Formulation and evaluation of clarithromycim oral microspong

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ABSTRACT

Clarithromycin is a broad-spectrum antibiotic and extensively absorbed orally. It is be used in the eradication of H. Pylori infection combined with an acid suppressing agent. The purpose of this study was to develop controlled release clarithromycin floating micro sponges for gastro retentive drug delivery. The micro sponge's formulations were prepared by quasi-emulsion solvent diffusion method employing eudragit RS 100 as a polymer and poly vinyl alcohol (PVA) as a surfactant. The compatibility of the drug with formulation components was established by Fourier Transform Infra-Red (FTIR) spectroscopy. The prepared microsponge were evaluated for angle of repose, Carr's Index ,particle size, floating time production yield, drug loading efficiency of micro sponges. Shape and surface morphology of the micro sponges were been examined using scanning electron microscopy. formulation F5 with drug to polymer ratio 8:1,10 ml of internal phase volume (ethanol: dichloromethane) and 0.25%pva solution was the best formula showing the highest degree of sustained release that is 79.59% at the end of 12 hours with floating time for 12 hr. Eudragit RS 100 could control drug release in stomach.

تحضير وتقييم عقار الكلارثرومايسين المايكرو سفنجي الفموي

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مفتاح الكلمات: المايكرو اسفنجي، كلارثرومايسين، طريقة انتشار المذيبات الشبه مستحلبه

الخلاصة

الكلارثرومايسن هو مضاد حيوي مايكرولايد مع طيف واسع من النشاط، يستخدم لعلاج التهابات الجهاز التنفسي والجلد والتهاب الأنسجة الرخوة. كما استخدم العقار للقضاء على البكتريا في نظم علاج امراض القرحة المعوية. وكان الغرض من هذا البحث هو تحضير كبسول مايكرو اسفنجيه عائم من مضاد الكلارثرومايسين من □ل تمديد تحرر الدواء بواسطة استخدام نوعين من البوليمرات يودر□يت RS100و .RL100. تم تحضير العديد من □يغ الكلارثرومايسين المايكرو اسفنجي بطريقه انتشار المذيب في شبه المستحلب بواسطة استعمال اليودر□يت بوليمر (RS100و (RL100 المبطئ للتحرر، واستخدام البوليفنيل الكحول كمثبت. ايضا تمت دراسة تأثير نوع المذيب ,تركيز المثبت PVA ,حجم المذيب في الوسط الداخلي ,سرعه الدوران وتركيز نسبه الدواء الى البوليمير. تم تقييم التركيبات لخصائص التدفق، توافق الدواء مع باقي مكونات التركيبة بواسطه (DSCو FTIR) ،الطفو ,حجم الجزيئات وتحرر المادة الفعالة .اظهرت النتائج ان افضل □يغه هي التي حضرت بتركيز نسبه الدواء الى البوليمر (8:1) ,10 مل من حجم المذيب (الايثانول: الدايكلورميثان (1:1)) والمثبت بتركيز 0.25 % من PVA . الصيغة رقم 5 اظهرت أفضل تدفق وطوفان الكلارثرومايسين المايكرو اسفنجي لمده 12 ساعة. اظهر تحرر الكلارثرومايسين المايكرو سفنجي العائم تمديد وبطيء تحرر الدواء حيث انه عند زيادة تركيز البوليمر سوف تودي الى قله تحرر الدواء.

1. INTRODUCTION

Microsponge are polymeric drug delivery systems composed of porous microspheres. They are tiny, sponge like spherical particles that consist of a myriad of interconnecting voids within a non-collapsible structure with a large porous surface. Moreover, they enhance stability, reduce side effects and modify drug release. [1] Clarithromycin (CLR) is a macrolide antibiotic with broad spectrum of activity. It is be given in the treatment of respiratory tract infections and in the skin and soft tissue infections. CLR may been given to eradicate *H. pylori* in treatment regimens for peptic ulcer diseases. CLR is rapidly absorbed from the gastrointestinal tract and undergoes first pass metabolism. The bioavailability of the drug is about 55%. It is be given in the doses of 250 mg and 500 mg as tablets and suspension. The terminal half-life of CLR is reportedly about 3-4 hours. Thus, CLR has all the requisites of gastro retentive drug delivery system.

Eudragit polymers have an ability to resist the acid environment of stomach and retain there for prolonged period. Hence, an attempt was be made to develop floating micros pong of CLR using Eudragit polymers with an aim to retain the micro sponges in the stomach for prolonged period. [2]

2. MATERIALS AND METHODS

Materials:

Clarithromycin (gifted by Samarra Drug industries (SDI), Iraq), Evonik Degussa Ltd., India provided Eudragit RS 100 and Eudragit RL 100 as gift samples. All other chemicals used were of analytical grade.

Methods:

Clarithromycin floating microsponges were prepared by quasi- emulsion solvent diffusion method. The internal phase was eudragit RS-100 dissolved in 10 ml mixture of Dichloromethane and Ethanol (1:1) at room temperature. This was, followed by addition of drug with sonication for 15min. The internal phase was then poured into polyvinyl alcohol (PVA) solution in water, the external phase with stirring, and the micro sponges then washed and filtered, then dried at 40°C for 12 hours. [3]

The composition of the prepared formulation is shown in table (1).

Table1: Composition of all the formulations (Batch F1 – Batch F11)

Formulation cod	Drug: polymer ratio	ethanol:dichloro-methame volume	PVA (%w/v)	water (ml)
F1	1:1	10	0.25	200
F2	2:1	10	0.25	200
F3	4:1	10	0.25	200
F4	6:1	10	0.25	200
F5	8:1	10	0.25	200
F6	10:1	10	0.25	200
F7	12:1	10	0.25	200
F8	2:1	10	0.5	200
F9	2:1	10	0.75	200

F10	2:1	5	0.25	200
F11	2:1	15	0.25	200

Note: Formulations 8to 11 is prepared for variables study (effect of solvent and emulsifying agent on production yield, particle size and encapsulation efficiency. F5 is optimized formulation, which was be used for variables study.

Microsponge Characterization:

Morphological examination : The morphology and surface characterization of the micro sponge formulation were evaluated by scanning electron microscope (SEM) analysis using JSM 840A SEM analyzer after the sample had been gold sputtered coated with 25nm gold film thickness .[4]

Particle Size Analysis.

The particle size and size distribution of the prepared microsponges was determined by using optical microscopy method .approximately 200- 300 microsponge were counted for particle size using a calibrated optical microscope (Olympus Pvt. Ltd., India) .[5]

Drug-excipients compatibility studies

Fourier transform infrared (FTIR) spectroscopic

Drug-excipient interaction is one of the most important compatibility studies, FTIR study used for this purpose on samples of pure clarithromycin and blend powder of selected formula. Spectra obtained by using (Shimadzu 8300, Japan) according to KBr disk method. About 2-3 mg sample were mixed with dried IR grade potassium bromide powder and the spectra were in between the wave number range of 4000-400 cm^{-1}

Production yield and loading efficiency

Microsponge's equivalent to 100 mg of CLR were dissolve and made up to the mark in 100 volumetric flask with 0.1 N HCL. The absorbance was measured spectrophotometriclly at 275 nm. Then Percentage yield can calculated using the following equation:

Production yield (PY) = (Final obtained mass of microsponge / initial mass of polymer and drug) × 100. ---------eq. (1)

The drug loading efficiency of the microsponge can computed using the following equation:

Loading Efficiency (LE %) = (Actual drug content / Theoretical drug content) × 100 --- eq. (2). [6]

Determination the Flow ability of powder

A. Bulk density and tapped density.

Both loose bulk density (LBD) and Tapped bulk density were determined. Powder was be taken in a 10ml measuring cylinder and initial volume was noted and tapped at height of 2.5cm at 2-second intervals until no further change in volume was noted. LBD and TBD calculated using the following formula.

LBD = Weight of the powder/volume of the packing

TBD= Weight of the powder/Tapped volume of the packing. [7]

B. determination of Carr's Index:

The compressibility index of the powder determined by the Carr's Compressibility index

Carr's index (%) = (TBD-LBD) x100/TBD. [8]

C. Determination of the Angle of Repose

Angle of repose was measured for the microsponge powder, to observe the flow properties of powders. Funnel method was be used; the powder were allowed to pass through a funnel and poured onto a horizontal plane, fixed base diameter (D), free of vibration petri dish to

form a cone. The funnel height was maintained at approximately 2-4 cm from the tip of the powder pile in order to minimize the impact of the falling powder on the tip of the cone. The tan of angle of repose (θ) was calculated after measuring the height (H) of the cone of the powder utilizing equation:

$$\text{Tan } \Theta = h/r \quad \ldots \quad \text{equation (3)}$$

The accepted limit of good flow properties is (20-30). [9]

Optimization of formulation parameters and process factors: The effect of drug: polymer ratio, concentration of emulsifying agent and volume of solvent was determined and show effect on particle size, production yield and drug loading efficiency

In Vitro Buoyancy Study:

Buoyancy test was determined by using USP type II apparatus at 50 rpm maintained at 37±0.5°C. The capsules were be placed in 900 ml jar containing 0.1N HCl as dissolution medium. The time required the capsule to rise to the surface and float was determined as floating lag time, while the time during which the capsules remained buoyant was the floating time. [10]

In Vitro Dissolution Studies:

The release rate of Clarithromycin from floating micros pong capsules (n=3) was determined using USP dissolution test apparatus Type II (paddle method). The dissolution test was carried out by using 900 ml of 0.1NHCl at 50 rpm. The temperature of the medium maintained at 37±0.5ºC and the study carried out for 12 hrs. Samples of 5 ml were be withdrawn at an interval of every hour; the withdrawn samples were replaced with fresh dissolution medium. The samples filtered through Whatman filter paper, and then analyzed spectrophotometrically at 275 nm. [11]

3. RESULT AND DISCUSSION:

Preparation of Microsponges:

The clarithromycin floating microsponge was prepared by quasi-emulsion solvent diffusion method. This method found to be very easy, reproducible, rapid and avoid solvent toxicity. [12]

In quasi-emulsion solvent diffusion method, the formation of microsponge described in the following processes: the formation of quasi-emulsion droplets, the diffusion of (ethanol and dichloromethane) and the solidification of the droplets. The rapid diffusion of ethanol and dichloromethane (good solvent for the polymer and drug) into the aqueous medium might reduce the solubility of the polymer in the droplets, since the polymer was insoluble in the water. The instant mixing of the (ethanol: dichloromethane) and water at the interface of the droplets induced precipitation of the polymer, thus forming the shell enclosing the ethanol: dichloromethane and the dissolved drug. Counter diffusions of ethanol: dichloromethane and water through the shell promoted further crystallization of the drug in the droplets from the surface inwards. The dispersed droplets of polymer solution of drug solidified in the aqueous phase via diffusion of solvent. [13, 14]

Shape & surface morphology:

SEM (Figure 1) showed the shape of microsponges prepared by quasi-emulsion solvent diffusion method. It was observed that the micro sponges were spherical, and uniform with no drug crystals on surface. The surface morphology reveals that the microsponges were porous due to the rapid escape of the volatile solvents during formulation. Inward dents were seen on the surface probably due to collapse of the walls of the micro sponges during the in situ drying process. [15]

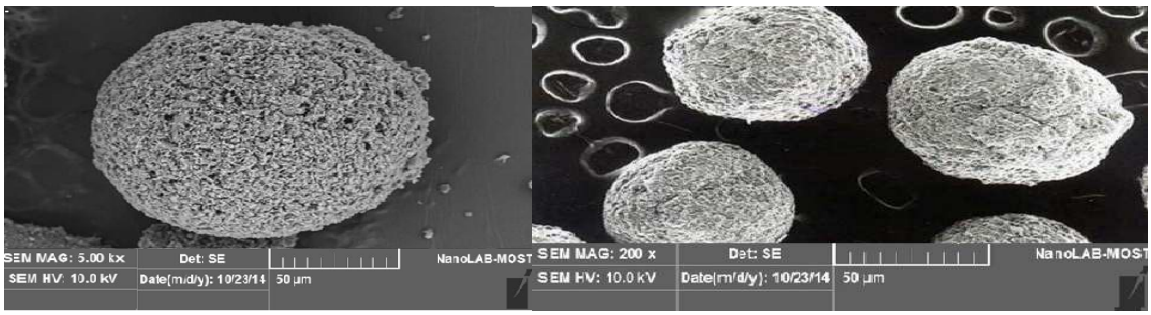

Fig. 1: Scanning electron microscope photo of microsponge formulation (F5).

Particle size: The microsponges found to be uniform in size**.** Particle size of prepared microsponges was in the range of 77 ± 12.5 to 35.5 ± 12.5 (Table 2**)** the sizes of microsponges affect the encapsulation efficiency and the release rate of the drug. It observed that as the ratio of drug to polymer was increased, the particle size decreased. This could probably be due to the fact that in high drug to polymer ratio, the amount of polymer available per microsponge was comparatively less. Probably in high drug-polymer ratios, less polymer amounts surround the drug were obtained which reduce the thickness of polymer wall and microsponges with smaller size.
We have studied the effect of concentration of poly vinyl alcohol (PVA) on size of microsponges for optimized formulation. The selected concentration of PVA was 0.25% since 0.5 % of PVA (F8) the particle size increased from 50 ± 10.3 µm to 67± 12.5 µm. Further increasing the concentration to 0.75 % of PVA (F9) the particle size increased to 75± 15 µm. The dispersion of the solution of the drug and polymer into droplets affected by the concentration of polyvinyl alcohol in the external phase. When the concentration of PVA increased, the size of microsponges increased which may be explained to be due to the increased viscosity wherein larger emulsion droplets formed resulting in larger microsponges.[16]

In addition it was found that increasing the solvent volume from 10 ml (F2) to 15 ml F(11), particle size was decreased from 50 ± 10.5 µm to 38.23 ± 10.4 µm due decrease in viscosity of solvent system ,The reason investigated that the particle size was directly proportional to the apparent viscosity of dispersed phase.[17]

Fourier transform infrared (FTIR)

Fourier transform infrared (FTIR) spectral study was done to find out the chemical stability or interaction of the excipients. In FTIR studies, the prominent peaks in the FTIR spectrum of pure drug CLR (figure2) at 1730.18 for O-C=O stretching vibration in a lactone ring, characteristic peaks of C=O stretching vibration from ketone group in a lactone ring at 1691.03 cm−1 , 3468.85 for Tertiary –N stretching, which are the same as the reported one. Hence, it can be concluded that there was no interaction between drug and excipients, since similar peaks of specific functional groups were be obtained as shown in figure (3). [18]

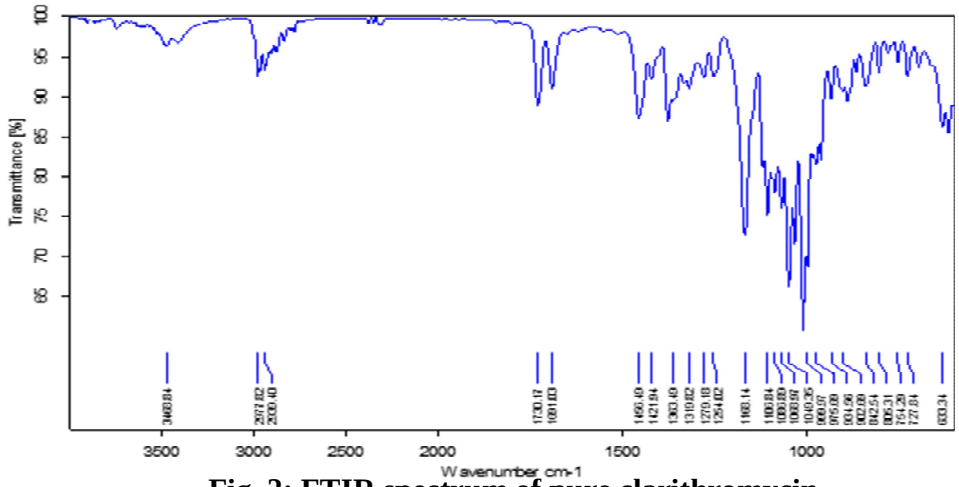

Fig. 2: FTIR spectrum of pure clarithromycin

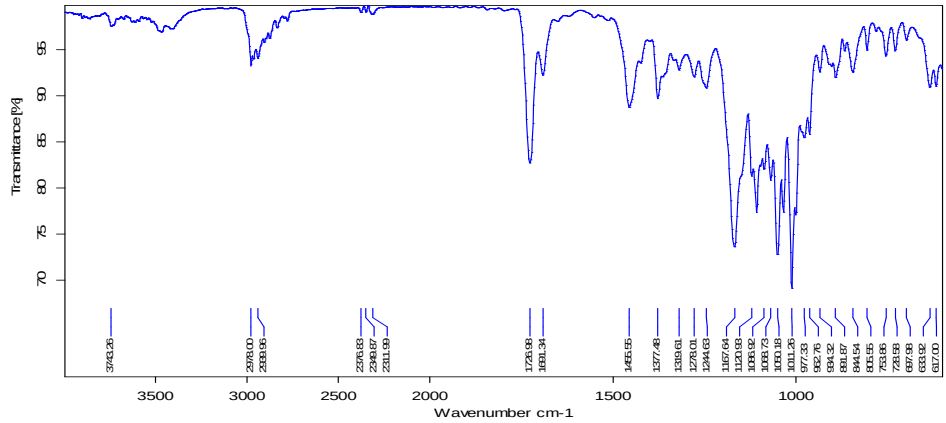

Fig. 3: FTIR spectrum of clarithromycin microsponge

Production yield: The production yield of the prepared microsponges of clarithromycin was in the range of (55.60 ± 1.60 % to 93 ± 1.4 %). The loss of product may be due to the formation of some agglomerates and polymer adherence to the container because of viscous nature of slurry.

Drug loading efficiency: Drug content in different formulations estimated by UV spectrophotometric method. Loading of the drug depends on the successful molecular association of the drug with the polymers. The drug loading efficiency of the micro sponges found in the range of (58.38 ± 0.95 to 92.5 ± 1.60 %). The best drug encapsulation efficiency found for the formulation F5 and F6 with the drug polymer ratio of 8:1 and 10:1 respectively. On further increasing drug polymer ratio, the loading efficiency decreased. This could be due to high concentration of drug molecule in comparison to low concentration of polymer molecules, which decreased the capability of polymer to coat the drug molecule and caused the reduction in encapsulation efficiency.[19]

Table 2: production yield, particle size and drug loading efficiency of Prepared micros pong.

Formulation code	Production Yield (%) (mean ± S.D)	Particle size (µm) (mean ± S.D)	Drug loading efficiency (%) (mean ± S.D
F1	55.60 ± 1.60	77± 12.5	58± 0.95
F2	70.00 ± 1.80	67 ± 10.5	66.2 ± 1.05
F3	75.00 ± 1.50	60 ± 12.5	76.6 ± 0.85
F4	77.50 ± 1.60	55. ± 11.5	80.4 ± 0.92
F5	92.00 ± 1.3	50 ± 10.3	91 .98± 1.25
F6	93.40 ± 1.4	43. ± 11.5	92.5 ± 1.60
F7	84.00 ± 1.80	35.5 ± 12.5	82.4 ± 0.87

Determination of the Angle of Repose:

The results of angle of repose were shown in table (3) .the angle of repose were ranged between 24.34 ±1.91 to 26.70 ± 2.02, which indicates good flow properties of powder. [20]

Determination of Carr's Index:

The result of the carr's index are show in table (3), its indicted that the carr's index of all the formulation were less than 20 from (12.231±0.25 to 13.974±0.27) which indicate good flow properties and good compressibility.[21]

In Vitro Buoyancy Study:

The in vitro floating test showed that the floating lag time observed in case of all formulation was zero. After placing of the clarithromycin capsules in 0.1N HCl acid, the capsules did not reach even in the bulk of the floating medium and they showed to remain at the surface. The capsules also showed a total floating time greater than 12 hours. These results exhibited satisfactory floatable ability because of their low density and internal voids. [22]

Table 3: Balk density, tapped density, angle of repose and Carr's index % of batches (f1 –f7).

Formula no.	Balk density	Tapped density	Angle of repose	Carr's index %	Flow character
F1	0.462±0.0065	0.528±0.0084	24.5±1 12.3	12±0.16	Good
F2	0.463±0.0113	0.537±0.0144	25.8±1.09	12.531±0.25	Good
F3	0.502±0.0077	0.581±0.0102	24.34±1	13.13±0.132	Good
F4	0.450±0.0061	0.538±0.0086	24.72±1.72	13.684±0.27	Good

F5	0.463±0.0113	0.527±0.0144	26.30±1.10	12.231±0.25	Good
F6	0.502±0.0077	0.571±0.0102	26.70±2.02	13.732±0.19	Good
F7	0.614±0.0124	0.714±0.0167	25.10±1.12	13.974±0.27	Good

In-vitro drug release studies:

In-vitro drug release studies was carried out in 0.1N HCl for 12 hours. The release profiles obtained for the formulation (F5 to F7) shown in Figure (4). It was be observed that the drug release increases with increase in drug polymer ratio. This may be due to the fact the polymer concentration was be kept constant for each formulation while the concentration of drug molecules was increasing which results in reduced thickness of polymer coat surrounding micro particles.

The in-vitro performance of Clarithromycin floating microsponge (F5) showed prolonged and controlled release of clarithromycin in predictable manner as the polymer concentration increases the drug release from the floating microspong decreases. This may be explained to be due to the less water permeability of Eudragit RS100 and increase in polymer thickness will increase in diffusion and erosion pathways. [23]

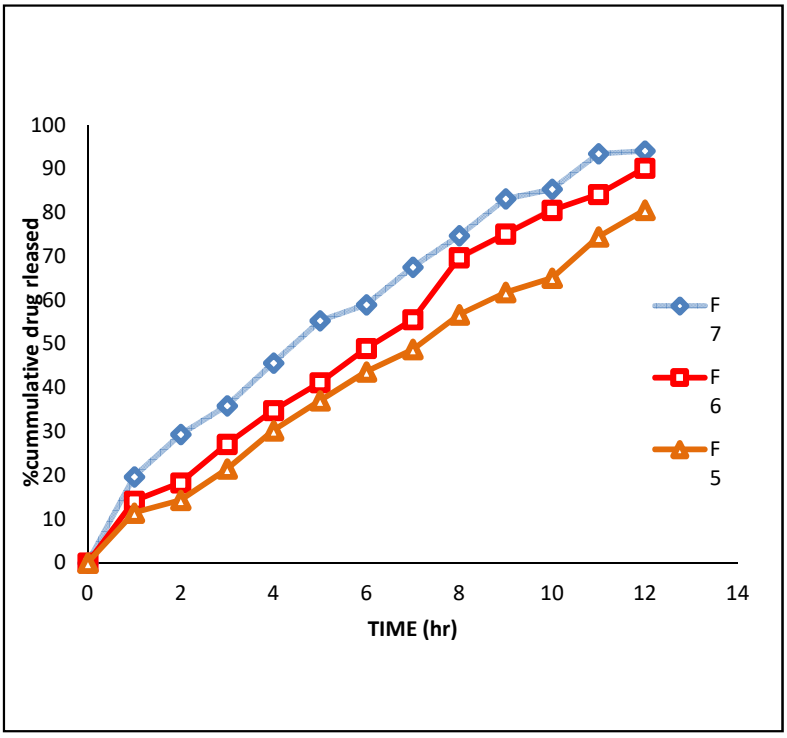

Fig. 4: In vitro drug release of (F5-F7)

CONCLUSION:

Floating microsponges of clarithromycin successfully prepared by quasi-emulsion solvent diffusion method using eudragite RS100. As the drug to polymer ratio was increased, the percentage yield of clarithromycin floating microspong was also increased. The average particle size of clarithromycin floating microspong have decreased with an increase in its drug to polymer ratio. By SEM studies, microspheres show porous spherical uniform structure. Buoyancy time more than 12 hr. Microspong containing eudragite RS100 in the ratio of 8:1 show high drug loading efficiency with particle size 50 µm. In-vitro release studies showed that microsponges containing eudragite Rs100 in formulation F5 showed a good degree of sustained release. As the polymer, concentration increases the amount of drug-released decreases.

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