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Formulation and Evaluation of Floating Microspheres of Clarithromycin

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Abstract

In this work we prepare, characterize and evaluatefloating microspheres of Clarithromycinby ionic gelation method with an aim of increasing the gastric residence time and for controlled release.Gelatin, polymeric mixture of gelatin and Hydroxy ethyl cellulose (HEC) were used as polymers. Sodium bicarbonate was used as the gas-forming agent. The production microspheres were characterized by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), and photomicrographs microscopy. The prepared floating microspheres evaluated by particles size distribution, floating behavior, drug content, entrapment efficency, morphology and in vitro release werestudied.The micros pheres diameters range322.49µm-584.64 µm.The results the release rate decrease with increaseing coeting material concentration. The floating microspheres followed zero order kinetics and drug mechanisam releasedtoPeppas model. The microspheres exponential coefficient values between 0.559 and 0.911 that indicatied to non fickian diffusion controlled release mechanism.

Key word: Clarithromycin, gelatin,Hydroxy ethyl cellulose,floating microspheres ,controlled release.

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مديرية تربية البصرة

الخلاصة:

تم في هذا البحث تحضير وتشخيص وتقييم الإطلاق المتوازن للكرات المجهرية العائمة بتقنية الجيلاتين الأيونية. حضرت الجسيمات الكروية الدقيقة العائمة للكلايثروميسين بطريقة الجيلاتين الأيونية ،بوليمر الجيلاتين وخليط من هيدروكسي اثيل سليلوز والجيلاتين استخدمت كبوليمرات . استخدمت بيكربونات الصوديوم كعامل لتكوين غازثنائي اوكسيد الكاربون . شخصت الجسيمات الكروية الدقيقة بتقنية الأشعة تحت الحمراء وتقنية المسح التفاضلي ألمسعري. تم تقيم الجسيمات الكروية الدقيقة بالشكل المورفولوجي وحجم الجسيمات والنسبة المئوية للطفو ونسب تحرر الدواء من الجسيمات الكروية بينت النتائج إنأقطار الجسيمات الكروية العائمة تراوحت بين(584,64-322,49) مايكرومتر،وإنمعدل تحرر الدواء يزداد بتقليل نسب البوليمر المستخدم ،وأشارت النتائجإن معدل تحرر الدواء كان ضمن حركية المرتبة الصفرية وفق نموذج بيباس إذ تراوحت قيم المعامل الأسي(0,559 و 0,911) مشيرا إلى إن التحرر لا يتموفق آلية الانتشار.

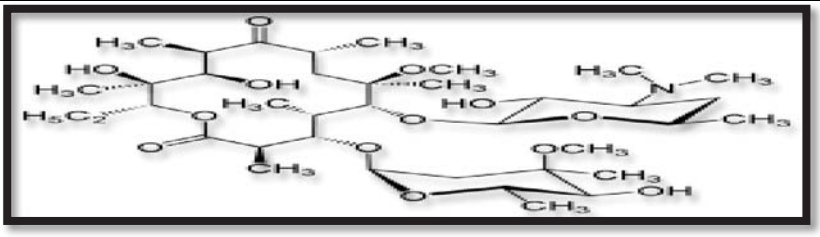
1-Introduction

The goal of any drug delivery system is provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired drug concentration. The aim of the present study was to design oral controlled release floating compressed natural polymer matrixes of clarithromycin which would remain in stomach for prolonged period of time thereby maximizing the drug release at desired site before Gastro-retentive drug formulation leaves the stomach.[1].

Floating drug delivery systems (FDDS) have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents. The drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from the stomach. This results in an increased gastric residence time(GRT) and a better control of the fluctuations in plasma drug concentration[2],Floating properties based on the mechanism of buoyancy are divided into: non effervescent systems with inherent low density or low density due to swelling; and effervescent systems with low density due to gas generation and entrapment. Effervescentsystem includes the use of gas generating agents, carbonates or any other organic acids (e.g. citric acid and tartaric acid) present in the formulation to produce carbon dioxide (CO_2) gas, are usually incorporate in the dosage form, thus reducing the density of the system and making it float on the gastric fluid. An alternative is the incorporation of matrix containing portion of liquid, which produce gas that evaporates at body temperature. [3, 4]

Clarithromycin is a novel macrolide anti-toxin with a methoxy bunch (- OCH3) connected to the C-6 position of erythromycin, which makes it more corrosive stable than erythromycin [5] Clarithromycin is an expansive range anti-infection agents it is dynamic against both gram positive and gram negative bacteria like staphylococcus aureus, E.coli, Klebsiella, Proteus [6] Clarithromycin is utilized to treat delicate tissue and skin diseases, Clarithromycin is likewise used to treat both upper and lower respiratory tract contamination, Helicobacter pylori infections.[7,8].

Tabe1: Properties Of Clarithromycin

structure	
Chemical formula	C38H69NO13
Physical	Clarithromycin was obtained as a white to off-white crystalline

appearance	powder.
Solubility	Clarithromycin was insoluble in water, soluble in acetone, slightly soluble in acetonitrile, ethanol, and methanol.
Pharmacodynamics	Gram- positive(Staphylococcus,S.pneumoniae,and S.pyogenes) Gram-nagative aerobic bacteria(Haemophilus influenza, H.parainfluenzae and Moraxella caatarrhalis)

Gelatin derived from collagen is a biodegradable and biocompatible natural polymer that has recently been extensively investigated as a biomaterial for use in biomedical applications such as tissue engineering and drug delivery[9]

Hydroxyethyl cellulose (HEC) is a plant-derived amino acid and a modified cellulose (natural poly saccharide) polymer. It is a nonionic, water-soluble polymer that can thicken, suspend, bind, emulsify, form films, stabilize, disperse, retain water and provide protective colloid action[10] The excellent properties of HEC allow it to be used in many biotechnological, biophysical and industrial fields. The HEC was existence of abundant reactive –OH groups, that is is liable to be modified by grafting polymerization with hydrophilic vinyl monomers to derive some new materials with improved properties[11] The floating microspheres can be used as a carriers of drugs calledabsorption windows, antiviral, antibiotic agents.

2- Material and Methods:-

2-1 Chemicals

Hydroxy ethylcellulose(HEC) and Gelatin were supplied by (B.D.H. Co., England). Clarithromycin was supplied by(Bristol-Myers Squibb,USA) All other chemicals were of reagent grade.

2-2 Preparation of floating microspheres

Gelatin beads of Clarithromycin were prepared by ionotropic gelatin method. The gelatin and hydroxy ethyl cellulosewere dissolved in distilled water with gentle agitation followed by the addition of 50 g of Sodium carbonate. The mixture kept aside for 5 minutes for the escape of air bubbles. Then drug (250mg) was added and mixed thoroughly. This solution was extruded into 1% w/v calcium chloride solution containing 10% w/v acetic acid. The beads collected and washed with distilled water and dried in an oven 45°C[12]. Gelatin-hydroxyl ethylcellulosemicrospheres formulated with different ratios in Tabe2:

Tabe2:prepared gelatin and hydroxy ethylcellulosemicrospheres containing Clarithromycin

Batch code	F1	F2	F3	F4	F5	F6
Clarithromycin (mg)	500	500	500	500	500	500
Gelatin(mg)	500	1000	1500	250	500	750
HEC(mg)	-	-	-	250	500	750
Sodium carbonate (%W/W)	50	50	50	50	50	50
calcium chloride(%W/V)	10	10	10	10	10	10
acetic acid	1.5	1.5	1.5	1.5	1.5	1.5
Drug:polymer ratio	1:1	1:2	1:3	1:1	1:2	1:3

2-3 Analytical Method

2-3-1 Morphological Study

Photomicrographs of microspheres portrayed utilizing a computerized optical magnifying lens . A little amount of dry microspheres was ultras .Two hundred microspheres were seized by the aforementioned technique and the mean breadth and in addition size dissemination of microspheres were resolved.[13]

2-3- 2Particle size analysis[14]

Particlesize was measured utilizing an optical magnifying lens and the mean molecule size was ascertained by measuring 300 particles with the assistance of an adjusted visual micrometer.

2-3-3 Determination of drug loading (%) and entrapment efficiency (%)[15]

50 mg of the microspheres were taken for assessment. The measure of drug entangled was assessed by pulverizing the microspheres and removing with aliquots of 0.1N HCl over and again. The concentrate was exchanged to a100 ml volumetric jar and the volume was made up utilizing 0.1N HCl. The arrangement was sifted and the absorbance was measured after reasonable weakening spectrophotometrically (UV 1700, Shimadzu, Japan) at 283 nm against fitting clear. The medication stacking (%) and entanglement effectiveness (%) were ascertained by taking after relationship

Drug loading (%) = Actual drug content/ Weight of powdered microspheres) × 100

Drug entrapment efficiency (%)=(Actual drug content/ Theoretical drug content) × 100

2-3-4Floating behavior (buoyancy %)[16]

50 mg of the microspheres were placed in 100 ml of simulated gastric fluid (pH 1.2) containing 0.02% w/v tween 80. The mixture was stirred at 100 rpm on a magnetic stirrer. After 12 h, the layer of buoyant microspheres was pipette and separated by filtration; particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in desiccators. Both the fractions of microspheres were weighed and buoyancy percentage was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microspheres

Buoyancy (%) =(Weight of floating microspheres after time t/Initial weight of microspheres)) × 100

2-3-5Fourier Transform Infrared Spectroscopy Study

FTIR spectrum of the drug, polymers, drug-loaded microspheres, were recorded using a FTIR (model 4100 type A,Perkin-Elmer, Norwak, CT,USA) spectrometer using KBr pellets in the range (400–4.000cm−1) .

2-3-6 DSC Analysis

Clarithromycin(Cla) thermotropic behavior inside the microspheres was investigated by a differential scanning calorimeter(DSC). Samples of pure, Clarithromycin ,polymers and Clarithromycinloaded microspheres were scanned at 5C°/min heating rate in the range(10°C to 300C°). In addition, DSC scans were run for drug, polymers and mixtures of (the drug with polymers)used in the preparation of microspheres. All the samples were freeze-dried dried over night before the measurements

2-3 -7 In Vitro Release Studies

100mg of each preparation of microspheres were placed in the synthetic dialysis bags and were immersed into 500 ml 0.1N HCl PH=1.2maintained at 37 ± 1 and stirred at 100 rpm. Samples (5 ml) were withdrawn at suitable interval of time and volume was adjusted. It was then assayed spectrophotometrically at 278 nm.Each beaker was accurately covered with glass watch and was fixed on a magnetic stirrer at 100rpm and 37±1C°. 3mlaliquot of the dissolution fluid was withdrawn at regular time interval and was replaced with fresh quantity dissolution fluid. The samples were analyzed spectrophotometrically at 283 nm to determine the dissolved drug concentration (content drug) using UVspectrophotometer. All the experimental units were analyzed in triplicate (n=3).[17]

2-3 -8 In Vitro Release Kinetic Studies

In order to study the exact mechanism of drug release from the prepared formulations, the drug release data was analyzed according to zero order, first order, Higuchi square root and Korsmeyer-Peppas model [18]

3-Results and Discussion

3-1FTIR Spectrums Of Drug and polymer interaction

The compatibility of Clarithromycin with various polymers was investigated by FT-IR-spectroscopy study. The FT-IR spectra of the drug and polymer combination were compared with the spectra of the pure drug[19] Show in (Fig 1,2,3,4) and spectrum data gathered in Table 3. In which no shifting of peaks was significantly found, indicating the stability of the drug during encapsulation process.

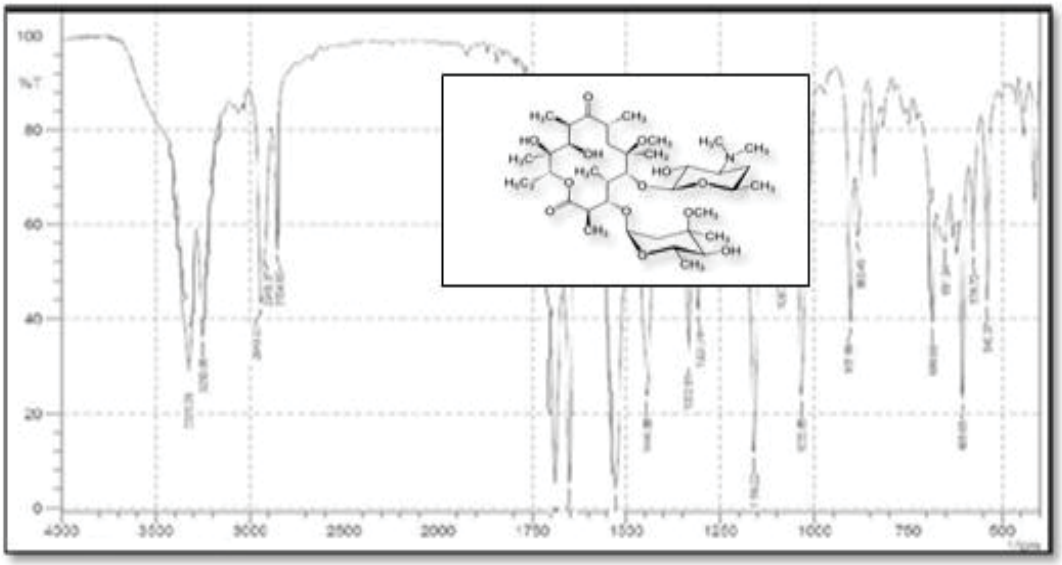
The spectra are included as figure 1and spectrum data gathered in Table3

spectrum observed that all characteristic peaks of clarithromycinpresent in the combination spectrum, thus indicating compatibility of theclarithromycin and polymerFTIR spectra of pure clarithromycinand F_4, FTIR spectrum ofF_4 showed that the mixture between clarithromycin and polymers was a physical because of the non-existence of chemical interaction (unbound form) between them, viz., no significant difference in the characteristic peaks of F_4 as compared to puredrug and polymers. This clarithromycinresult confirmed that the drug was chemical stability in the chosen polymeric mixture

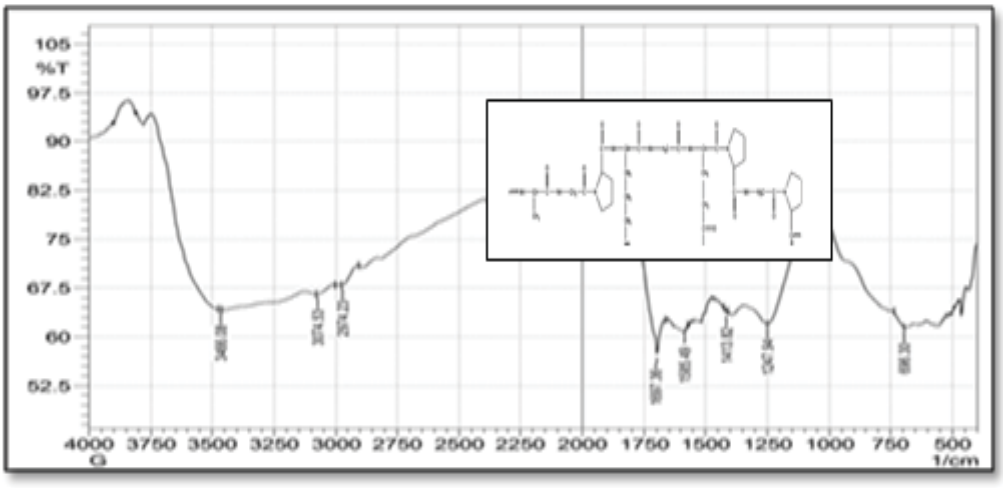
Table 3: Important data of FT-IR spectrums

S.no	IR Spectrum	Peaks(cm^{-1})	Groups	Stretching / Deformation
1	clarithromycin	3450.15	Hydrogen bonding between OH Groups	Stretching
		3109	O-H(carboxylic acid)	Stretching
		1690.50	(Ketone carbonyl),)	Stretching
		1729.22	Lactone carbonyl	Stretching
		1373.32	CH2	Stretching

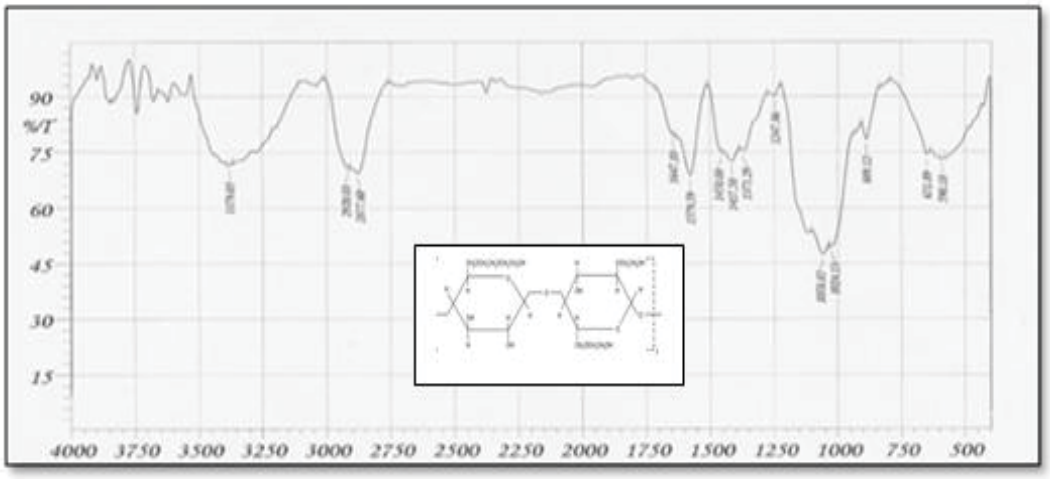
2	Gelatin	3492	NH_2	Stretching
		2871&2934	C- H(alkyl)	Stretching
		1703	Amid 1	Stretching
		1516	N-H ,Amid 11	bending
		1444	Amid 111	bending
		1331	C-N	Stretching
3	Hydroxyethyl cellulose (HEC)	3650-3600	O-H	Stretching
		3109	O-H(carboxylic acid)	Stretching
		2877.&2920	C- H(alkyl)	Stretching
		1458.08	C-O-H	Stretching
		1058.85	C-O-C	Stretching
4	microspheres of clarithromycin loaded with Hydroxyethyl cellulose (HEC)and Gelatin	3373	NH_2	Stretching
		2866.&2958	C- H(alkyl)	Stretching
		1741	Lactone carbonyl	Stretching
		1662	C=O	Stretching
		1539	COOH(unsaturated carboxylic acid)	Stretching
		1317	c-o	Stretching



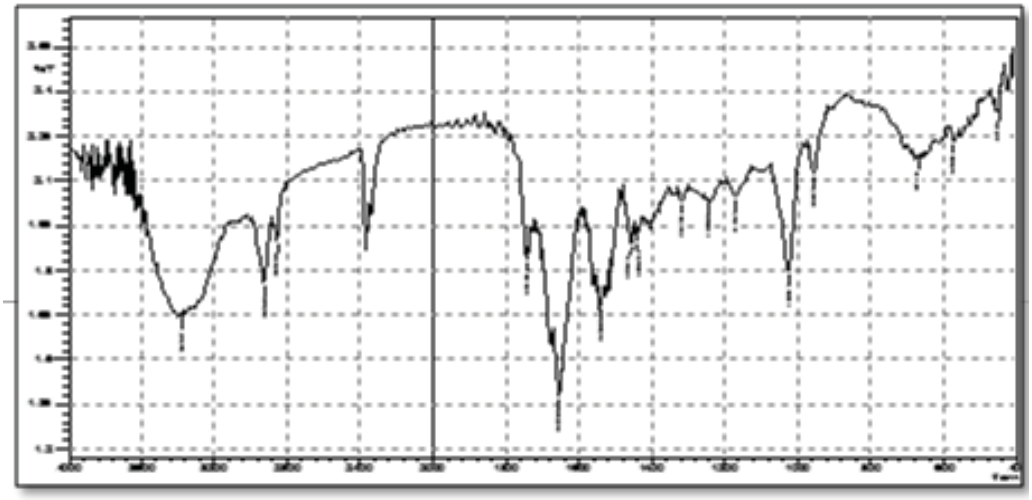
Fig(1) FTIR spectrum of pureclarithromycin



Fig(2) FTIR spectrum of pure gelatin



Fig(3) FTIR spectrum of pure Hydroxyethyl cellulose (HEC)

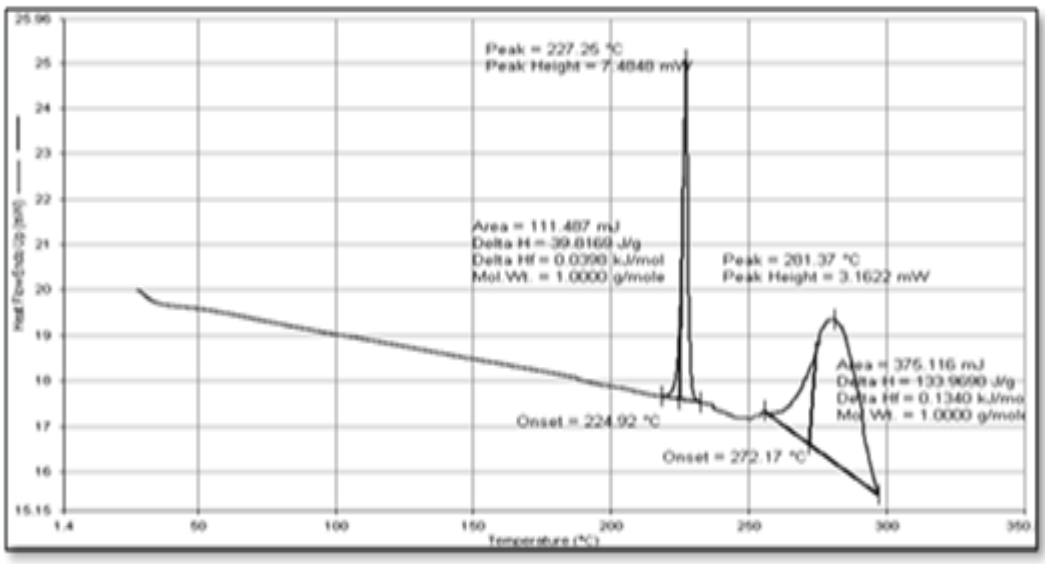


Fig(4) FTIR spectrum of drug loaded gelatin & Hydroxy ethyl cellulose (HEC)

3-2 Differential Scanning Calorimetry (DSC)

The thermogram of the drug alone, polymer, and drug-loaded microspheres were carried out shown in (Fig. 5-8). DSC thermogram of clarithromycinwas shown in figure (5).

A single sharp endothermic peak (melting endotherms) that corresponds to T_m of pure drug clarithromycin in the range of (222-227°C)[20], the DSC curve indicated the initiation of an exothermic process with a positive slope, resulting in a complete degradation of the drug at 281.37°C , DSC thermogram of pureHEC was shown in figure (6). It can be noticed that HEC showed two-thermal steps, as follows: the initial endothermic peak at 62.02°C corresponds to T_g according to the water content and the plasticizing effect in the composite[21]At temperatures over 100°C the plot rises, possibly indicating the loss of moisture which is physically adsorbed. After the loss, the endothermic peak at 243.62°C showed and due to the absorbed moisture in determined rate. The steps of the loss and absorption of the moisture were correlated with the decomposition process which continues to complete this process. This explanation of the thermal analysis for HEC agrees with the recent researches[22]The DSC thermograms of gelatin found to exhibit two endotherms at 105.37C° and 224 Co respectively. First endotherm may be due to water contect and the second melting point[23] ,DSC thermogram of F_4 confirmed that the mixture between clarithromycin and the polymers was physical because the appearance endothermic peak at 222.13°C and which corresponds to T_m of pure clarithromycinin the range of (222-227°C). This result indicates that clarithromycin is compatible, molecular dispersion and does not show any interaction with the chosen polymers . Also, these results of DSC support the results obtained from FTIR spectrum ofF_4.



Fig(5) DSC thermogramof clarithromycin

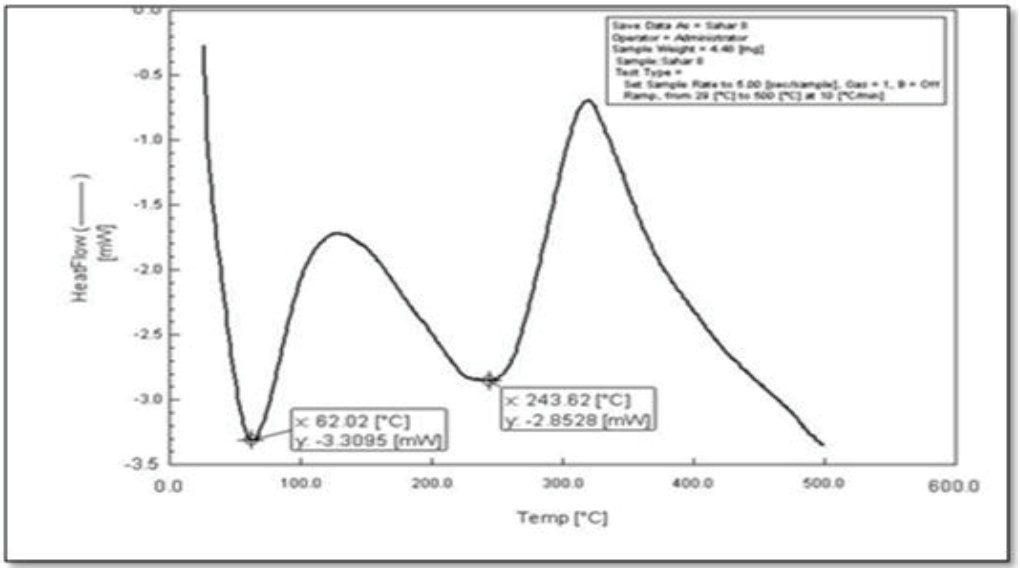


Fig (6) DSC thermogram of pure HEC

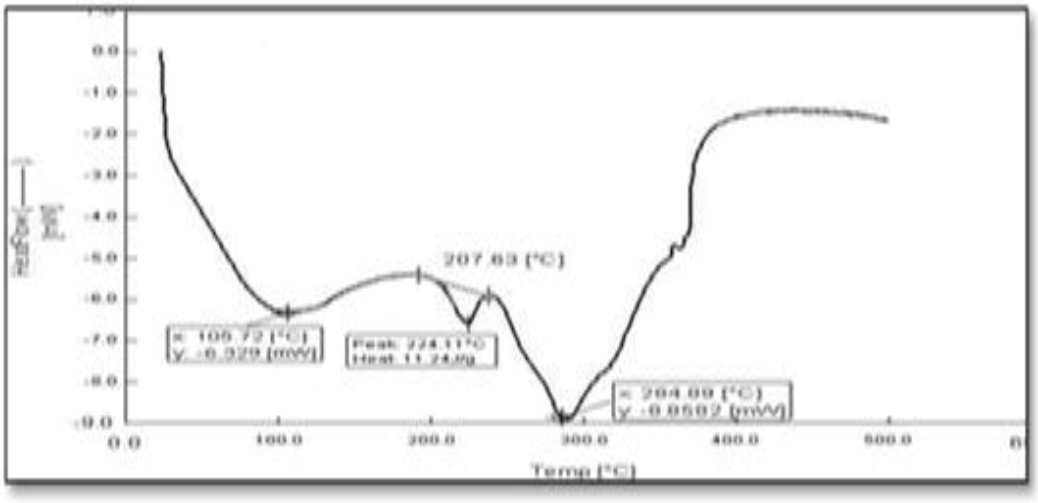


Fig (7) DSC thermogram of pure gelatin

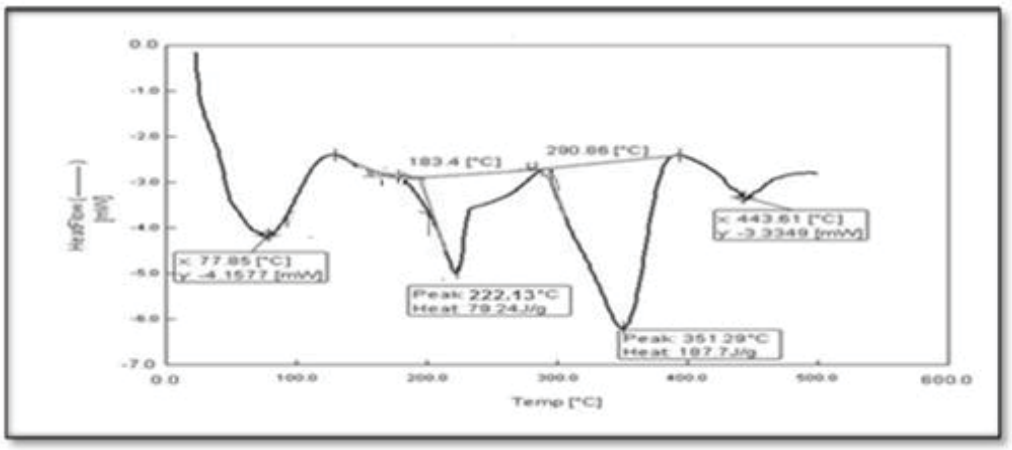
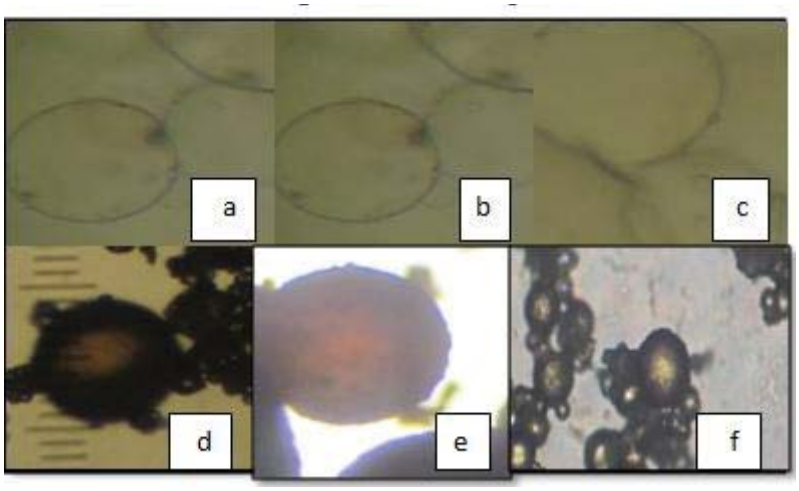


Fig (8) DSC thermogram of clarithromycin loaded gelatin&HEC

3-3Surface morphology

The surface morphology of the floating microspheres was studied by digital optical microscopeof the various formulations were shown in the Figure 9. Surface smoothness of theclarithromycin floating microspheres was increased by increasing the polymer conc. At lower polymer conc. (1:1) rough and wrinkled surface of clarithromycinfloating microspheres was obtained Fig9 and at higher polymer concentration (1:3) the clarithromycin floating microspheres with smooth surface was obtained Fig9. Microspheres with gelatin contain smooth surface and smaller in size compare to the microspheres with ethyl cellulose.

All gelatin microparticles had deflated shapes and smooth surfaces with fine dispersibility this may be due to them containing of an internal porous structure. [24,25]



Fig(9): Optical micrograph clarithromycinmicrospheres , (a–c) clarithromycinmicrospheres gelatin , (d–f) clarithromycinmicrosphereswithgelatin&HEC

The microspheres also evaluated for size analysis, Drug Content and % Encapsulation Efficiency. The results are given in Table 4. The increase in concentration of gelatin solution (F1,F2and F3) resulted in an increase in particle size from322.49-561.66µmmay be because of the increase of viscostity of droplets, With increasing HEC content of the blend (F4, F5and F6), particle size increases from 340.48-584.64 µm, because the presence of HEC would enhance the viscosity of the blend matrix, thus facilitating the formation of bigger emulsions, producing higher particle sizes. As the polymer concentration increases the buoyancy time increases. Percentage buoyancy of the Clarithromycin floating microspheres with HEC was in the range 86.36% to 88.16% after 12 hrs.

The drug content in the formulations with HEC was found to be in the range of49.12 %to74.69%The percentage entrapment efficiency for the formulations with HECwas found to be in

the range of98.48 % to 99.58%. The technique also showed good entrapment efficiency. as well as extent of clarithromycin loading.

Table 4: Particle size of Drug content, Entrapment efficiency, Wall thickness of clarithromycin microspheres prepared with gelatin alone and incombination with Hydroxy ethyl cellulose (HEC)

Invitro Buoyancy	Entrapment Efficiency	% Drug Content	Mean Diameter (µm)	Formulation
84.15	98.24	49.12	322.49	F1
86.24	99.16	65.45	441.94	F2
87.12	99.41	74.56	561.66	F3
86.36	98.48	49.24	340.48	F4
87.76	99.33	65.58	463.99	F5
88.16	99.58	74.69	584.64	F6

3-4 In Vitro Release Study of clarithromycin

100 mg sample of drug-loaded microspheres were placed in the synthetic dialysis bags and were immersed into100 ml of phosphate buffer (pH=1.2) after they were fixed in sterilized beakers . The system was placed in the Lap-Shaker at constant temperature 37˚C. Three millilitres of the dispersion medium was withdrawn and filtered through 0.22 µm Millipor filters. The drug concentration was measured at (λ =278 nm)using UV spectrophotometer .The measurements were carried out each one hour in the first hours then each 2 hrs .The drug release was evaluated using the following definitions [26]:

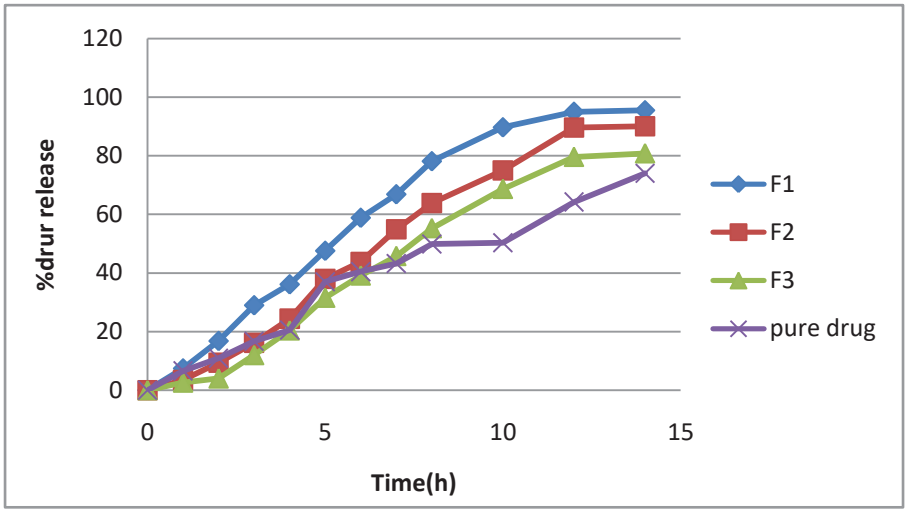
Drug Release =[Amount of drug release (mg) / Total amount of drug loaded (mg)]x 100 %

The in vitro release profile of differentclarithromycin concentrationsmicrosphereformulations is shown in Fig(10,11)andTable(5,6). The drug release rate becomes lower when the polymer concentration increases due to the smaller specific surface area of formulated larger microspheres The cumulative release of clarithromycin was decreased with increasing polymer concentration. The increased density of the polymer matrix at higher concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. **Figure 10 (Table 5)**. It can be seen that clarithromycin release is quite faster and higher at lower amount of gelatin , but the release follows a slower trend at higher amount of gelatin(F3). At higher amount of GA,

crosslink density of the matrix would be higher, making the matrix more rigid due to the contraction of micro-voids, thus hindering the movement of micro particles through the polymer matrix and thus, decreasing its % cumulative release.**Figure 11(Table 6)**, from which it is observed that % cumulative release increases from F6 to F4 formulations i.e., release rate increases with increasing HEC content of the blend matrix, because of the more hydrophilic nature of the matrix at higher concentration of the hydrophilic HEC, leading to higher swelling and higher release rates. Compared to our earlier study[27,28]The in vitro release profile of two different polymersmicrospheresare shown in Table(5,6). .

Table (5): Evaluation of drug release from clarithromycin microspheres with gelatin

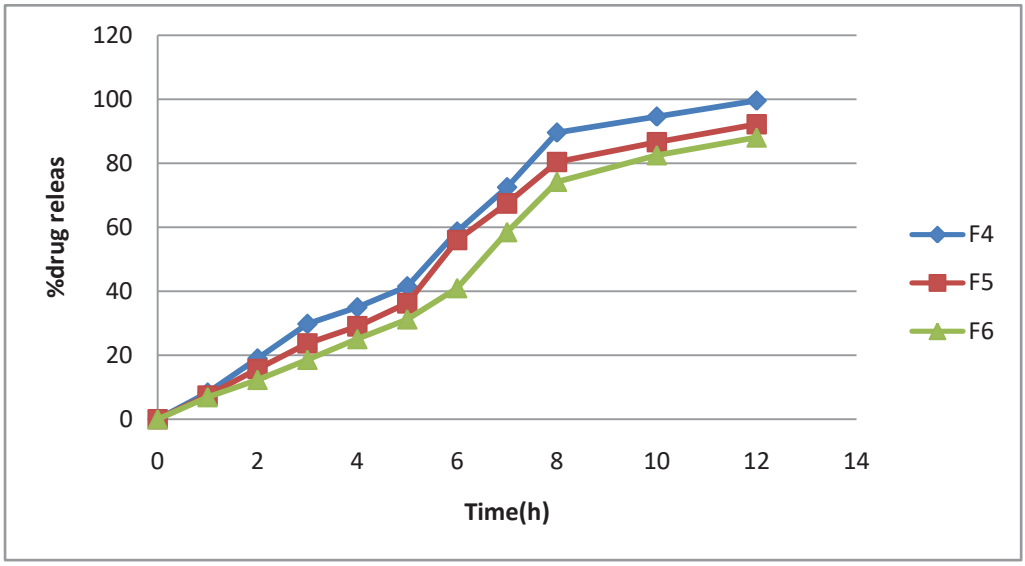
Time(h)	F1	F2	F3	pure drug
0	0.00	0.00	0.00	0.00
1	6.90	7.40	8.30	6.60
2	11.71	15.45	19.4	10.90
3	19.59	23.29	27.56	16.65
4	24.35	28.09	33.66	20.53
5	30.56	35.24	40.42	36.98
6	41.98	50.87	56.72	40.43
7	59.58	65.05	70.48	43.22
8	72.64	78.85	83.39	49.87
10	80.59	84.81	90.54	30.34
12	87.07	90.45	99.62	20.12



Fig(10):Average percentage clarithromycin released from geltin microspheres in pH 1.2

Table (6): Evaluation of drug release from clarithromycin microspheres with gelatin &HEC

Time(h)	F4	F5	F6
0	0.00	0.00	0.00
1	8.30	7.40	6.90
2	18.98	15.65	12.31
3	29.78	23.67	18.59
4	34.96	29.03	25.05
5	41.52	36.24	31.22
6	58.65	55.97	40.98
7	72.5	67.43	58.48
8	89.59	80.34	74.22
10	94.59	86.54	82.52
12	99.58	92.15	88.07



Fig(11):Average percentage clarithromycin released from geltin&HEC microspheres in pH 1.2

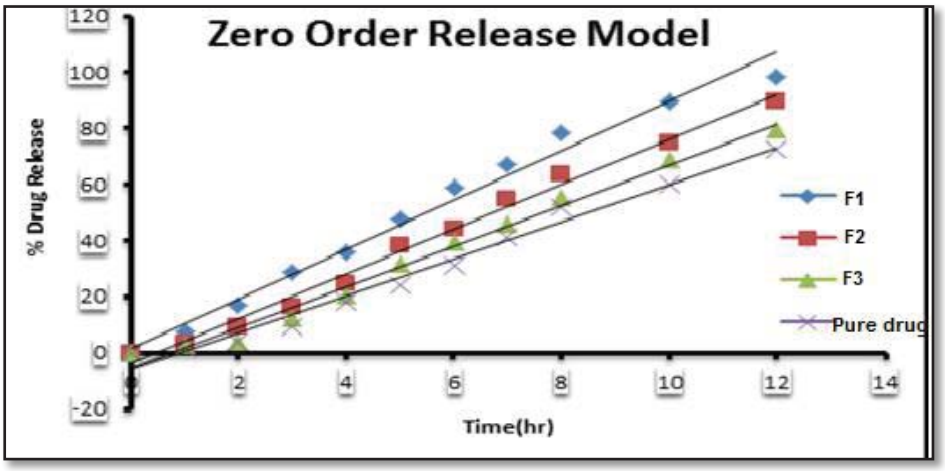
3-5 Kinetics of Drug Release

The slopes and the regression coefficient of determinations(R^2) are listed in (Table7). The coefficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. Additional evidence for the diffusion controlled mechanism was obtained by fitting the Korsmeyer–Peppas equation to the release data. The diffusion exponent n value was found to be inrange of (0. 911 to 0.559) for different drug–

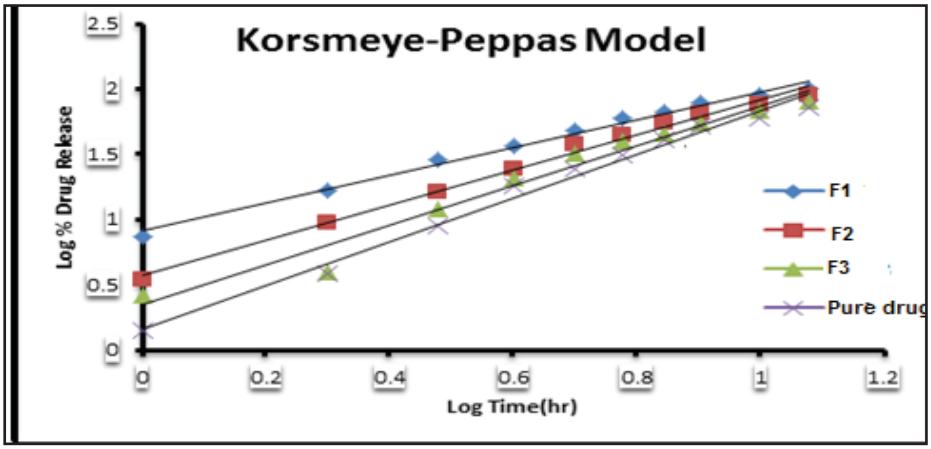
polymer compositions, indicating Fickian diffusion for (lass 0.5) and Non-Fickian diffusion for (above 0.5) of drug through microspheres

Table7: Regression co- efficient (R^2) values of different kinetic models and diffusion exponent (n) of Peppas model forclarithromycin microspheres

Formulation	Zero order	First order	Higuchi model	Peppas model	
				R^2	'n'value
F1	0.993	0.817	0.988	0.990	0.911
F2	0.983	0.829	0.973	0.992	0.576
F3	0.980	0.823	0.966	0.970	0.348
F4	0.991	0.819	0.954	0.987	0.114
F5	0.967	0.893	0.936	0.979	0.776
F6	0.965	0.864	0.948	0.984	0.559



Fig(12) Zero order Plots of clarithromycin Floating Microspheres Prepared with gelatin



Fig(13) Peppas Plots of clarithromycin Floating Microspheres Prepared with gelatin

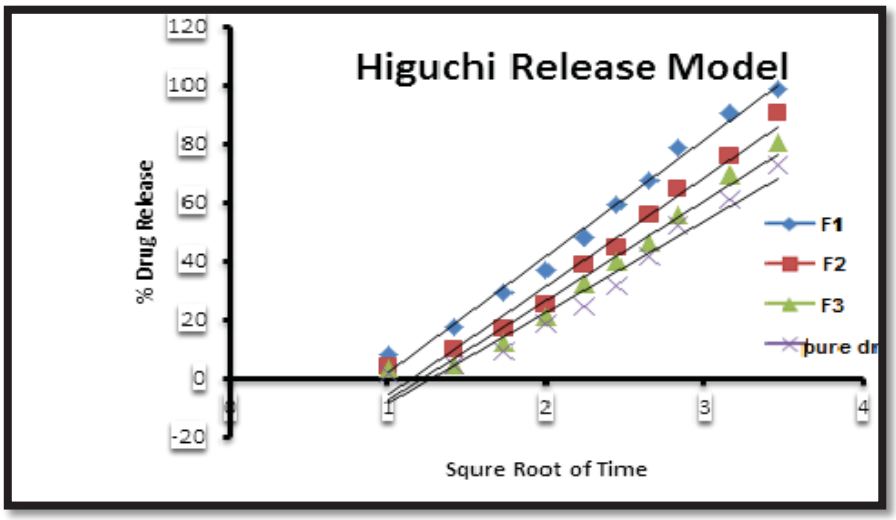
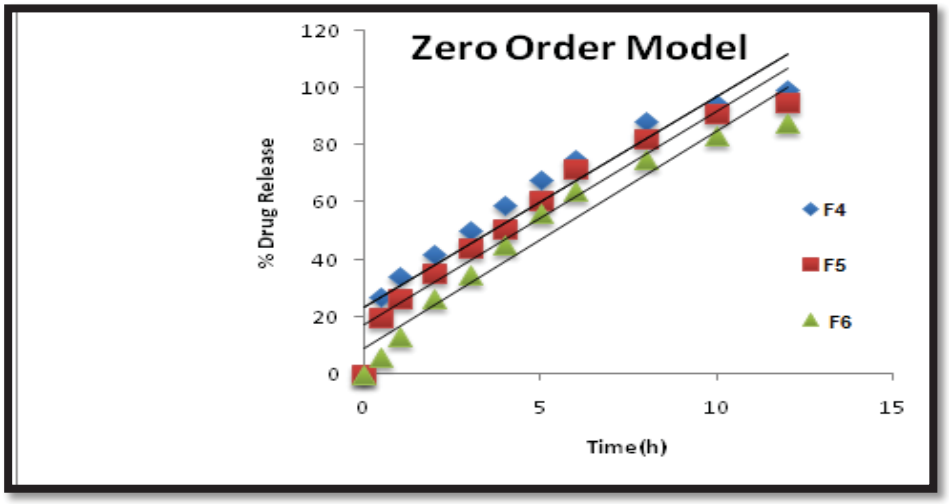
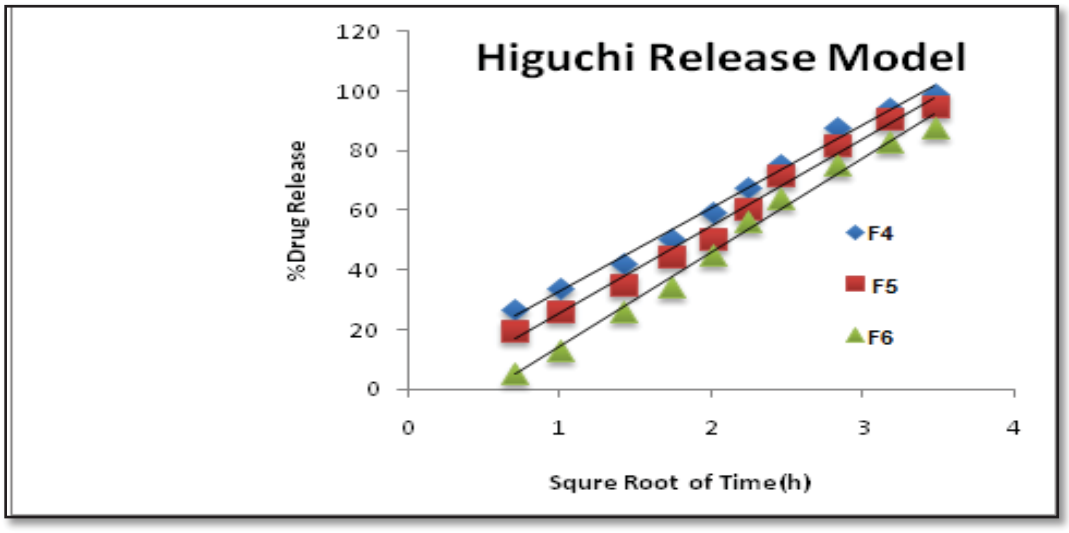


Fig (14) Higuchi plot of clarithromycin Floating Microspheres Prepared with gelatin



Fig(15) Zero order Plots of clarithromycin Floating Microspheres Prepared with gelatin&HEC



Fig(16) Peppas Plots of clarithromycin Floating Microspheres Prepared with gelatin&HEC

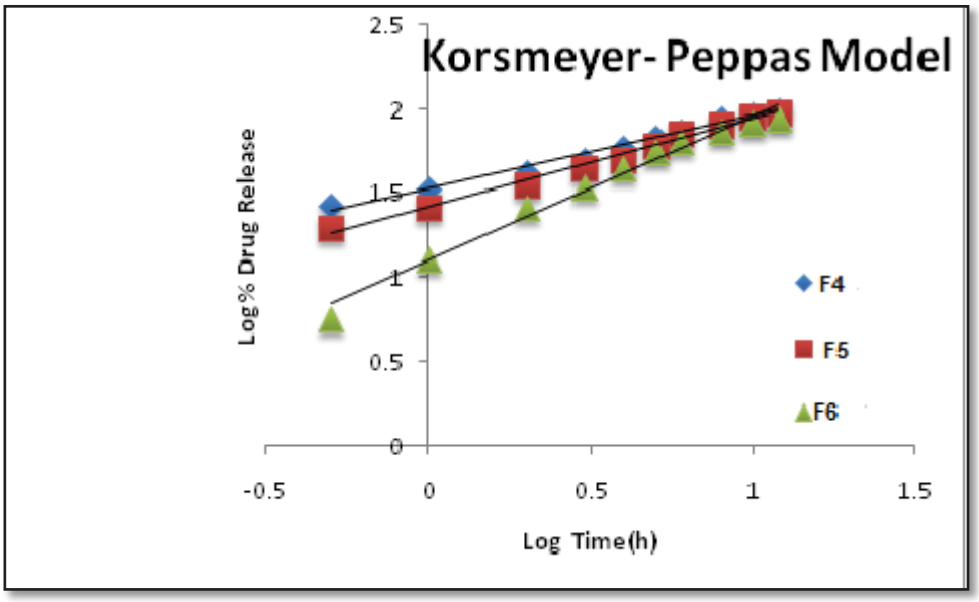


Fig (17) Higuchi plot of clarithromycin Floating Microspheres Prepared with gelatin&HEC

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