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Formulation and evaluation of biodegradable microspheres of tinidazole

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Abstract

The aim of present study is to develop biodegradable microspheres of Tinidazole. Bovine Serum Albumin was used for the preparation of microspheres. They were made in four batches. The emulsion cross-linking method was used for the preparation. The quantity of BSA varies for each formulation. Formulations were evaluated for particle size, Melting point, TLC, entrapment efficiency and *in vitro* release studies. Depending upon the drug to polymer ratio, the entrapment, loading were found to range between 48, 55, 75 and 78 (in %) respectively. Particle size of prepared microspheres was measured using a compound microscope. The surface topography and internal textures of the microspheres was observed by scanning electron microscopy. The microspheres were spherical, discrete and compact and size distribution was between 33.28 to 36.25 µm. *In vitro* studies were carried out at different pH for a period of 18 h and compared with marketed formulation. From all the batches it is concluded that when concentration of polymer increases microspheres shows more controlled and prolonged release. The drug release was between 66, 51, 48, 42 (in %). The drug release from 1:4 is most prolonged and constant. Both the IR spectra of drug and formulation were almost same. Combination multitone recorded due to N=O stretching and S=O in the IR region of 1500-1250 cm^{-1}.

Keywords: Biodegradable microspheres, BSA, Tinidazole, *In vitro* release.

Introduction

Microspheres come under controlled drug delivery system. Microspheres are characteristically free flowing powders consist of protein/synthetic polymer which are biodegradable in nature and ideally having particle size less than 200µm. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for the control release of drug [1].

Microspheres have been extensively studied for use as drug delivery systems, where they have been shown to protect sensitive macromolecules from enzymatic and acid degradation, and allow controlled release and tissue targeting of the formulated drug [2]. Tinidazole is a nitroimidazole derivative, antiparasitic drug used against protozoan infection. It has broad spectrum cidal activity against protozoa including Giardia Lamblia many anaerobic bacteria such as fragilis,

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fusobacterium, clostridium Perfrigens, cldifficile, Helicobacter pylori. Its elimination half life is 12 hours [3].

Materials and methods

Tinidazole was obtained as gift sample from FDC limited Mumbai. Bovine serum albumin, Span 80, glutaraldehyde and toluene were obtained from commercial sources and were of analytical grade.

Preparation of microspheres of tinidazole

BSA microspheres containing tinidazole were prepared by the emulsion technique. An aqueous solution of BSA (20 % w/v) was adjusted to PH 10.Take 1ml.of BSA solution and to this add 0.1gm of tinidazole and dissolve. This solution was dispersed in a 10ml.solution of span 80 in toluene (13 %) by vortexing. This dispersed solution is stirred at 300rpm and gradually added to 0.5-1ml.of glutaraldehyde in distilled water (10 %), which was maintained at PH 10.After initial cross linking, add 20 ml. of acetone with continuous stirring for 5 hours. The microspheres formed were washed with acetone five times and with water three times and then dried to get free flowing microspheres in a powder form. By varying the drug: polymer ratio, five batches of microspheres were prepared [4].

Table1. Composition of tinidazole microspheres.

Batch code	Drug: polymer
A-1	1:1
A-2	1:2
A-3	1:3
A-4	1:4

Physicochemical evaluation of the microspheres

Size analysis

Particle size of prepared microspheres was measured using a compound microscope and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer.

Morphology

The surface topography and internal textures of the microspheres was observed by scanning electron microscopy.

Melting point

Microspheres were taken and placed within melting point apparatus inside the capillary. Microspheres were sufficiently heated to get their melting point.

Thin layer chromatography

A pure sample of tinidazole 5 mg was dissolved in 0.5%w/v solution of ammonium carbonate to produce 4.17ml.Similarily, 5 mg equivalent tinidazole microspheres and 5 mg of BSA were separately dissolved in 0.5 % w/v solution of ammonium carbonate. These solutions were spotted in a TLC Plate and marked. Then the plate was placed in closed vessel containing 15.6 % w/v solution of sodium dihydrogen phosphate adjusted to PH 4.8 with sodium hydroxide solution as the mobile phase. The developed spots were noted and Rf value were measured. The drug stability was studied by means of comparison of Rf values [4].

Drug content determination

Microspheres thoroughly triturated and suspended in minimal amount of alcohol. This suspension is diluted with water and this is then filtered to separate shell fragments and drug content was analyzed by UV Spectrophotometer at 366.4 nm [5].

$$\% \text{ incorporation} = \frac{\text{Drug content in microspheres}}{\text{Drug included in the formulation}} \times 100$$

***In vitro* drug release**

In vitro release profile of tinidazole microspheres from the preparations was examined in pH 1.2 buffer from 0-2 hr, in pH 4.5 phosphate buffer from 2 to 4 h and in phosphate buffer pH 7.2 from 4 to 12 h using the rotating basket method specified in USP XXI at 100 rpm. Microspheres equivalent to 100 mg of drug were placed in the basket and the medium was maintained at 37 ± 0.5^0C. An aliquot of 10 ml were withdrawn periodically at intervals of one h and same volume of fresh medium was replaced. The concentration of the drug released at different time intervals was determined by measuring the absorbance at 366.4 nm [6].

IR Analysis

IR studies of drug and microspheres were carried out by IR Spectrophotometer and record the spectra in the IR region of 4000-625 cm^{-1}. Compare the position and relative intensity of the absorption band of the microspheres and pure tinidazole.

Result and discussion

Morphology

The result shows microspheres were spherical in shape with smooth surface [Figure 1].

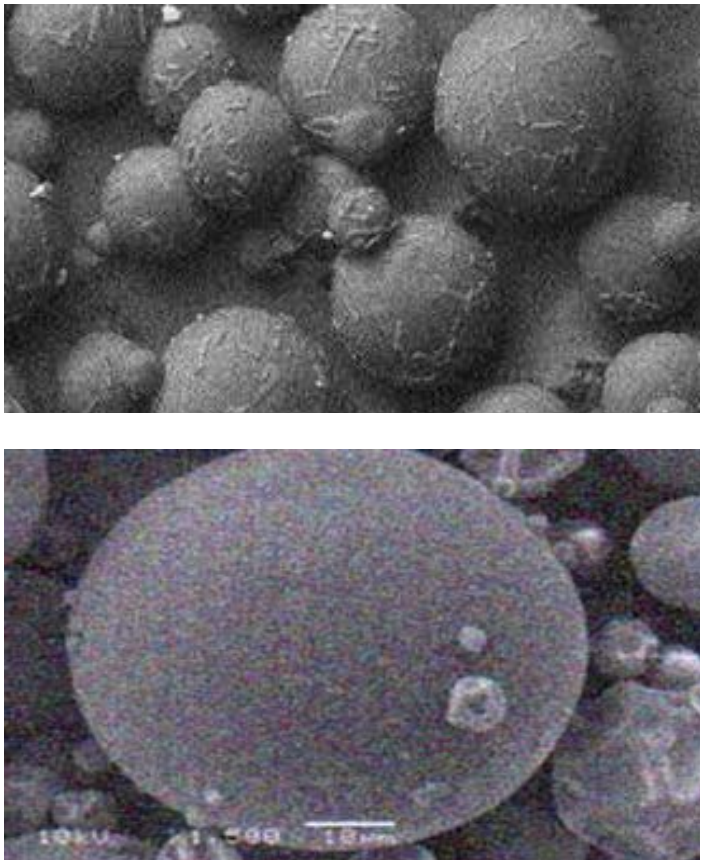

Figure 1. SEM images of microspheres

Size analysis

Results of all four formulation showed that particle size of prepared microspheres was in the range of 33.28 µm to 36.25 µm so it was concluded that particle size of prepared microspheres increases with increase in polymer concentration as shown in table 2.

Melting point

The melting point of free drug and drug inside the microspheres were found to be 126^0C and and 131^0C respectively. The melting point of free drug and microspheres are very close, this shows that after entrapment inside the formulation drug retain its behavior and nature as its original free state i.e. no change in the nature of drug.

Table 2. Data of particle size analysis.

Batch code	Particle size (in µm)
A-1	33.28
A-2	35
A-3	35.14
A-4	36.25

Thin layer chromatography

TLC was done to check the drug stability in the microspheres. The Rf value of drug and microspheres were found to be 0.820 and 0.811 respectively. So they show that process does not affect drug stability.

Drug content determination

First two batches shows entrapment of drug near to 50% whereas last two batches shows entrapment more than 70%.This shows that drug entrapment increases with increase in amount of the polymer [Table 3].

Table3. Data of drug content.

Batch code	% Drug Entrapment
A-1	48
A-2	55
A-3	75
A-4	78

In vitro Drug release studies

The drug release is controlled over 18 hours from all batches. The release from 1:4 batch is most constant and prolonged over time. From all the batches it is concluded that when concentration of polymer increases. Microspheres show more controlled and prolonged release. Drug release study shows that for a fixed time hours, microspheres varies its drug release, after that drug release become stagnant [Table 4 and Fig 2 (a-d)].

Table 4. Data of drug release analysis.

Batch code	% Drug release after 18 h
A-1	66
A-2	51
A-3	48
A-4	42

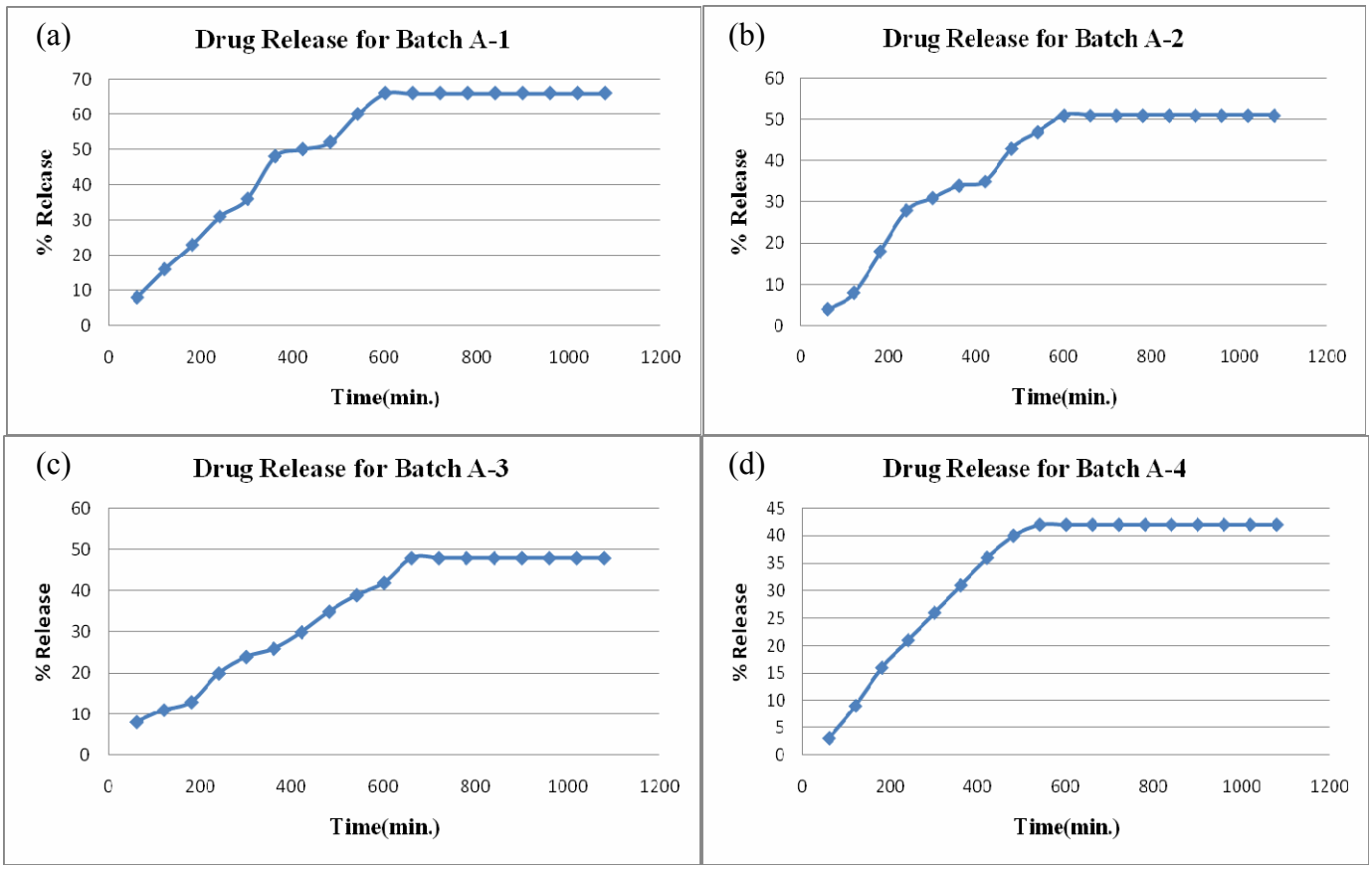

Figure 2. Release profile of batch (a) A-1, (b) A-2, (c) A-3 and (d) A-4

IR analysis

Both the spectra of drug and formulation were almost same. Combination multitone recorded due to N=O stretching and S=O in the IR region of 1500-1250 cm^{-1}. In between 1750-1500 cm^{-1} peaks recorded due to C=C.

Conclusion

From the present study it was concluded that biodegradable microspheres of tinidazole can be prepared using emulsion cross linking method. The drug entrapment is around 50% in first two batches while in last two batches it reaches up to 78%. This concluded that when the polymer concentration increases, drug entrapment efficiency also increases. Drug release from all the batches varies between 42 to 66%. Batch 1:4 shows more constant release. This concluded that when the polymer concentration increases drug release will be more constant and prolonged.

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