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Preparation ,Characterization and Evaluation of controlled release microspheres Containing Amphotericin B

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Abstract

The purpose of this research work was to prepare , characterize and evaluate microspheres for controlled release delivery of Amphotericin B (AmB) , as a model drug, with two natural polymers like Bovine Serum Albumin (BSA) and gelatin by a solvent/ evaporation method, induced by addition of glutaraldehyde (GA) as a cross-linking agent. The microspheres were characterized by Fourier transform infrared spectroscopy FT-IR, differential scanning calorimetry (DSC), and photomicrographs microscopy. The effect of different process parameters, such as drug/polymer ratio and Bovine Serum Albumin (BSA) / gelatin ratio, on the morphology, size distribution, in vitro drug release and release kinetics of microspheres were studied. The microspheres had mean diameters between 16-60 µm. The results show that the drug release rate from smaller microspheres was faster than from larger microspheres. Also the increasing the drug content of microspheres from 5 mg to 15 mg led to significantly faster drug release from microspheres. The total amount of drug released from microspheres after 12h in phosphate buffer saline, pH 7.4 at 37°C were 72% - 98% . The coefficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. Finally , The results show that biological activities for the microspheres loaded with AmB exceed biological activities against three types of fungal isolates including *Candida albicans, Trichophyton rubrum, and Aepergillus fumigates* in comparison with activity of AmB alone.

Key words: Albumin microspheres, Amphotericin B, Natural polymers, Drug release, Higuchi equation

1-Introduction

Amphotericin B (AmB) is still considered the gold standard of antifungal drugs because of its great therapeutic efficacy. This member of the macrolide polyenes family of antibiotics has been formulated as a selfmicellar system [1] . Nevertheless, the major problem with this drug is its great acute toxicity, its instability and its insolubility in water. Drug delivery systems such as(nano and micro) particles have been developed to avoid these problems. These systems protect drugs against breakdown[2] and reduce adverse effects[3] .

The choice of vector depends on the lipophilic character of drugs, which assumes that these molecules could be delivered by lipidic vectors, micro or nano particles or in the hydrophobic cavity of a supramolecular system. Rajagopalan and Chow [4] used β-cyclodextrin as a carrier for AmB. AmB has also been encapsulated in nanoparticles of poly (l-lactide-co-glycolic acid) (PLGA),[5] however the inclusion rate determined no exceed 2%. Tabosa Do Egito et al.[6]have recently developed a new system for AmB based on a lipid emulsion with high concentration of AmB. Fig.(1) shows the structure of Amphotericin B.

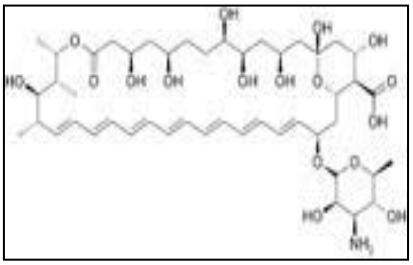

Fig.(1) : The Structure of Amphotericin B

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 μm [7]. They are made from polymeric, waxy, or other protective materials such as starches, gums, proteins, fats and waxes and used as drug carrier matrices for drug delivery. Albumin and gelatin are among the natural polymers which are used in preparation of microspheres. Preparation of uniformly sized Albumin microspheres was first reported in the late 60's and early 70's [8, 9, 10]. Albumin microspheres have received wide attention during the recent decades due to their specificity [11], biodegradability [12] and other desirable characteristics such as non-toxicity and biocompatibility [13] as an ideal drug carrier. More than 100 therapeutic and diagnostic agents have been incorporated into Albumin microspheres and drugs of various therapeutic categories such as nifedipine [14, 15], mitoxantrone [16], dexamethasone [17], salbutamol sulfate [18]have been prepared and characterized as Albumin microspheres delivery systems. Two methods have been developed for the preparation of Albumin microspheres which include heat stabilization and chemical cross-linking by using glutaraldehyde [19].

The aim of the present study was to prepare and characterize Bovine Serum Albumin (BSA) and gelatin microspheres loaded with AmB. Microspheres were prepared by an emulsification technique and stabilized by heat denaturation. The particle size, size distribution, release characteristics, and drug diffusion were studied .

1- MATERIALS AND METHODS

2-1 Chemicals

Bovine Serum Albumin(BSA) and Amphotericin B was supplied by (NDI Co.-Iraq). Gelatin was supplied by (B.D.H. Co., England). Glyceraldehyde was supplied by (Fluka Co., Switzerland). Muller – Hinton Agar was supplied by (Titan Biotech Co., Rajasthan, India). All other chemicals were of reagent grade.

2-2 Preparation of Microspheres

2-2-1 preparation BSA microspheres

BSA microspheres containing AmB were prepared by solvent / evaporation stabilization technique described previously(with modifications) [20]. BSA 1 g was dissolved in 10 ml of distilled water and then was added drop it wise using syring into 200 ml of sun flower oil with 0.5ml Tween 80 (as emulsifier) preheated to 60°C and stirred at 600 rpm for 30 min. Then glutaraldehyde 1.0 ml (25%v/v) was added to water /oil (W/O) emulsion with continuous stirring at the same speed. Stirring was stopped after 1 hr of the final addition of glutaraldehyde. Suspension of BSA microspheres in sun flower oil thus obtained was allowed to stand for 1 hr to let the microspheres settled down. Clear supernatant was decanted and washed free oil with (20 ml) of dimethylether for 2 min at 1500 rpm .The microspheres then washed 3 times with 20 ml of acetone at 2000 rpm . Finally microspheres were allowed to drying at room temp .

A 0.5 gram of dried microspheres was allowed to swell in 10 ml phosphate buffered saline (PBS) solution containing various concentration (as shown in Table 1)of AmB at 37 ± 0.2°C for 30 min under continuous magnetic stirring . Microspheres were then rapidly collected using paper filtration , washed with acetone and dried at room temp .

Table 1. Prepared BSA microspheres containing AmB

Batch code	Drug : Polymer (g)
AmB1	0.005:0.5
AmB2	0.01:0.5
AmB3	0.015:0.5

Microspheres were prepared with different polymer ratios were shown in Table 2

Table 2: Formulations code of various polymer concentration

Batch code	Drug : Polymer (g)
BSA1	0.01:0.25
BSA2	0.01:0.5
BSA3	0.01:0.75
BSA4	0.01 : 1.0

2-2-2 Preparation BSA microspheres with gelatin

BSA microspheres with gelatin containing AmB were prepared by solvent / evaporation stabilization technique described previously(with modifications) [20]. As shown in Table 3, the required quantity of BSA was dissolved in 10 ml of distilled water**.** On the other hand, quantity of gelatin was dissolved in 10 ml of distilled water preheated to 60°C. Next, both suspensions were mixed together and then was added it drop wise using syring into 200 ml of sun flower oil with 0.5ml Tween 80 (as emulsifier) preheated to 60°C and stirred at 600 rpm for 30 min.

Then glutaraldehyde 1.0 ml (25%v/v) was added to W/O emulsion with continuous stirring at the same speed. Stirring was stopped after 2 hr of the final addition of glutaraldehyde. Suspension of microspheres in sun flower oil thus obtained was allowed to stand for 1 hr to let the microspheres settled down. Clear supernatant was decanted and washed free oil with (20 ml) of dimethylether for 2 min at 1500 rpm .The microspheres were then washed and dehydrated 3 times with 20 ml of acetone at 2000 rpm . Finally microspheres were allowed to drying at room temp .

A 0.5 gram of dried microspheres was allowed to swell in 10 ml phosphate buffered saline (PBS) solution containing 10 mg of AmB at 37 ± 0.2°C for 30 min under continuous magnetic stirring . Microspheres were then rapidly collected using paper filtration , washed with acetone then dried at room temp .

Table 3 Formulation of the BSA / gelatin microspheres

Batch code	BSA : G (g)
AG1	0.75:0.25
AG2	0.5:0.5
AG3	0.25:0.75

2-3 Analytical Method

2-3 -1 Morphological Study

Photomicrographs of microspheres characterized using a digital optical microscope . A small amount of dry microspheres was ultras .Two hundred microspheres were seized by the above mentioned method and the mean diameter as well as size distribution of microspheres were determined.

2-3 -2 Fourier Transform Infrared Spectroscopy Study

FTIR spectrum of the drug, drug-loaded microspheres, blank microspheres , and physical mixture of drug and empty microspheres were recorded using a FTIR (model 4100 type A,Perkin-Elmer, Norwak, CT,USA) spectrometer using KBr pellets in the range (400–4.000 cm^{-1}) .

2-3 -3 DSC Analysis

AmB thermotropic behavior inside the microspheres was investigated by a differential scanning calorimeter(DSC). Samples of AmB loaded microspheres and blank microspheres were scanned at 5 °C/min heating rate in the range(10°C to 300°C). 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 -4 In Vitro Release Studies

Briefly,100mg of each prepared microspheres were placed in the synthetic dialysis bags and were immersed into 100mL phosphate buffer solution pH=7.4 after they were fixed in sterilized beakers[21] . Each beaker was accurately covered with glass watch and was fixed on a magnetic stirrer at 100rpm and 37±1^{o}C. 3mL aliquot of the dissolution fluid was withdrawn at regular time interval and was replaced with fresh quantity dissolution fluid. The samples were analyzed spectrophotometrically at 406 nm to determine the dissolved drug concentration (content drug) using UV-spectrophotometer. All the experimental units were analyzed in triplicate (n=3).

2-3 *-5 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[22]. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test.

2-4 **Biological Part**

2- 4 **-1 Microbial Isolates:**

Three clinical Fungal isolates which include *Aepergillus fumigates* , *Candida albicans* and *Trichophyton* rubrum . Were tested for antifungal susceptibility. Fungal isolates were grown on Sabouraud dextrose agar (SDA) medium.

2-4 **-2 Microbial Inocula** [23]**:**

A suspension for each isolate was prepared and adjusted to 1×10^{6} cfu/mL standardized by (0.5MacFarland scale) and by using haemocytometer.

2- 4 **-3 Drugs*:***

The anti fungal drugs that were used to test their activity include different concentrations of AmB alone, polymeric microspheres loaded with different concentrations of AmB and polymeric microspheres alone .

2- 4-**4 anti- fungal Assay:**

This test was performed by agar diffusion method [24,25]. 0.2 ml of fungal inocula was placed on the surface of SDA media and then spread with L–shape glass rod. The plate was left for 10 minutes. Antifungal drug was placed in central pore which included (0.5g) for polymeric microspheres loaded with different concentrations of AmB, (0.5ml) of AmB solutions and also used microspheres alone (0.5g) as control plates for the polymer. A control experiment was set up by using an equal amount of phosphate buffer pH 7.3 in place of different antifungal formulations. *Candida albicans* plates were incubated at (30 Cº) for 1-3 days, while *Aspergillus fumigatus* and *Trichophyton rubrum* plates were incubated at (30 Cº)for 3-7 days.

The inhibition zones of each isolate were recorded according to growth of their controls. duplicate plates were used for each test.

3-Results and Discussion

3-1 The FT- IR spectrum of BSA / gelatin microspheres:

The FT-IR spectra of AmB, AmB-loaded microspheres, blank microspheres , and physical mixture of BSA and Gelatin 1empty microspheres, shown in(Fig. 2 - 5) and spectrum data gathered in Table 4.The spectrum observed that all characteristic peaks of AmB present in the combination spectrum, thus indicating compatibility of the AmB and polymer .

Table 4. Important data of FT-IR spectrums

IR Spectrum	Peaks(cm^{-1})	Groups	Stretching / Deformation
AmB	3400.27	O-H(carboxylic acid)	Stretching
	2866.&2933	C- H(alkyl)	Stretching
	1649.62	C=O(aromatic ketone)	Stretching
	1629.6	COOH(unsaturated carboxylic acid)	Stretching
	1535	Amid 11	Stretching
Blank microspheres of Bovine serum albumin	3365.66	O-H(carboxylic acid)	Stretching
	2856&2927	C- H(alkyl)	Stretching
	1739.67	C=O(aromatic ketone)	Stretching
	1665	COOH(unsaturated carboxylic acid)	Stretching
	1560	Amid 11	Stretching
AmB microspheres of Bovine serum albumin	3440	O-H(carboxylic acid)	Stretching
	2872.&2958	C- H(alkyl)	Stretching
	1680	C=O(aromatic ketone)	Stretching
	1745	C=O(aldehyd)	
	1629.6	COOH(unsaturated carboxylic acid)	Stretching
	1535	Amid 11	Stretching
Blank microspheres of Bovine serum albumin and Gelatin	3440	O-H(carboxylic acid)	Stretching
	2872.&2958	C- H(alkyl)	Stretching
	1680	C=O(aromatic ketone)	Stretching
	1629.6	COOH(unsaturated carboxylic acid)	Stretching

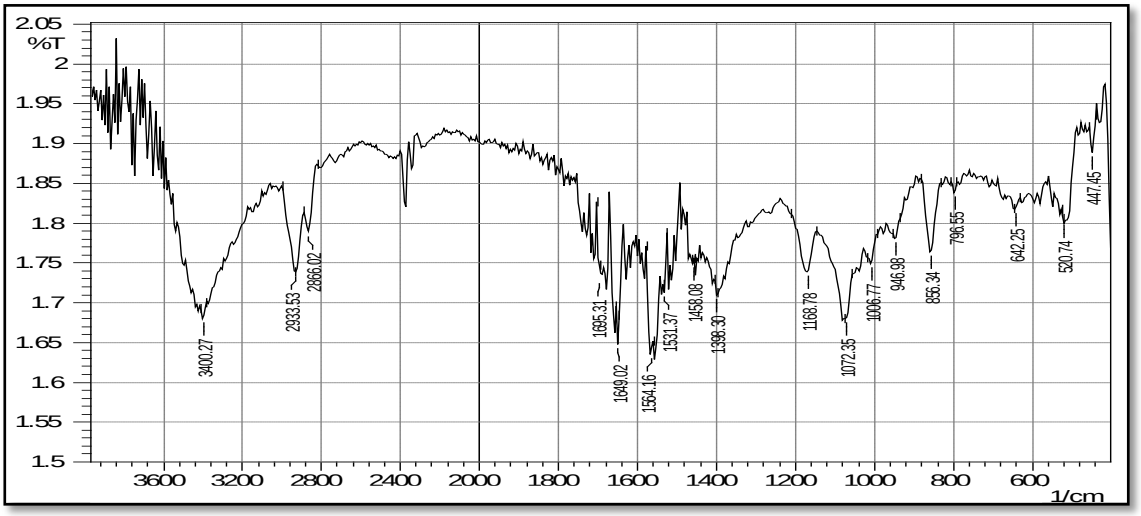

Fig 2 The IR spectrum of AmB

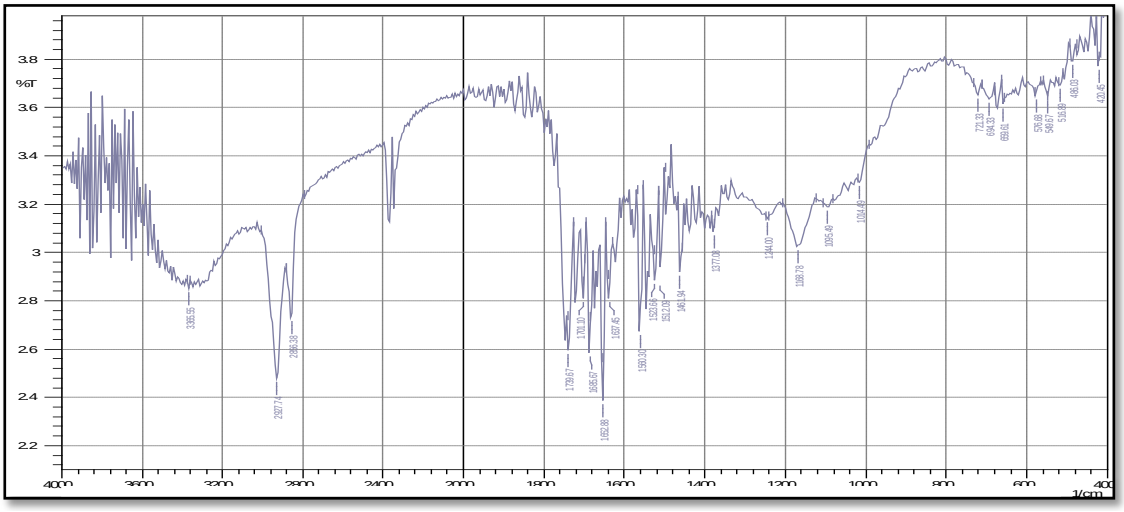

Fig 3 The IR spectrum of BSA

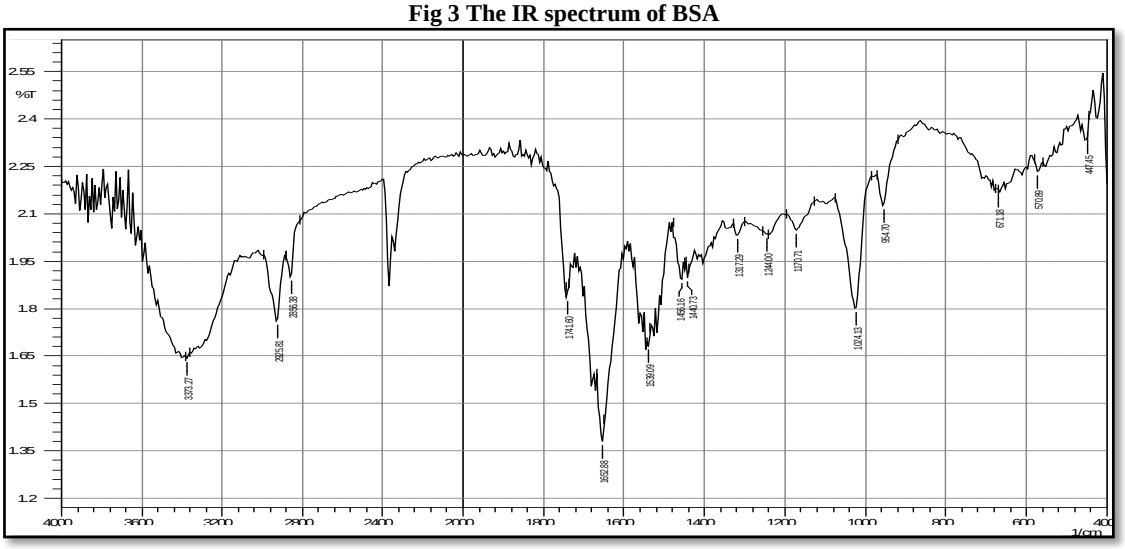

Fig 4 The IR spectrum of BSA+AmB

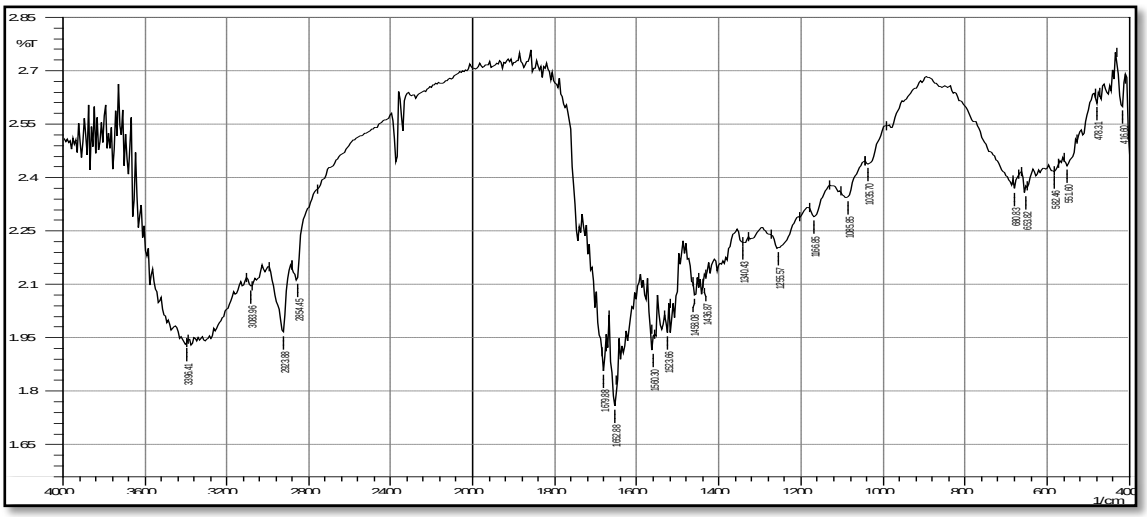

Fig 5 The IR spectrum of BSA+Gelatin

3-2 Differential Scanning Calorimetry (DSC)

The thermogram of the drug alone, blank microspheres, and drug-loaded microspheres were carried out and shown in (Fig. 6 to 9). The DSC thermogram of drug showed the broad endothermic due to the loss of moisture starting from 30°C to 100°C, which is attributed to the loss of adsorbed water (AmB being hygroscopic in nature),the another endothermic peak at 167.55°C, its melting point. BSA has a denaturation temperature at 66°C thus presence of this endothermic peak in blank as well as drug-loaded MS starting from 65°C indicates that process has not affected the denaturation temperature of BSA. the endothermic peak at 82°C, its melting point of Albumin. The peak at 225.82°C demonstrated intense glass transition, the high temperature transition was attributed to the devitrification of blocks rich in imino acids (rigid blocks)[26].

The DSC thermogram of drug-loaded microsphere (Fig 8) where no prominent degradation of AmB was observed at 210°C and the corresponding peak was shifted toward higher values at 234°C is increase in pyrolysis temperature was probably due to presence of AmB in Albumin matrix that provides stabilization of AmB.

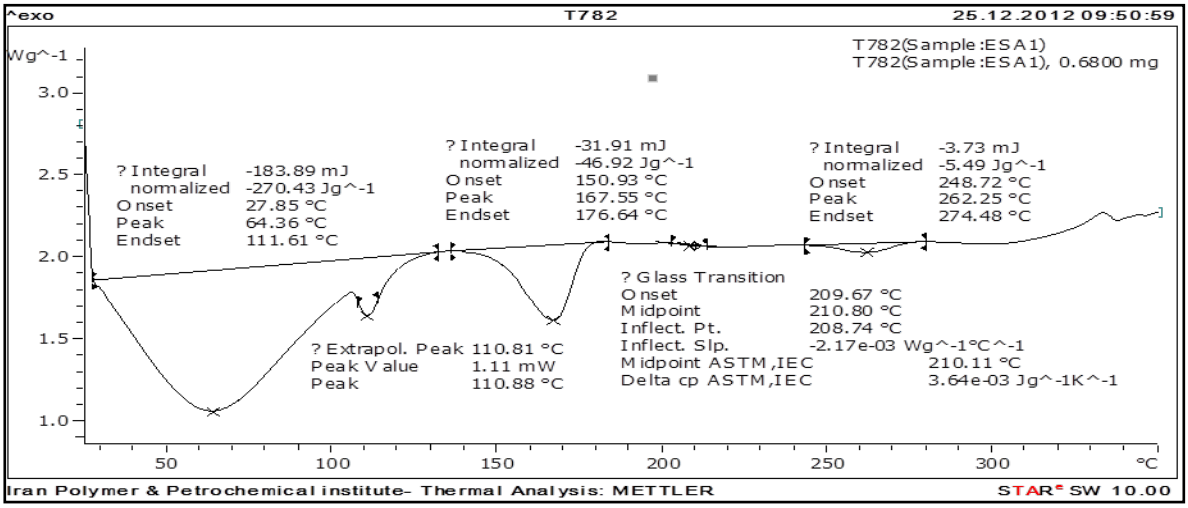

Fig 6 the DSC thermogram of AmB

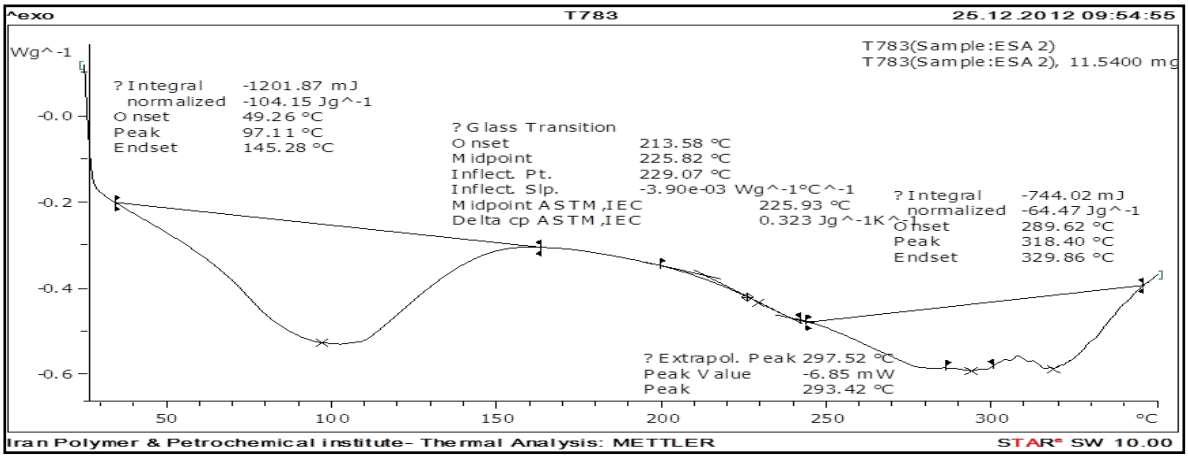

Fig 7 the DSC thermogram of BSA

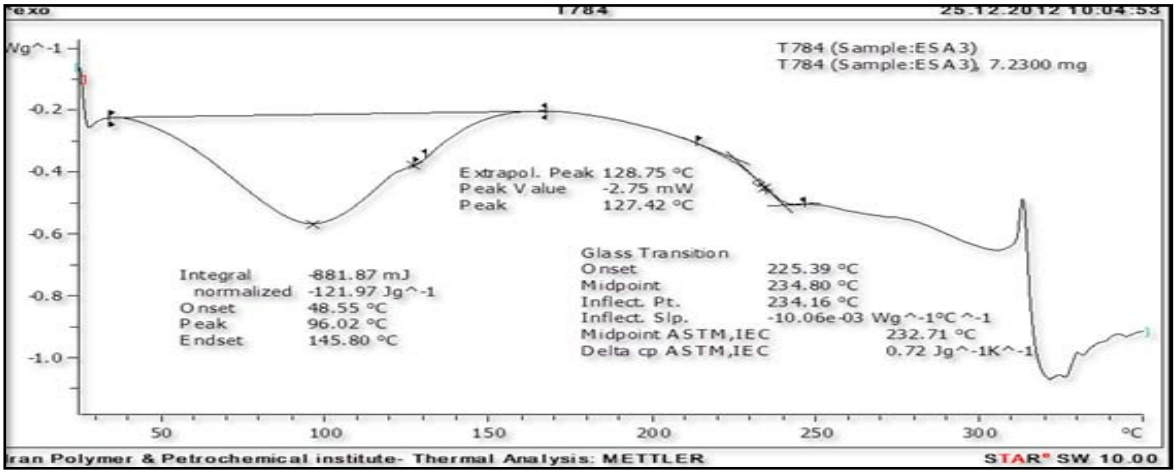

Fig 8 the DSC thermogram of BSA+AmB microspheres

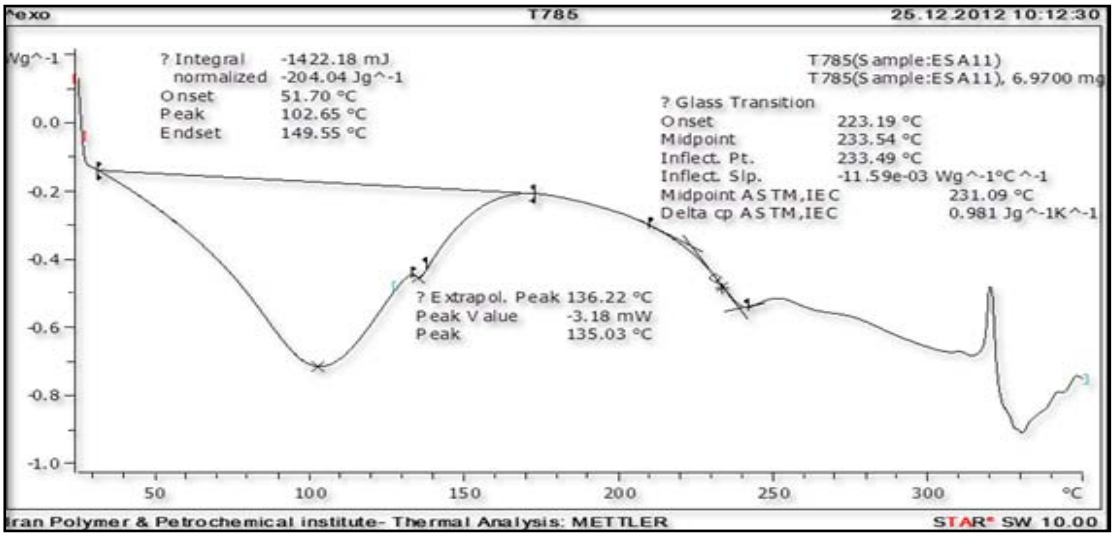

Fig 9 the DSC thermogram of BSA+Gelatin microspheres

3-3 Morphology and Size Distribution

The release of morphological analysis of AmB microspheres Fig10 , Table (5) showed that smooth and solid microspheres .Increase in drug concentration of AmB resulted in increase in mean particle size (MPS) of microspheres. Increase in MPS may be as a result of the release in viscosity of the droplets present in the internal phase caused by the increase in drug concentration

Table 5 Effect of Drug Conc. On MPS of AmB microspheres

AmB Conc. mg	5.0	10.0	15.0
MPS µm	16	25	34

The Effect of polymer solution concentration on morphology, mean particle size shown in Table (6) an increase in concentration of Bovine serum Albumin solution resulted in an increase in MPS of AmB microspheres . This significant increase may be because of the increase in viscosity of droplets (due to the increase in concentration of Bovine serum Albumin solution) .This increase is high enough to

result in difficult dispersion in emulsification and subdivision of droplets. Increase in mean particle size was due to increase viscosity of the polymer solution which has also reported by Jeyanth [27]

Table 6 Effect of polymer concentration on characteristics of microsphere

BSA Conc. g	0.25	0.5	0.75	1.0
MPS µm	18.2	25.6	29.4	36.5

The mean size and morphology of microspheres of two different polymers are shown in Table (7) . The BSA and gelatine used here ,it is seen that increase in concentration of gelatine, microspheres have a larger size than that of the BSA microspheres. This is attributed to higher viscosity of gelatine solution, therefor it is more difficulty in dispersing in to the aqueous phase during emulsification process , the morphology of microspheres can be affected by the phase separation kinetics during solvent evaporation.

Table7 Effect of different types of polymer on characteristic microspheres

Batch code	AG1	AG2	AG3
MPS µm	30	43	60

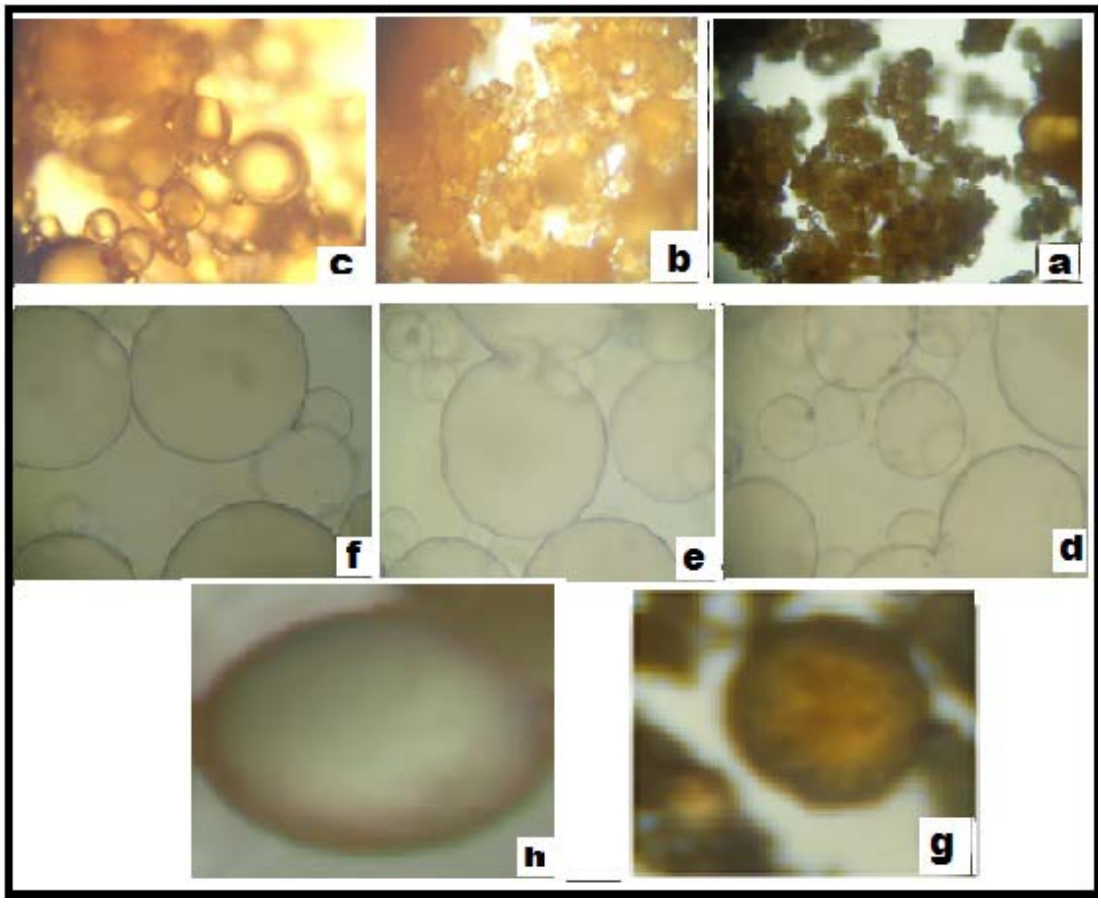

Fig10: Optical micrograph of BSA-AmB microspheres , (a–c) unloaded BSA microspheres , (d–f) BSA –gelatin microspheres. g and h show the particle size distribution of loaded(BSA ,BSA –gelatin)microspheres, respectively.

3-4 In Vitro Release Study of AmB

100 mg sample of drug-loaded microspheres were placed in the synthetic dialysis bags and were immersed into 100 ml of n-saline phosphate buffer (pH=7.4) 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 =406 nm)using UV spectrophotometer .The measurements were carried out each hour in the first eight hours then each 2 hrs .The drug release was evaluated using the following definitions :

$$\text{Drug Release (\%)} = \frac{\text{Amount of drug release (mg)}}{\text{Total amount of drug loaded (mg)}} \times 100$$

The effect of drug loading of microspheres on AmB release fror microspheres is shown in Fig(11).It can b seen that by increasing the amount of drug loading from(5 to 15 mg), the rate of drug release from the microspheres increase dramatically .With higher drug loading more drug molecules are available at the surface of microspheres leading to higher initial release

Also , by increasing the amount of drug loading ,appoint will be reached when the solid drug particles will begin to from continuous pores or channels within the matrix .Under these , the path of least resistance for drug molecules will be diffused within the channels formed areas where drug has previously leached out from the matrix. Therefore, as the amount of drug content is increased and drug leaches out from the polymer, the matrix becomes more porous and , a faster drug release rate occurs. The releasing data is shown in Table(8).

Table 8 Evaluation of drug release from BSA microspheres

AmB3	AmB2	AmB1	Time(hr)
0	0	0	0
6.89	3.5	1.28	1
13.3	9.35	3.13	2
25.75	16.16	6.7	3
43.49	24.36	10.28	4

52.99	37.98	18.17	5
65.5	43.77	26.45	6
74.17	54.91	39.56	7
83.76	63.82	55.67	8
91.7	74.97	70.82	10
98.5	89.56	83.71	12

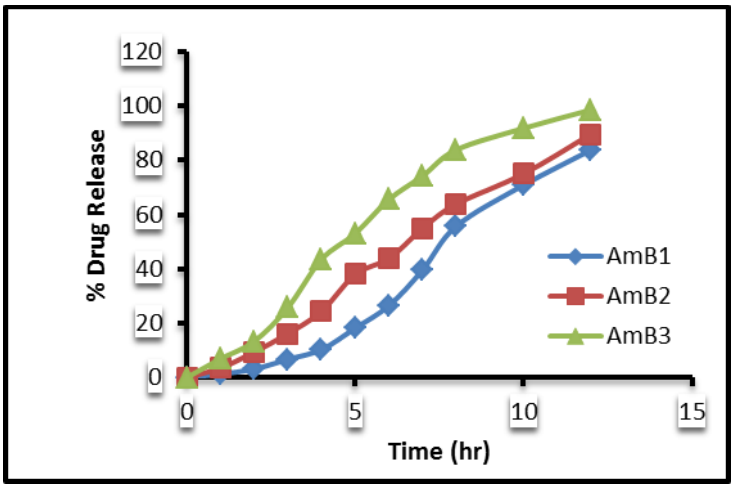

Fig. 11 Average percentage AmB released from BSA microspheres in PBS pH 7.4 at 37 °C

The in vitro release profile of different BSA concentrations microsphere formulations is shown in Fig (12)and Table(9). The drug release rate becomes lower when the polymer concentration

creases due to the smaller specific surface ea of formulated larger microspheres .It snows that size is one of the effective keys for controlled release of microspheres.

Table 9 Evaluation of drug release from different BSA concentration microspheres

BSA4	BSA3	BSA2	BSA1	Time
0	0	0	0	0
1.4	2.6	3.5	7.4	1
3.83	4.01	9.35	16.77	2
9.007	11.98	16.16	28.95	3

18.01	20.39	24.36	36.09	4
24.1	31.55	37.98	47.53	5
31.11	39.12	43.77	58.79	6
40.86	45.78	54.91	66.81	7
51.8	55.27	63.82	78.08	8
60.23	68.66	74.97	89.65	10
72.3	79.59	89.56	98.34	12

AG3	AG2	AG1	Time(h)
0	0	0	0
8.3	7.4	6.9	1
19.4	15.45	11.71	2
27.56	23.29	19.59	3

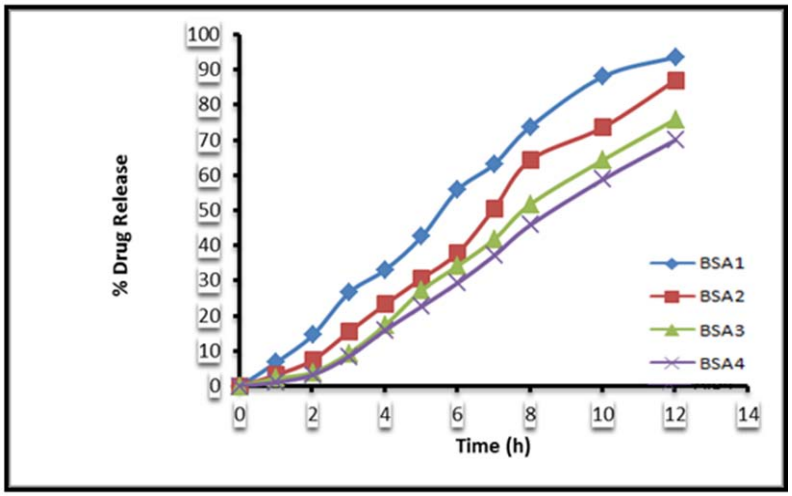

Fig 12 Average percentage AmB release from different BSA concentration on microspheres in PBS pH 7.4 at 37 °C

The in vitro release profile of two different polymers microspheres are shown in Table (10), Fig. 13 .

Table 10 Evaluation of drug om two different polymers concentrat spheres

33.66	28.09	24.35	4
40.42	35.24	30.56	5
56.72	50.87	41.98	6
70.48	65.05	59.58	7
83.39	78.85	72.64	8
90.54	84.81	80.59	10
95.62	90.45	87.07	12

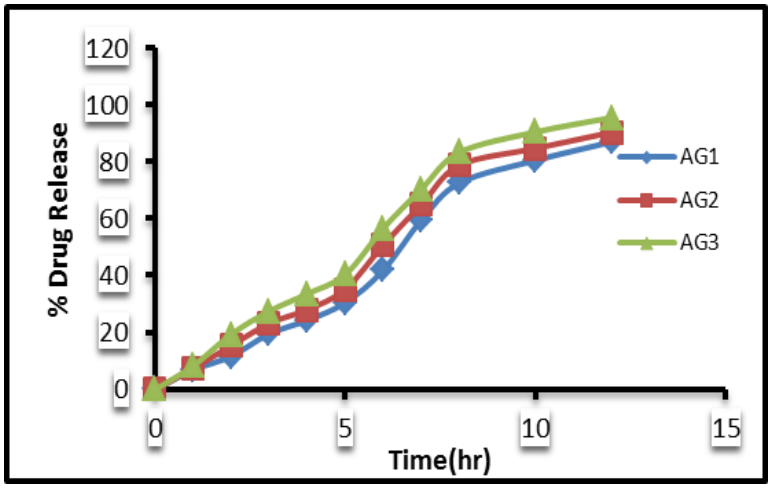

Fig. 13 Average percentage AmB released from two different polymers concentration microspheres in PBS pH 7.4at 37 °C

In principle , diffusion of a drug through a polymer matrix is dependent on the particle size, since the diffusion distance is longer in larger particle size. In addition ,dissolution velocity also influenced by the particle size as the specific area increases when particles become smaller.

3-5 Kinetics of Drug Release

The slopes and the regression coefficient of determinations(R^2) are listed in (Table 11). 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 in range of (0.014 to 0.985) for different drug–polymer compositions, indicating Fickian diffusion for (less 0.5) and Non- Fickian diffusion for (above 0.5) of drug through microspheres.

Table 11 Regression co- efficient (R^2) values of different kinetic models and diffusion exponent (n) of Peppas model for AmB microspheres

Formulation	Zero order	First order	Higuchi model	Peppas model	
				R^2	'n'value
AmB1	0.962	0.895	0.890	0.988	0.014
AmB2	0.991	0.829	0.973	0.992	0.576
AmB3	0.945	0.777	0.979	0.972	0.861
BSA1	0.984	0.817	0.988	0.990	0.911
BSA2	0.990	0.829	0.973	0.992	0.576
BSA3	0.986	0.823	0.966	0.970	0.348
BSA4	0.981	0.819	0.954	0.987	0.163
AG1	0.967	0.893	0.936	0.979	0.776
AG2	0.965	0.864	0.948	0.984	0.859
AG3	0.967	0.842	0.960	0.985	0.985

3-6 Anti-fungal activities:

The antifungals activities were given in Table (12) and Fig (14). This formulation was evaluated for its *in vitro* antifungal activity.

Table 12: The Inhibition Zone Diameter (cm) for Different microspheres against three fungal species

Microspheres	*Candida Albicans*	*Trichophyton* rubrum	*Aepergillus fumigates*
Drug	0.2,0.2	2.7,2.7	0.5,0.4
BSA (m)	0	0	0
BSA+G (m)	0	0	0
(BSA+drug) s	2.5 , 2.8	3.2 , 3.4	3.6 , 3.6
(BSA+G+drug) s	2.5 ,2.5	3.2 , 3.4	2.8 , 3.0
(BSA+drug) l	3.5 ,3.5	4.2 ,4.3	3.9 , 4.0
(BSA+G+drug) l	2.8 , 3.0	3.6 , 3.8	3.0 , 3.0

Note: BSA (m), BSA+G (m) [Albumin and Albumin+gelatin microspheres alone] ,(BSA+drug) s and (BSA+G+drug) s [microspheres loaded with AmB In the solid state], (BSA+drug) land BSA+G+drug) l [microspheres loaded with AmB In buffer solution](

The results show that biological activities for the microspheres loaded with AmB exceed biological activities against three types of fungal isolates including *Candida Albicans*, *Trichophyton rubrum, and Aepergillus fumigates* in comparison with activity of AmB alone respectively in inhibiting all three types of fungal isolates (Fig 14). This is due to the increase in the activity of the polymeric microspheres. Table (12) show that the BSA microspheres loaded with AmB in buffer solution is most effective in inhibiting the growth of fungal isolates and then BSA+Gelatin microspheres loaded with AmB in buffer solution. While the activity of microspheres alone was studied against same isolates we showed that it has un active against all isolate fungal .

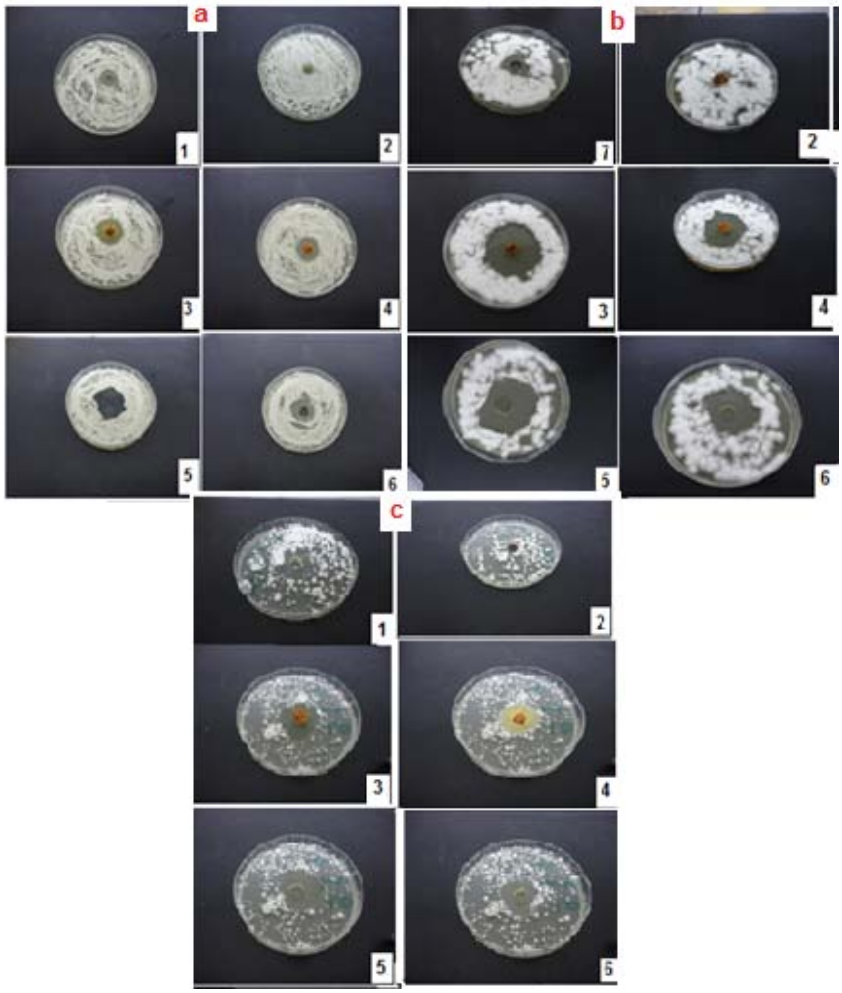

Fig.14 plates showing (1) drug ,(2) microspheres ,(3) ,(BSA+drug) s, (4) (BSA+G+drug) s ,(5) (BSA+drug) l,(6)(BSA+G+drug) l, against fungal isolates including(a) *Candida Albicans* ,(b) *Trichophyton rubrum, and(c) Aepergillus fumigatesn*

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تحضير وتشخيص وتقييم الجسيمات الكروية الدقيقة ذات الإطلاق المتوازن لعقار امفوتريسين B

الخلاصة

تم في هذا البحث تحضير وتشخيص وتقييم الاطلاق المتوازن للكرات المجهرية من خلال تحميل امفوتريسين B كنموذج للدواء على بوليمرات طبيعية مثل ألبومين المصل البقري والجيلاتين بتقنية تبخير المذيب باستخدام كلوتارالدهيد كعامل شابك. شخصت الجسيمات الكروية الدقيقة بتقنية مطيافية الاشعة تحت الحمراء وتقنية المسح التفاضلي المسعري على شكل وحجم والمجهر الضوئي , ثم دراسة تأثير تغيير نسب الدواء/ البوليمر وألبومين المصل البقري / الجلاتين وتحرر الدواء خارج الجسم الحي وحركية تحرر الدواء من الجسيمات الكروية الدقيقة . بينت النتائج ان اقطار الجسيمات الكروية الدقيقة تراوحت بين (16–60µm) , وكان معدل تحرر امفوتريسينB من الجسيمات الكروية الدقيقة الاصغر حجما اسرع من الجسيمات الكروية الدقيقة الاكبر حجما كذلك اظهرت النتائج ان نسب امفوتريسين B المتحرر يزداد كلما يزداد تركيز الدواء المحمل في الجسيمات الكروية الدقيقة.واشارت النتائج ان معدل تحرر الدواء كانت ضمن حركية المرتبة الاولى, وان تحرر الدواء من هذه الجسيمات يتم غالبا وفق الية الانتشار. وأخيرا، اظهرت النتائج أن الفعالية البيولوجية تجاه *Candida Albicans, Trichophyton rubrum, and Aepergillus fumigate* هي افضل مقارنة من الدواء لوحده.