

## FORMULATION AND EVALUATION OF EGG ALBUMIN BASED CONTROLLED RELEASE MICROSPHERES OF METRONIDAZOLE

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### ABSTRACT

**Objective:** The objective of this study was to formulate controlled release microspheres of metronidazole using natural polymer egg albumin.

**Methods:** In this study egg albumin microspheres loaded with metronidazole were prepared by a chemical cross-linking method with the help of glutaraldehyde as a cross-linking agent. Microspheres were prepared by using different drug/polymer ratios. Egg albumin was used as a release retarding agent in the formulation to get controlled release of metronidazole.

**Results:** The drug-excipients compatibility studies reveal that there is no physical change observed in the drug and egg albumin mixture. Microspheres were found to be spherical, with porous outer skins and quite smooth surfaces when viewed microscopically. Best Results of the carr's index, Hauser's ratio as well as the angle of repose of the M5 batch were 10.2%, 1.11 and 35.37 respectively Maximum Drug entrapment efficiency (78%) and % yield (94%) was obtained with formulation M5. Drug release for the batch M5 (drug: polymer ratio-1:4) was 77.09% up to 10 h.

**Conclusion:** The hypothesis of this research work i.e. Controlled drug release by using natural, biodegradable polymer was well promoted by results of its evaluation. The *in vitro* release studies showed that drug release was controlled (best with M5 formulation) for more than 10 h. This study reveals the utility of microspheres in extending the drug release. This may, in turn, reduces the dosage frequency, thereby improving the patient compliance.

**Keywords:** Microspheres, Metronidazole, Egg Albumin, Glutaraldehyde, Chemical cross-linking method

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### INTRODUCTION

The controlled release provides expected delivery of drugs from polymeric matrices to match specific needs for physiological functions. Controlled or sustained drug release provides many advantages in comparison to its conventional forms including decreased side effects, drug concentration is kept in effective levels in plasma, improved utilization of drug and decrease the dosing times.

Microencapsulation is a process by which one can formulate controlled/sustained action dosage form for drugs with a short half-life. Microencapsulation increases the drug absorption and minimizes side effects due to the localized buildup of drugs in against of gastrointestinal mucosa. Microspheres constitute an important part of these particulate DDS. Microspheres are characteristically homogeneous, free flowing particles made from natural/synthetic polymer and ideally they have a particle size less than 200µm [1-3]. The microspheric drug delivery systems by the virtue of their controlled drug release help in reducing the administration frequency of the drug to the patient and also helpful in the delayed release of less bioavailable drugs.

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol) is a synthetic oral nitroimidazole antibiotic medication which is used for the treatment of infections caused by anaerobic bacteria and protozoa [4]. In addition to antibiotic it is also an amebicide, and antiprotozoal. Metronidazole is sparingly soluble in alcohol and water and slightly soluble in ether, chloroform, acetone, and methanol. Since it has a low plasma elimination, Half-life ranging from 6 to 7 h wide range of controlled release formulation have been developed from hydrophilic polymers such as hydroxyl propyl methyl cellulose (HPMC), sodium alginate, hydroxypropyl cellulose (HPC), chitosan and xanthan gum [5-8]. In the present study, an attempt was made to develop controlled release Metronidazole microspheres that will be capable of delivering acceptable bioavailability.

The use of Natural biodegradable polymers for drug delivery continues to be an area of active research despite the advent of synthetic biodegradable polymers. Egg albumin is a naturally obtained polymer

and is biodegradable in nature with good aqueous solubility. As well as it has a property of protein binding and physical entrapment. It also supports passive as well as the facilitated release of various types of incorporated drugs from the polymer matrix [9]. Egg albumin microspheres are widely accepted for drug delivery, fabrication of bio smears and delivery of both hydrophilic and lipophilic drugs.

The present study focuses on the formulation and evaluation of metronidazole microspheres containing a different ratio of drug and egg albumin polymer, cross-linked with Glutaraldehyde, for oral controlled release.

### MATERIALS AND METHODS

#### Materials

Metronidazole was received as a gift sample from Meyer Healthcare Ltd, Bangalore, India. Tween 80, Span 80, paraffin liquid light was obtained from Lobachemi Pvt. Ltd. Mumbai, egg albumin, n-hexane, petroleum ether, glutaraldehyde solution was obtained from Merck specialties Pvt. Ltd. Mumbai. All solvents and chemicals used are of analytical grade.

#### Preparation of egg albumin microspheres of metronidazole

Microspheres were prepared by using the chemical cross-linking method. In this method, various solutions of albumin (having a different concentration) in 15 ml of distilled water was prepared, with the addition of 0.5% Tween 80 and kept overnight. Then after 10-15 minute stirring, the drug was added to the above albumin solutions. The formulation was carried out with 1:0.5, 1:1, 1:2, 1:3, 1:4 drug: polymer ratios. Then above drug-polymer solutions were slowly added dropwise by injection to a beaker containing 150 ml of liquid paraffin containing 1% of span 80 as an emulsifying agent and stirred for 30 minutes at 250 RPM. The resulting microspheres were solidified using glutaraldehyde solution and stirred for a period of 1hr. to this add 5 ml. of n-hexane. The microspheres were collected by decantation and then washed with distill water followed by petroleum ether and dried at room temperature.

Table 1: Composition of microspheres

S. No.	Formulation code	Drug (mg.)	Polymer (mg.)	Drug: polymer Ratio	Light Liquid Paraffin (ml.)	Span 80	Gluteraldehyde (ml)
1.	M1	500	250	1:0.5	150	1%	20
2.	M2	500	500	1:1	150	1%	20
3.	M3	500	1000	1:2	150	1%	20
4.	M4	500	1500	1:3	150	1%	20
5.	M5	500	2000	1:4	150	1%	20

## Evaluation of microspheres

### Standard curve of metronidazole

100 mg of Metronidazole was accurately weighed and dissolved in 100 ml of PBS pH 7.4 to Prepare stock solution. Take 10.0 ml of this stock solution & dilute further to 100.0 ml with pH 7.4 buffer. From this sub-stock solution Pipette out 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml. and 1.4 ml of this solution & diluted to 10.0 ml in separate 10.0 ml volumetric flask to make 2, 4, 6,8, 10, 12 and 14 µg/ml concentration solutions. Then the absorbance was measured in a UV spectrophotometer at 319 nm against pH 7.4 buffers as blank. Calibration curve was constructed and shown in fig. 1.

### Drug-polymer interaction studies

Drug-polymer interactions were determined by FT-IR spectroscopy. The spectra were recorded for Metronidazole, egg albumin and physical mixture of Metronidazole: albumin (1:1). Samples were prepared in KBr disks (containing 2 mg sample in 200 mg KBr) with a hydrostatic press at a force of 5.2 τ cm<sup>-2</sup> for 3 min. Scanned in range of 7800 to 350 cm<sup>-1</sup>. and the resolution was 4 cm<sup>-1</sup>.

### Micrometrics properties of microspheres [10]

The particle sizes of the microspheres are determined by using an optical microscope fitted with an ocular micrometer which is calibrated with a stage micrometer. A total no. of 100 microspheres was evaluated, and its mean diameter was noted. The average particle size for each formulation ( $n = 3$ ) is shown in table 2

The flow properties and packing properties were determined by measuring the angle of repose, Carr's index, Hauser's ratio and bulk density (table 1).

#### i). Bulk density

Bulk density was determined by measuring the volume of 30 g of powder sample taken in a 100 ml graduated cylinder and then tapped it on a hard surface 3 times from a height of 1 inch using a tapped density apparatus.

$$U = M/Vb$$

Where, M = Mass of microspheres in gram

Vb = volume of microspheres (after three tapping)

#### ii). Tapped density

Tapped density was determined by mechanically tapping a 100 ml measuring cylinder containing the 30 g powder sample. After observing the initial volume, the cylinder was tapped 100 times from a height of 1inch on a hard surface using a tapped density apparatus.

$$b = m/vt$$

Where, m = mass of microspheres in gram

Vt= volume of microspheres (final tapped volume)

#### iii). Carr's Index

The Carr index (Carr's Compressibility Index) is an indication of the compressibility of powder. It is named from the pharmacologist Charles JelleffCarr (1910–2005). The Carr's Index is calculated by the formula-

$$\text{Carr's Index} = (\text{Tapped density} - \text{Bulk density}) \times 100$$

## Tapped density

### iv). Hausner's ratio

The Hausner ratio is a number which is correlated to the flowability of a powder or granules. It is named from the engineer Henry H. Hausner (1900–1995). The Hausner ratio is calculated by the formula:

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

### v). Angle of repose

The angle of repose is a characteristic property related to inter-particulate friction or resistance for movement between particles. The angle of repose is the constant, three-dimensional angle (relative to the horizontal base) formed by a cone-like pile of material formed while falling from the funnel.

$$\theta = \text{Tan}^{-1}(h/r)$$

Where,  $\theta$  = Angle of Repose, h= height of the pile, r= radius of the pile formed

From the study of micrometric properties of the microspheres, the conclusion was drawn regarding the nature of the flow of the microspheres prepared.

### Entrapment efficiency [11]

For the drug entrapment efficiency of microspheres, 50 mg of microspheres were accurately weighed and dissolved in methanol in a 50 ml volumetric flask to get a solution containing one mg drug per ml. The resulting solution was filtered by whatman filter paper and then suitably diluted to check for the absorbance on the UV spectrophotometer. The absorbance was measured at 319 nm using UV spectrophotometer (table 3).

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Amount of drug actually present}}{\text{Theoretical weight of the drug}} \times 100$$

### Percentage yield

Determining whether the preparation procedure used for incorporating a drug into the polymers is efficient and is of first importance. The raw materials, amount of active material, polymer(s) and other process parameters are deciding factors for the %yield of the product at the time of microspheres preparation. The yield is determined by weighing the microspheres and then calculated the percentage yield with respect to the total weight of materials taken, i.e., the weight of drug and polymers used (table 3). The formula for calculation of % yield is as follows;

$$\% \text{ Yield} = \frac{\text{Weight of Microspheres}}{\text{Theoretical weight of drug and polymer}} \times 100$$

### Scanning electron microscopy

Scanning electron microscopy (SEM) is one of the most common methods used to characterize drug delivery systems, having a large part of simplicity of sample preparation and ease of operation. Scanning electron microscopy was performed in order to characterize surface morphology of the microspheres. In this study, the morphological observations were carried out to study the surface morphology of metronidazole microspheres. SEM micrographs and typical surface morphology of the microspheres

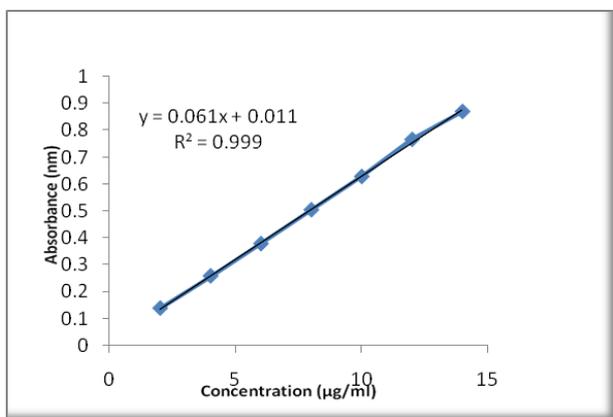
are shown in fig. 2. It was observed that microspheres were spherical in nature.

**In vitro release studies**

The *in vitro* release studies of Metronidazole from microspheres of all 5 formulations were performed out using USP 6 basket Digital dissolution test apparatus (Veego, Mumbai). 100 mg of microspheres were weighed accurately and filled into tea bags. These tea bags were tied using a thread with a paddle and loaded into the basket of the dissolution test apparatus. The dissolution test was performed by using 500 ml of 7.4 phosphate buffer, at 37±0.5 C and at 75 rpm. Sampling (5 ml) was at done 30, 60, 90... min and it was replaced with equal volume of fresh dissolution medium. After suitable dilution, the sample is analyzed for the drug content by scanning the sample at 319 nm using UV spectrophotometer (Shimadzu UV1700 japan) (table 4).

**RESULTS AND DISCUSSION**

Calibration curve of pure drug Metronidazole was done at λ max 319 nm in pH 7.4 with a UV-VIS spectrometer (UV-1700, Shimadzu Corporation). It obeyed Beer's law. The calibration curve was done in the concentration range of 2-14µg/ml.



**Fig. 1: Calibration curve of pure drug metronidazole in 7.4 pH buffer**

**Preparation of microspheres**

In the present work controlled release microspheres of Metronidazole were formulated using egg albumin polymer by chemical cross-linking technique. The microspheres obtained under these conditions were spherical, free flowing and without aggregation in the size range of 15-41 µm, 5 batches prepared with different polymer ratios were evaluated for physical properties like FTIR, SEM, particle size, Micromeritic properties Percentage yield, encapsulation efficiency, *in vitro* dissolution, of Metronidazole microspheres.

**IR spectroscopy**

The FT-IR spectra of the free drug and the microspheres were recorded. The drug-excipients compatibility studies reveal that there is no physical change observed in the drug and polymer mixtures. The IR spectrum of the drug, drug-albumin mixture and microspheres formulation were compared to find any change in the frequency of functional group in microspheres with a respective functional group of the drug. The spectral observations indicated that the principle IR absorption peaks observed in the spectra of the drug were close to those in the spectra of the microspheres indicates that there is no interaction between the drug and the polymer. The identical peaks corresponding to the functional groups and features confirm that neither the polymer nor the method of preparation has affected the drug stability.

**SEM analysis**

Surface morphology was using scanning electron microscopy (SEM). The examination of the internal structure of M5 microspheres shows that the interior of microspheres structure was solid in appearances with no pores or perforation. The microspheres were found to be spherical, with porous outer skins and quite smooth surfaces when viewed microscopically.

**Micrometrics studies**

The micrometric studies carried out, and the results of the carr's index, Hauser's ratio as well as the angle of repose of the M5 batch were 10.2%, 1.11 and 35.37 respectively, which were better than the results of other four batches i. e M1, M2, M3, M4. The results of the batch M5 were found to be in the acceptable range (table 2) and indicate that the batch was having fair flow properties.

**Table 2: Micrometrics properties of microspheres**

Flow properties	M1	M2	M3	M4	M5
Angle of repose	26.56	28.68	30.54	32.71	35.37
Bulk density	0.428	0.439	0.452	0.461	0.480
Carr's index	10.83	11.49	11.0	11.0	10.2
Hausner ratio	1.12	1.129	1.12	1.12	1.11

**Drug entrapment efficiency of microspheres**

From the percentage drug efficiency study, it was inferred that the entrapment of the drug in the microspheres was variable and it was changed with the change in the polymer concentration. As the polymer concentration increases, the drug encapsulation was found to be increasing in albumin microspheres which can be attributed to the increased availability of the polymer for encapsulating the drug. It was also observed that in batch M5 least amount of drug was wasted as most of it was entrapped. (table 3)

**Percentage yield**

The experiments were carried out and the results of % yield of albumin microspheres were 48.2% to a maximum of 94%. The maximum yield was obtained with formulation M5. (table 3)

**Table 3: Percentage yield, drug entrapment efficiency and average particle size of metronidazole microspheres**

Parameters	M1	M2	M3	M4	M5
% Yield	48.2	57.15	67.8	83.0	94.0
Drug entrapment efficiency (%)	57.20	61.32	67.08	72.12	78
Avg. particle size (µm)	55.65	49.06	40.7.92	31.78	25.6

**Table 4: Data for In vitro drug release profile of metronidazole albumin microspheres (Time in H)**

Time (in Hrs.)	Cumulative % drug release*				
	M1	M2	M3	M4	M5
0	0	0	0	0	0
0.5	13.26	11.81	9.12	8.19	6.14
1	21.5	17.99	13	14.2	10
2	30.48	27.3	25.67	21	17.47
3	39.8	35.23	33.45	28.48	22.79
4	48.85	42	42.17	36	29.54
5	58.6	51.61	49.94	44.92	36.4
6	68.75	60.34	57.4	52.13	44.73
7	77.26	69.21	64.74	60.12	52.82
8	85.7	77.89	73.47	68.13	61.75
9	93.2	87.07	82.99	76.2	69.87
10	100	95.32	90	84	77.09

**In vitro dissolution studies of microspheres**

The *in vitro* dissolution studies were conducted to check for the rate and time of the release of the drug (table 4). In all the formulations, with the increase in the polymer concentration, the rate and amount of drug release was found to decrease, which can be attributed to the greater binding of the drug with the polymer.

As the concentration of polymer increases, larger amounts of drug got binded in the polymer matrix. As a result, the rate of drug release from the microspheres decreases. From the data obtained it could be inferred that the controlled release of the drug from the microspheres was almost uniform and the release was found to be 77.09% for M5 over a period of 10 h.

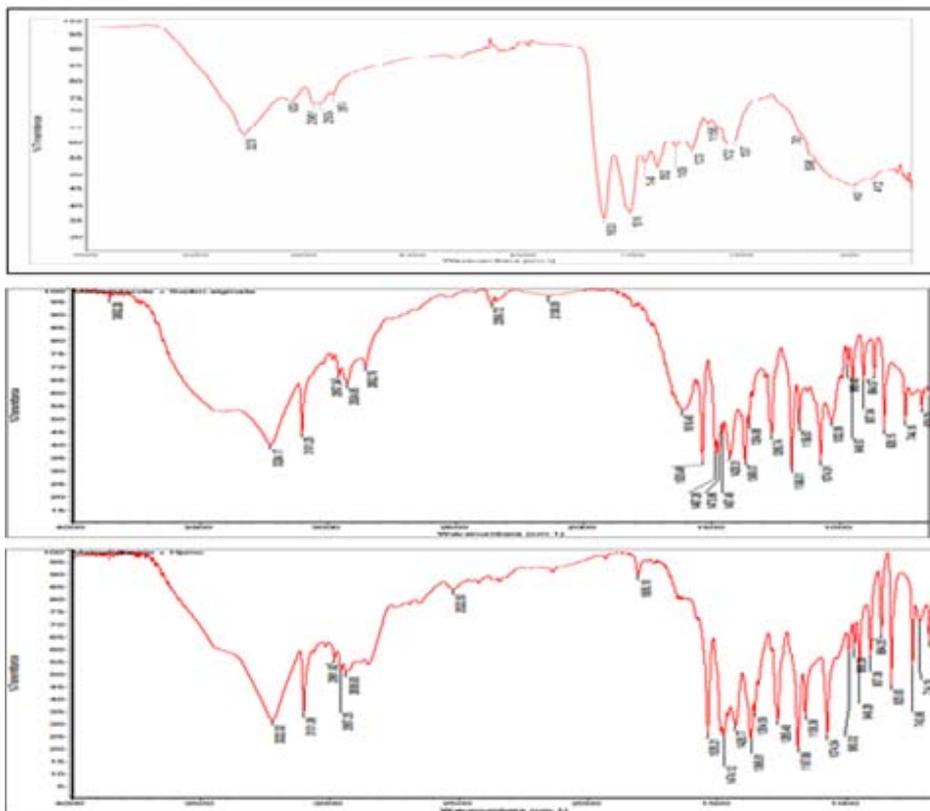


Fig. 2: FT-IR spectra for (A) egg albumin, (B) pure drug metronidazole and (C) metronidazole with egg albumin

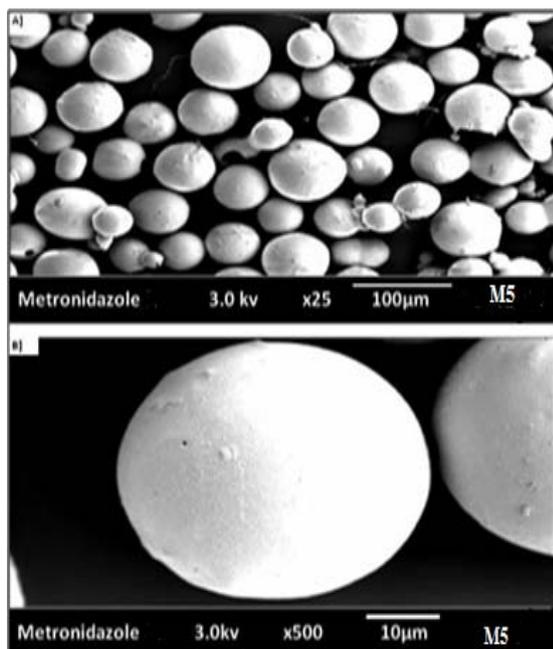


Fig. 3: SEM images of metronidazole loaded microspheres of formulation M5

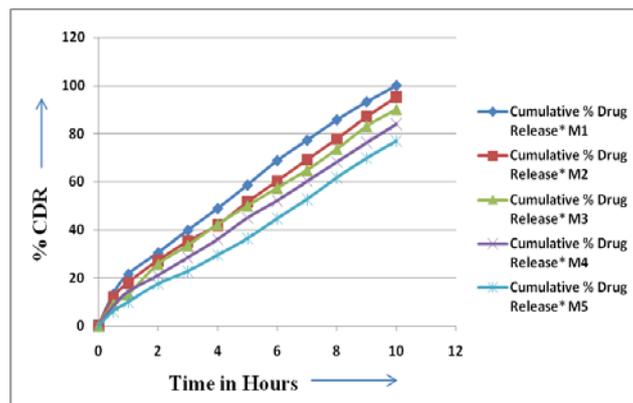


Fig. 4: Comparative release profile of metronidazole albumin microspheres

**CONCLUSION**

Microspheres for metronidazole by using natural polymer egg albumin were successfully prepared by using the chemical cross-linking method in order to improve the oral bioavailability with prolonged drug release. This method is very simple as compared to other tedious methods of microsphere preparation, as it requires simple manufacturing steps, as well as microspheres produced by

this method, are of good appearance and uniform sphere in shape. The concentration of the polymer influenced the particle size as well as the *in vitro* release. The *in vitro* release studies showed that drug release was controlled (best with M5 formulation) for more than 10 h. The present study signifies the utility of microspheres in retarding the drug release. This may, in turn, reduces the frequency of dosing, thereby improving the patient compliance. So from this, it could be concluded that the prepared microspheres can be used for the controlled drug delivery of the drug for a prolonged period.

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#### CONFLICT OF INTERESTS

Declare none

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