distilled Water (ml)	200	200	200	200	200

*prepared at different stirring speed (1000, 2000 and 3000 rpm).

Tabel-2: Effect of Drug: Polymer ratio

Formulation Code	Drug: Polymer Ration	Production yield (% ±SD)	Entrapment efficiency(%±SD)	Mean particle size (µm ± SD)
F3	1: 0.1	58.23±1.89	51.23±2.11	120.41±8.13
F2	1: 0.125	70.24±2.13	60.72±2.41	70.85±4.24
F1	1: 0.2	80.46±2.41	70.84±1.71	57.42±2.26

Table-3: Effect of Polymer Type

Formulation Code	Polymer (Eudragit)	Production yield (% ±SD)	Entrapment efficiency (% ±SD)	Mean particle size (µm ± SD
F4	RS	78.52±2.51	73.86±1.85	60.62±3.11
F5	RL	77.86±1.89	69.31±2.11	61.23±2.86
F1b	E	80.46±2.41	70.84±1.71	57.42±2.26

Table- 4: Effect of Stirring Rate on KTZ Microsponges

Formulation	Stirring rate (rpm)	Production yield (% ±SD)	Entrapment efficiency(% ±SD	Mean particle size (µm ± SD
F1a	1000	93.21±3.11	62.13±1.84	68.21±2.81
F1b	2000	80.46±2.87	70.84±1.71	57.42±2.97
F1c	3000	60.62±2.41	71.62±1.71	50.13±2.26

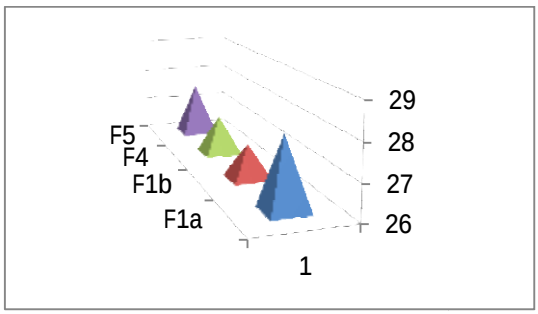

Figure 1: Angle of Repose of Formulated Microsponges

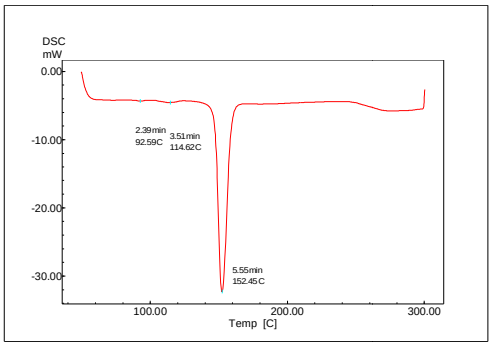

Figure -2: Thermogram of ketoconazole

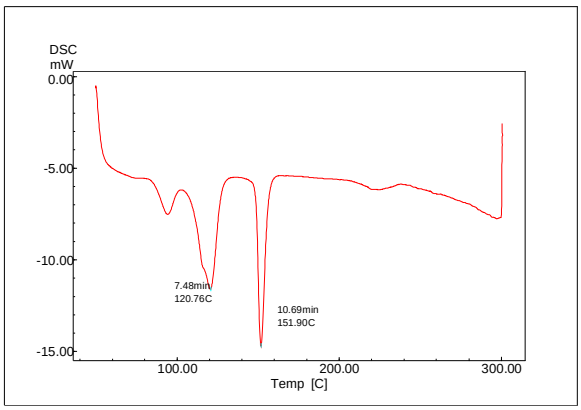

Figure-3: Thermogram of ketocanazole microsponage.

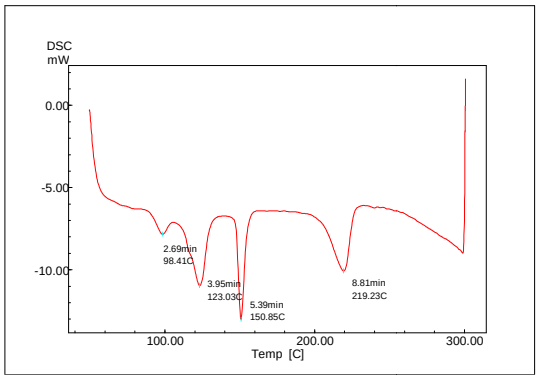

Figure- 4: Thermogram of physical mixture of ketoconazole microsponges components.

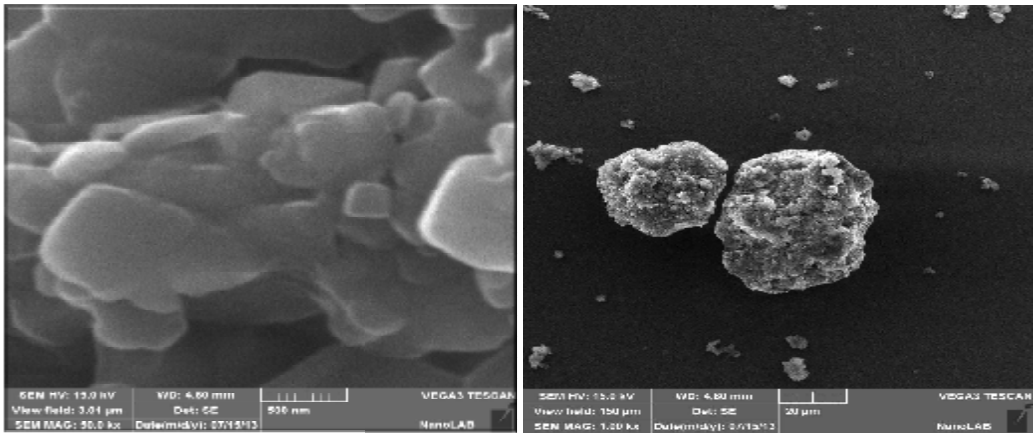

Figure- 5: scanning electron

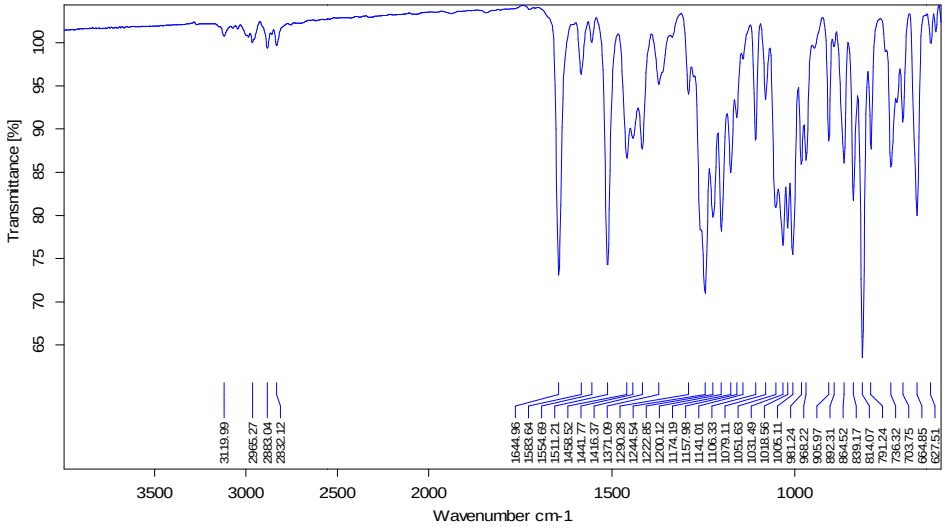

Figure (6): FTIR spectrum of ketocanazole.

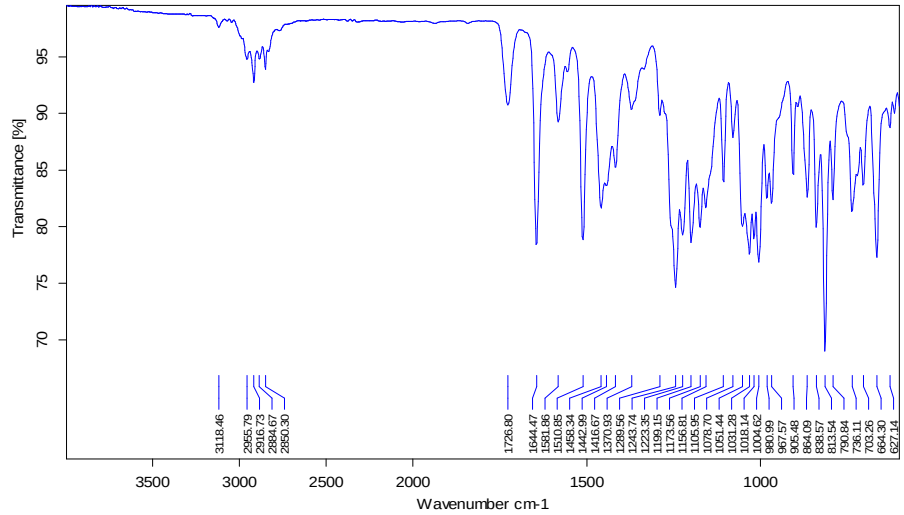

Figure- 7:FTIR spectrum of ketocanazolemicrosponage

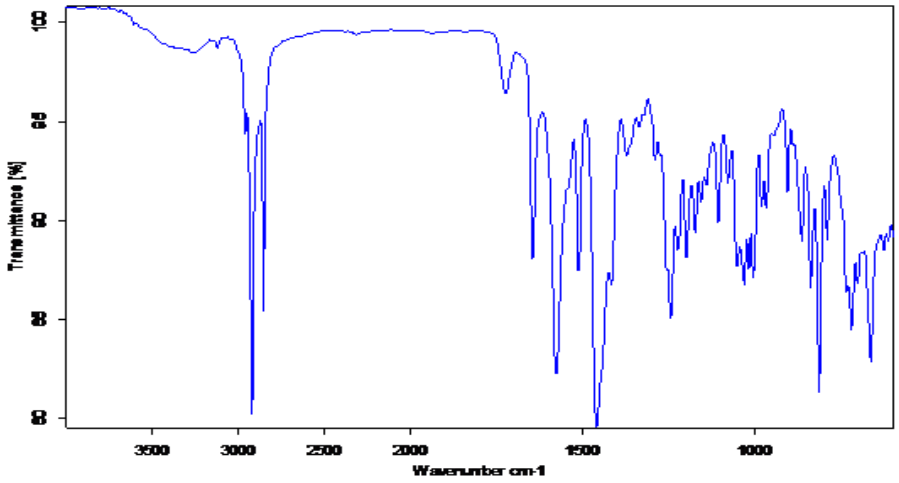

Figure- 8: FTIR spectrum of physical mixture of ketocanazole microspoages.

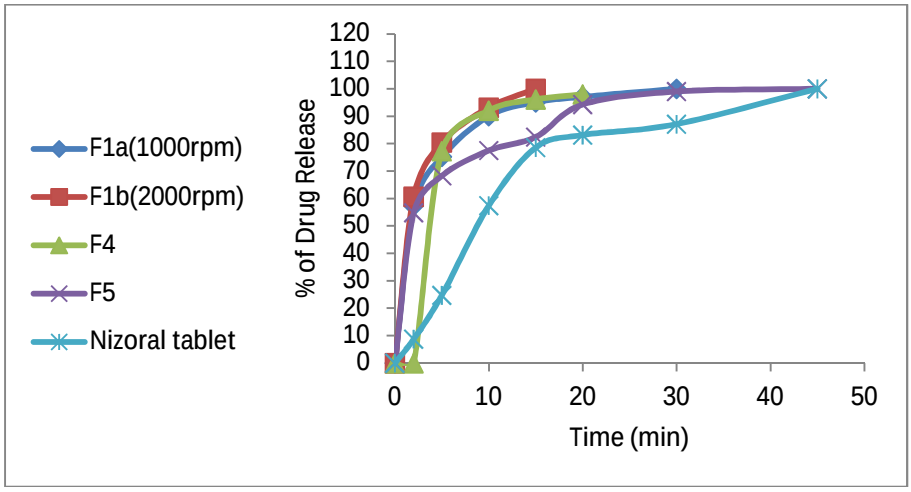

Figure- 9: Release profile of the prepared microsponges formulas in 0.1N HCl at 37 ^{o}C.