Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading

Formulation and evaluation of acyclovir microcapsules using bakers yeast

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ARTICLE INFO

Article history:
Received 6 April 2010
Received in revised form 23 May 2010
Accepted 2 June 2010
Available online 20 June 2010

Keywords:
Acyclovir
Yeast
Formulation

ABSTRACT

Objective: To formulate and evaluate acyclovir microcapsules using bakers yeast. **Methods:** Acyclovir, pretreated yeast and deionised water were taken at a volumetric ratio of 1:2:4 respectively. This suspension was agitated in a magnetic stirrer at 25 ℃, 30 ℃, 35℃, and 40 ℃ for 4 hours. The suspension was then centrifuged for 10 minutes at 2 000 rpm. The supernatant solution was decanted and the cells were washed 5 times with deionised water. Then the suspended drug entrapped yeast cells were dried in a lyophillizer for 48 hours. The yield was noted. **Results:** The first four formulations were done with 200 mg of the drug, followed by 400 mg for the next four formulations and 800 mg the last four formulations. SEM showed that the surface of the microcapsules was intact, with no burst characteristics. FTIR showed no interaction between acyclovir and the cell wall. DSC showed that the peak was within the standard values. The mean particle size for all the samples was 8 μm in diameter. The dissolution studies were done for all the twelve samples and showed a Fickian model of diffusion. **Conclusions:** From the results it is inferred that the samples prepared at 40 ℃ (FY-4, FY- 8, FY-12) show better entrapment and release. So these samples are formulated in the form of a suspension and compared with marketed acyclovir suspension using HPLC technique. The formulated suspensions with FY-4, FY-8 and FY-12 shows drug content in accordance with the standards of the pharmacopoeial limits.

1. Introduction

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body in order to promptly achieve and thereby to maintain the desired concentration[1]. Micro encapsulation is a process in which tiny particles or droplets are surrounded by coating to give small beads with many useful properties. In its simplest forms a microcapsule is a small sphere with a uniform wall around[2]. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell, coating or membrane[3]. A range of materials are suited for use as the capsule material: lipids, wax, crystals starch, modified starch, cellulose, phospholipids and other polymers. In general, microcapsules size ranges from 5-500 μm. They can be made below 1 μm and upto 5 000 μm in size. Micro-organisms offer certain advantages over conventional process, as microcapsules are preformed[4,5]. The technology is based on using yeast and other single micro organism as capsules to protect and deliver the active drug. Micro organisms were first used to encapsulate fat soluble materials[6]. Yeast contains very low level of fat (less than 10 percent) and used as micro capsules without lipid extending substances[7]. The technology of micro-encapsulation using yeast cell is unique as it involves the use of preformed walls and membranes of micro organisms to provide the capsules[8,9]. This method can improve the shelf life and bioavailability of active ingredients. Antiviral drugs are a class of medication used specifically for treating viral infections[10]. Like antibiotics, specific antivirals are used for specific viruses[11]. Most of the antiviral drugs available are designed to deal with HIV, Herpes virus, which is best known for causing cold sores but actually covers a wide range of diseases, hepatitis B and hepatitis C viruses which can cause liver cancer[12,13] Acyclovir is an analogue of 2-deoxy guanosine that exerts its antiviral effects after being metabolized to acyclovir tri phosphate. Acyclovir triphosphate is 30-50 times as potent inhibitor of herpes simplex type-1 DNA polymerase[14]. Their meager production of acyclovir triphosphate in uninfected cells and its specificity for viral DNA polymerase result in minimal

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cellular toxic effects. Acyclovir has proved effective for the treatment of infections caused by herpes simplex virus type 1 and 2 and varicella-zoster virus and for suppression of some forms of cytomegalo virus disease. Acyclovir when orally administered, peak plasma concentration occurs after 1-2 hours. It has a high distribution rate, only 30 percent is protein bound in plasma. The elimination half life of acyclovir is approximately three hours[15]. It is renally excreted partly by glomerlur filtration and partly by tubular secretion. It has not been shown to cause teratogenic and carcinogenic effects. Acyclovir is marketed as tablets (200 mg, 400 mg and 800 mg) topical cream(5%), intravenous injection (25 mg/mL) and ophthalmic ointment(3%).

2. Materials and methods

2.1. Pre-treatment of yeast cells

Pre-treatment of these cells was done prior to the encapsulation process to assess the importance of cell viability. A suspension of fresh yeast (100 mL of 50% solids) was treated overnight with sodium azide (2 g), a respiratory inhibitor used to prevent the cells from performing any energy dependant process. Sterilization by autoclaving is a thermally destructive process denaturing any carrier protein molecules likely to be involved in facilitated diffusion process which may be responsible for encapsulation process.

2.2. Preparation of acyclovir microcapsules using Baker's yeast

Acyclovir, yeast and distilled water were taken in the volumetric ratio of 1:2:4 respectively. This suspension was agitated in a magnetic stirrer for 4 hours. The suspension containing the cells were then centrifuged for 10 minutes at 2 000 rpm. The supernatant solution was decanted and the cells were washed 5 times with deionised water and dried in a lyophillizer for 48 hours.

The effect of temperature and the drug concentration on microcapsule formulations were studied. The above procedure was followed at 4 different temperatures (25 ℃, 30 ℃, 35 ℃ and 40 ℃) for 3 different doses of drug (200 mg, 400 mg, 800 mg) (FY1-FY12).

3. Results

The microcapsules of acyclovir was prepared using the drug, Baker's yeast and water at specific ratio and various temperatures and lyophilized and the yield was obtained. The surface morphology shown by scanning electron microscopy (SEM) was intact and with no burst on the surface. The SEM of FY-3 was considered to be the best which was done at 10 μm and 500 μm (Figure 1 & 2).

The standard graph of the pure sample was made as per the procedure. The particle size analysis showed size of 0-10 μm in diameter was common in most groups except group FY-9, where most particles were found to be at 10-20 micrometer. Particle size analysis of sample FY-8 microcapsules by optical microscopic method was shown in (Figure 3, 4, 5).

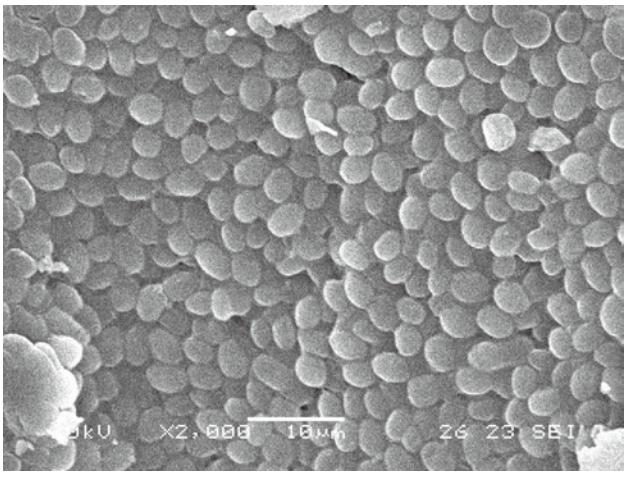

Figure 1. Morphology of FY-3(10 μm) shown by scanning electron microscopy.

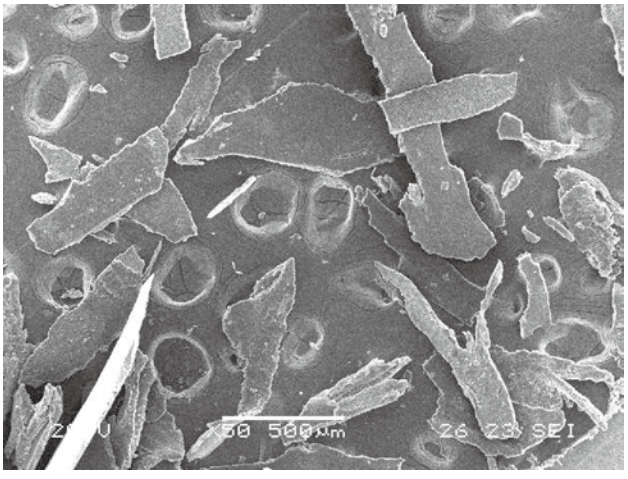

Figure 2. Morphology of FY-3(500 μm) shown by scanning electron microscopy.

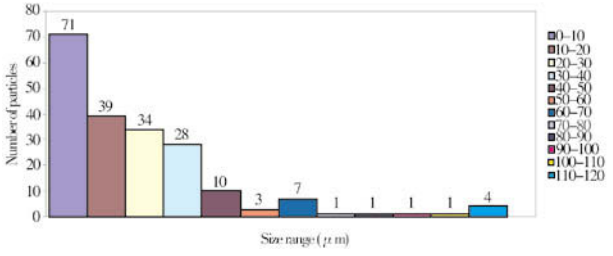

Figure 3. Particle size analysis of sample FY-8 microcapsules by optical microscopic method.

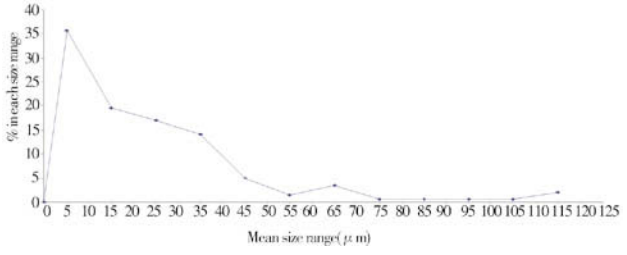

Figure 4. Frequency distribution curve of FY-8.

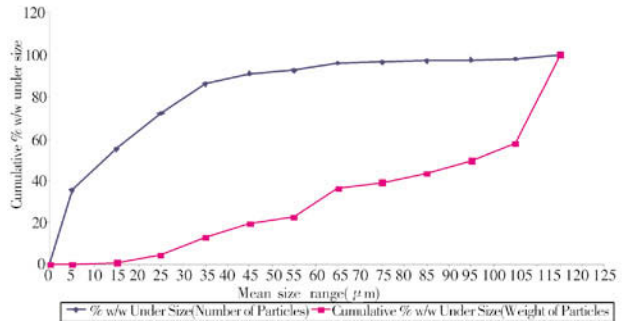

Figure 5. Cumulative under size curve of FY-8.

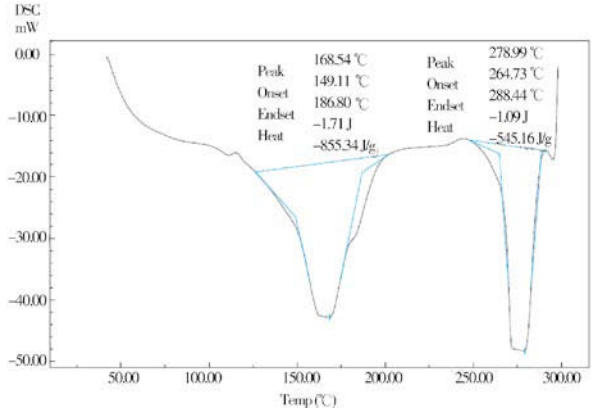

Figure 6. Thermal analysis of standard acyclovir.

The entrapment efficiency of the twelve samples was done for every 100 mg of the sample. FY-1 showed the least entrapment efficiency of 90.35 mg and FY-8 showed the most (97.61 mg) (Table 1). According to the amount of drug released from these samples (Table 1) it was shown that the amount released increases with temperature.

The release study of acyclovir was done for the first two hours at an acidic pH using hydrochloric acid (pH 1.2) and then it was continued at an alkaline pH using phosphate buffer (pH 6.8). This was carried out for all the twelve formulations. The cumulative release at the end of the 2nd hour in the acidic pH and at the end of the 8th hour in alkaline pH were 29.5 mg and 191 mg for FY-1, 30.5 mg and 195 mg for FY-2, 30 mg and 193 mg for FY-3, 31.5 mg and 197.5 mg for FY-4, 55 mg and 395 mg for FY-5, 59 mg and 392 mg for FY-6, 61 mg and 396 mg for FY-7, 115 mg and 398 mg for FY-8. From FY-9 to FY-12, the cumulative release at 6th hour was also calculated, at the end of 2nd, 6th and 8th hour, they were 118 mg, 156 mg and 776 mg for FY-9, 114 mg, 152 mg and 792 mg for FY-10, 124 mg, 136 mg and 798 mg for FY-11. For FY-12 it was 122 mg at the end of the 2nd hour in the acidic pH and 792 mg in the alkaline pH.

The FT- IR of the microcapsules FY-1 to FY-12 was done. The spectra shows a strong absorption band at 1716.78 for C=O group. An intense peak is observed at 1635.1 cm^{-1} for-C=C-aromatic nuclei. Two strong absorption band was seen at 1488.01 and 1541.68 cm^{-1} for 2 C=N, 8 C=N, stretching in ring. Absorption is seen at 1308.91 for C-N stretching for primary amino group. Absorption is seen at 902.01 for 5,6 alkene -CH=CH- group. Two strong absorption bands at 3441.87, 3480 cm^{-1} and this confirms primary amino group. Absorption at 3551 cm^{-1}, indicates presence of O-H (primary alcohol). Absorption is seen at 3186.66 cm^{-1} for C-H stretching at alkenes. It was concluded that there was no interaction with acyclovir and bakers yeast based on the

Table 1
Preparation of acyloir loaded microcapsules using bakers yeast.

Sl No	Sample No	Temperature	Drug (mg) in 5 mL buffer (pH5)	Yeast (g) in 10 mL water	Water (mL)	Volume taken for stirring (mL)	Stirring in rpm	Yield %	Entrapment efficiency		Amount of drug released (mg)
									Absorbance	Entrapment (%)	
	FY-1		200	0.4				69	0.759	90.35	191.0
1	FY-5	25 ℃	400	0.8	20	35	2 000	76	0.770	91.66	395.0
	FY-9		800	1.6				94	0.773	92.02	776.0
	FY-2		200	0.4				71	0.765	91.07	195.0
2	FY-6	30 ℃	400	0.8	20	35	2 000	75	0.779	92.73	392.0
	FY-10		800	1.6				93	0.784	93.33	792.0
	FY-3		200	0.4				68	0.782	93.09	193.0
3	FY-7	35 ℃	400	0.8	20	35	2 000	74	0.790	94.04	396.0
	FY-11		800	1.6				91	0.799	95.11	798.0
	FY-4		200	0.4				70	0.812	96.66	197.5
4	FY-8	40 ℃	400	0.8	20	35	2 000	77	0.820	97.61	398.0
	FY-12		800	1.6				92	0.810	96.42	792,0

above given data.

Table 2
Formulation studies of acyclovir microcapsules by HPLC method.

Sl. No.	Sample taken	Peak area	Amount of acyclovir present in 400 mg of sample (mg)
1	Standard drug	4462254	400.0
2	Marketed sample	4451954	397.0
3	Sample 4	4380939	390.7
4	Sample 8	4394568	391.9
5	Sample 12	4326804	386.0

The High Performance Liquid Chromatography (HPLC) was done for the pure drug acyclovir, FY-4(200 mg) and the peak found was 4380939, FY- 8(400 mg) the peak was 4394568, and FY-12 (800 mg) the peak was 4326804 and the content uniformity was ascertained (Table 2).

The Thermal analysis (DSC) was done for the standard (pure drug) acyclovir and sample FY-1, sample FY-3, sample FY-5 and sample FY-7. For the standard (pure drug-acyclovir) two peaks were obtained at 168.54 ℃ and 278.99 ℃. For sample FY- 1 a single peak was obtained at 263.70 ℃. Sample FY-3 showed a single peak at 116.72 ℃, sample FY-5 showed two peaks at 160.49 ℃ and 260.77 ℃, Sample FY-7 gave one peak at 263.59 ℃. As per the standard (pure sample) the peak value is between 168.54 ℃ and 278.99 ℃. Except sample FY- 3 which showed a peak value of 116.72 ℃ all the other samples are within the range of the peak values of the standard acyclovir (Figure 6).

4. Discussion

The microcapsules of acyclovir was prepared using the drug, bakers yeast and water at specific ratio, at various temperatures and lyophilized to obtain the yield. The yield was maximum in the case of FY-9 (94%). The entrapment efficiency was found to be maximum in FY-8 (97.61). The SEM analysis revealed there was no burst on the surface and it was intact. The SEM of FY-3 was considered to be the best which was done at 10 μm and 500 μm. The particle size analysis showed size of 0-10 μm in diameter was common in most groups except group FY-9, where most particles were found to be at 10-20 micrometer.

Entrapment efficiency for every 100 mg of the samples was done for all the twelve samples, FY-8 showed maximum entrapment of 97.61 mg.

The FTIR of the twelve samples showed no interaction between acyclovir and bakers yeast. The thermal analysis of standard (pure drug acyclovir) showed two peaks at 168.54 ℃ and 278.99 ℃, peaks of all samples were within the peak values of the standard one.

The dissolution study of the twelve samples showed the amount of release increased with temperature, and sample FY -8 had a cumulative drug release of 398 mg.

HPLC study showed FY-4, FY-8 and FY -12 were prepared in the form of suspension. It was found that the amount of acyclovir presented in 400 mg marketed sample was 397 mg, for FY-4, FY-8 and FY-12 was 390.7 mg, 391.9 mg and 386 mg, which are all above 95% and almost equal to the label claim of the marketed samples. So it has confirmed that sample FY- 8 is the best microencapsulated sample using acyclovir, yeast and water for micro encapsulation.

Conflict of interest statement

We declare that we have no conflict of interest.

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