FABRICATION OF TINIDAZOLE LOADED MICROSPONGE

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ABSTRACT:

Microsponge drug delivery system is a novel drug delivery system which is based on polymer-based microsphere which contains voids having large porous surface. Microsponges are spherical in nature. They are used for sustain drug delivery while entrapping the drugs in the small pores which regulates the flow of the drug out of the porous surface. The main aim of formulating this delivery system is to incorporate the drug in minimum dose with enhance stability, reducing the side effect without compromising its efficacy. Microsponges are formulated for oral route; microsponges can control its drug delivery upto 12 hours. The aim of the present study to develop HPMC fabricated microsponge where DCM used as internal phase and PVA used as external phase and the drug entrapped here is Tinidazole, it is an anti-parasitic and antibiotic classified drug. It is used to treat Giardiasis, Giardiasis is a parasite intestinal infection caused by *Giardia duodenalis,* this is also known as *G.lambia* & *G.intestinalis.* In conventional dosage forms there is chance of having colonic cancer, ulcerative colitis and due to frequent dose requirements, the patient compliance is very low, so microsponges are advantageous in this case. Microsponges are stable at pH range 1-11 and upto 130^0C temperature. As Microsponges are obtained from various methods the common being Quasi-Solvent emulsions diffusion method. It is obtained by preparing two phases i.e., external and internal phase and mixed together and stirred while giving heat as the solvent evaporates it leaves quasi emulsion globules. Once the microsponges are produced, they are dried in oven at 40°C and evaluation is done. Various Evaluation parameters such as particle size, morphology, percent yield, drug interaction etc. are evaluated.

Keywords: Microsponge, HPMC, tinidazole, PVA, Giardiasis, Quasi-emulsion solvent diffusion method.

1. INTRODUCTION

A Microsponge drug delivery system (MDS) is a unique drug delivery system utilized to deliver drugs from the system in a controlled manner. Microsponge is small (5-300 µm in diameter), spherical, porous (0.25 µm) polymeric molecule looks like a bathroom sponge. The polymers are interconnected with each other and form a porous non-collapsible cage, and the drug is entangled within this, and its pores allow the drug to diffuse from the structure at a controlled rate up to 12 hours [1, 2]. Microsponge reports high entrapment efficiency and high compatibility with a wide range of active ingredients. Many scientists and researchers showed keen interest in Microsponge technology because of the controlled delivery of active ingredients to a specific site at minimal dose, increased stability, reduced systemic exposure and toxic effect [3, 4]. Drugs like- Domperidone (gastro pro-kinetic and anti-emetic), 5-flurouracil (anti-neoplastic), curcumin (anti-ulcer) are incorporated inmicrospongesby Quasi-emulsion solvent diffusion method for controlling the rate of drug delivery intended to be applied into target site. Microsponges are stable at a high range of pH (1-11) and a high temperature up to 130°C and compatible with most of the ingredients. Just because of the lesser pore size of the microsponge, micro-organisms cannot enter the structure and reach the active agents. Therefore, the microsponge delivery system does not need any preservative for chemical stability; they are self-sterilizing formulation. For being porous in nature, microsponges are thermally, physically and chemically stable than any other lipid-based colloidal system [5, 6, 7].

Giardiasis is a parasite intestinal infection caused by *Giardia duodenalis*, this is also known as *G.lambia & G.intestinalis* [8]. Mostly it is occurred in early autumn and in the summer months. In clinical presentation there is a bimodal age is distributed like as 1 to 9 years and 45 to 49 years. This situation is most common in man. Giardia parasites exist in the intestines of the people and animal. At first this microscopic parasites are encased within cysts (hard cell) and then passed in stool. These cysts allow the parasite to endure outside the intestines

for month. Thereafter dissolved cysts and released the parasites. In giardiasis the expression of brush border enzymes is decreased, changes in morphological character to the microvillus, intestinal permeability increased. Transmission of this infection through hazardous food, water person to person contacts [9]. Sometime there are never developed signs or symptoms in some people with giardiasis, but parasite still carry by them and spread in other person through their stool. Otherwise after one- or two-weeks signs and symptoms may occur include fatigue, diarrhoea, gas, dehydration, stomach cramps, nausea, watery and weight loss. Medication of giardiasis include: Tinidazole, Metronidazole, and Nitazoxanide [10].

As an example of synthetic antiprotozoal and antibacterial agent is tinidazole. The nitro- group of tinidazole is reduced by cell extracts of Trichomonas. The free nitro- radical generated as a result of this reduction may be responsible for the antiprotozoal activity. Tinidazole tablets is a nitroimidazole antimicrobial used to treat giardiasis in patients age 3 and older. It also used in trichomoniasis; amebiasis & in adult women it is used as bacterial vaginosis [11].

The purpose of the study is to formulate & develop the colon drug delivery system by tinidazole microsponge. The drug is supposed to directly target into colon and drug reaches with its minimum concentration & the residence time of drug will increases in colon which will help to reduce dosing frequency and also reduce the local and systemic side effects. To reach the colon, the tablets should be prepared using excipients which dissolve and degrade only in the colon [12, 13, 14].

2. MATERIALS AND METHOD:

2.1 Materials:

Tinidazole was purchased from Wyeth Limited and HPMC, poly vinyl alcohol, Potassium Dihydrogen Phosphate, Sodium Chloride were procured from Loba Chemical pvt. Ltd and Dichloromethane, Potassium Di-hydrogen Phosphate was purchased from Merck life Science pvt.Ltd.

2.2. Method

2.2.1. Pre-formulation study

2.2.1.1. Partition coefficient determination
The partition coefficient was determined by Shake flask method, in this method the drug was dissolved in ethanol and solvent was shaken for 30 min. The mixture was allowed standing for 5 min. The aqueous solution was centrifuged and then assayed [15].
2.2.1.2. Solubility study: Tinidazole was dissolved in 1.5 pH acidic buffer and water then dissolution was carried out and absorbance of tinidazole was checked by UV spectroscopy at 318 nm wavelength [16].
2.2.1.3. Melting point determination: The melting point of tinidazole drug was determined by taking small amount of drug into a small capillary tube, attached with thermometer centred in a heating bath then slowly heating the bath, and initial and final temperature of melting point was recorded [17].
2.2.1.4. Calibration curve: 10 mg of Tinidazole was dissolved with 10 ml of ethanol. From this stock solution different aliquots were prepared. Absorbance's of every concentration of drug solutions were checked under UV spectroscopy at 300-400 nm range [18].
2.2.2. Fabrication of tinidazole loaded microsponge: The Tinidazole loaded microsponge was prepared by quasi emulsion solvent diffusion method [19, 20, 21]. The internal phase was prepared by adding HPMC (200 mg) and tri-ethylcitrate (1% w/v) dissolved in 5 ml of dichloromethane. Tri-ethylcitrate (TEC) was added for obtaining the plasticity. Tinidazole was added into the internal phase with continuous stirring (1000 rpm). Then the internal phase was added into external phase containing polyvinyl alcohol solution in water. After 6 hrs of continuous stirring the microsponges were formed due to complete evaporation of dichloromethane from the emulsion. After that the microsponges were filtered by filter paper and dried under vacuum dryer at 40°C for 12 h. The compositions of microsponge formulation are given in Table 1 [22, 23].

Table-1- Composition of microsponge

Components	**Formulations**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Tinidazole	100	100	100	100	100	100
HPMC	100	200	300	400	500	600
TEC	1	1	1	1	1	1
Dichloromethane	5	5	5	5	5	5
PVA	0.5	0.5	0.5	0.5	0.5	0.5

2.2.3. Evaluation of Tinidazole loaded microsponge

2.2.3.1. Particle size determination:-Particle size of microsponge was determined by optical microscope as well as ocular and objective microscope. Tinidazole loaded microsponge was suspended in glycerol and then placed on the slide to observe under optical microscope [24, 25].

2.2.3.2. Determination of entrapment efficiency, percentage yield, drug loading: 10 mg of prepared microsponge was dissolved in 10 ml of dichloromethane and filter the solution and then from the filtrate 1 ml was withdrawn then diluted with 10 ml of fresh solution and absorbance was checked under 318 nm in UV spectroscopy and drug content was determined by standard curve. By following formula encapsulation efficiency, percentage yield, drug loading was calculated [26, 27].

$$\textbf{Entrapment efficiency}=\frac{actual\ mass\ of\ drug}{mass\ of\ drug\ present\ in\ microsponge}\times 100$$

$$\textbf{Percentage yield}=\frac{actual\ mass\ of\ drug}{mass\ of\ drug+polymer}\times 100$$

$$\textbf{Drug loading}=\frac{\boldsymbol{mass\ of\ drug\ in\ microsponges}}{\boldsymbol{mass\ of\ microsponges}}$$

2.2.3.3. Determination of surface morphology: The surface morphology of prepared microsponge was determined by scanning electron microscopy (SEM) [28].

2.2.3.4. In-vitro dissolution study:

In-vitro drug release study was carried out by USP type-II paddle type dissolution apparatus. The prepared microsponges were weighed about 100 mg and zipped into muslin cloth and tied into paddle in immersed in 900 ml of dissolution medium. The content was allowed to rotate in 100 rpm at 37±0.5^{0}C by using 1.5 pH acidic buffer and sample was withdrawn after time intervals and fresh sample were added to maintain sink condition then samples were analysed under UV spectroscopy under 318nm [29,30].

3. RESULT

After preparation of Tinidazole loaded microsponge by quasi-emulsion solvent diffusion method they were subjected to different evaluation test.

3.1. Pre-formulation study

Before the formulation of microsponge, Tinidazole drug was allowed to preformulation study. The melting point of Tinidazole was found to be -127.5^{0}C and the solubility of pure drug in water was less as compared to acid medium. The calibration curve for Tinidazole was plotted by taking concentration v/s absorbance and regression equation was calculated. Following table represent the observed value of absorbance of different concentration of pure drug.

Table no-2: Calibration curve of Tinidazole.

Concentration (ppm)	Wavelength	Absorbance
5	318	0.2608
10	318	0.4352
15	318	0.5913
20	318	0.7059
25	318	0.7921
30	318	0.9085

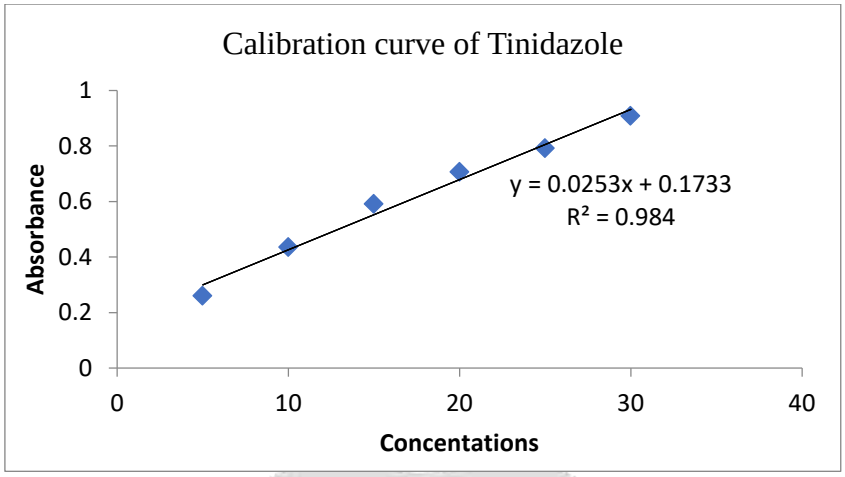

Fig.1 Calibration curve of Tinidazole drug

3.2. Evaluation study

3.2.1. Determination of entrapment efficiency, percentage yield, drug loading:

Table no-3: Evaluation parameters of Tinidazole loaded microsponge.

Formulation	Entrapment efficiency (%)	Percentage yield (%)	Drug loading (%)	Average particle size(µm)
F1	72.52	85.12	45.26	51.56
F2	82.85	84.58	49.82	53.23
F3	84.98	80.18	58.23	49.98
F4	87.52	83.68	57.28	46.45
F5	93.58	89.95	65.89	50.68
F6	92.94	84.74	64.32	52.18

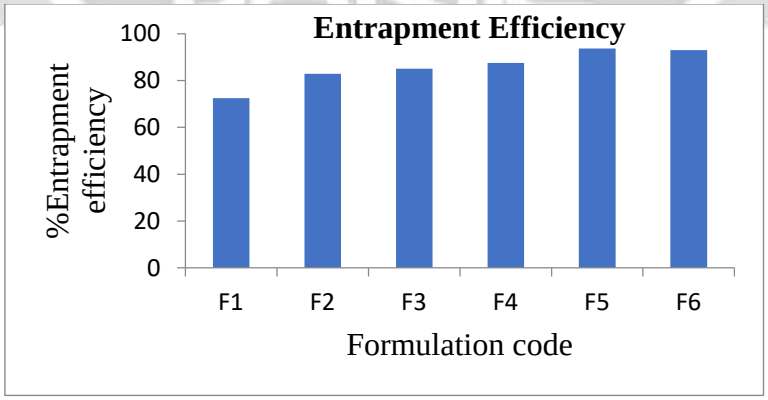

Fig. 2 % Entrapment efficiency of Tinidazole loaded microsponge

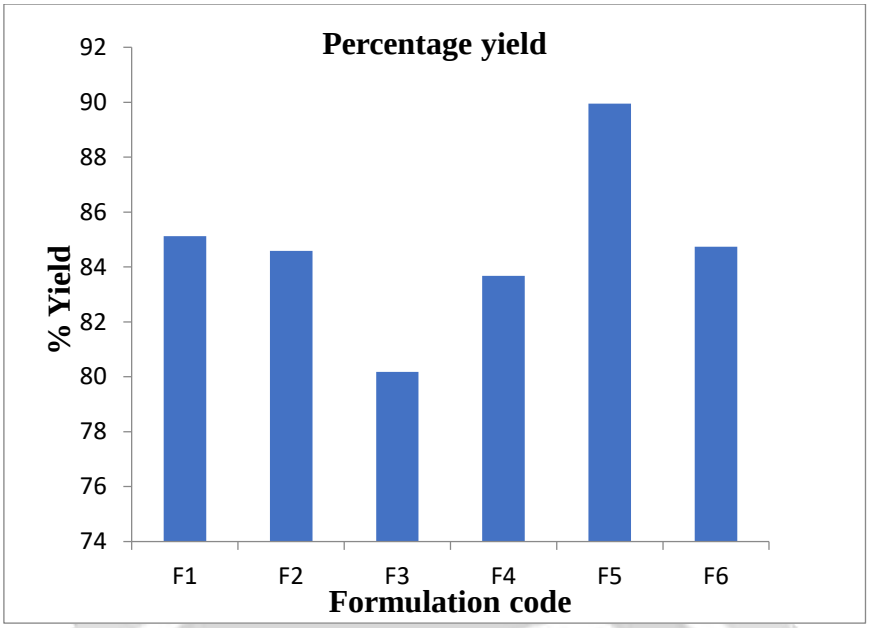

Fig. 3 Percentage yield of Tinidazole loaded microsponge

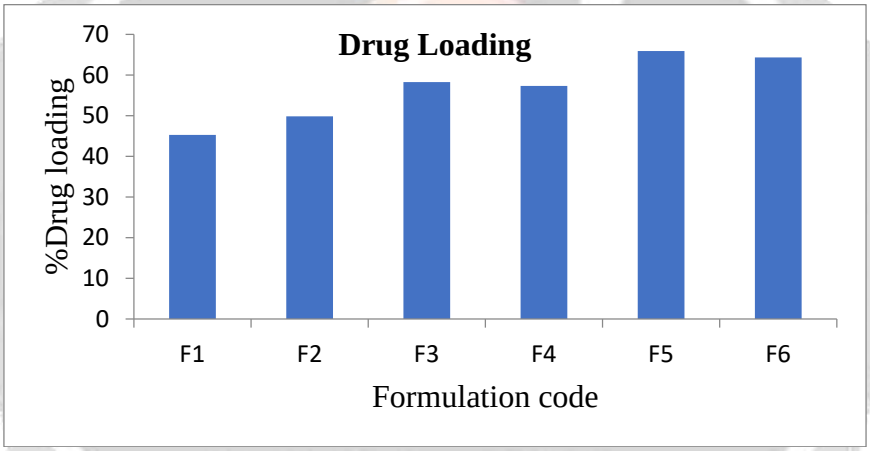

Fig. 4 % Drug loading of Tinidazole loaded microsponge

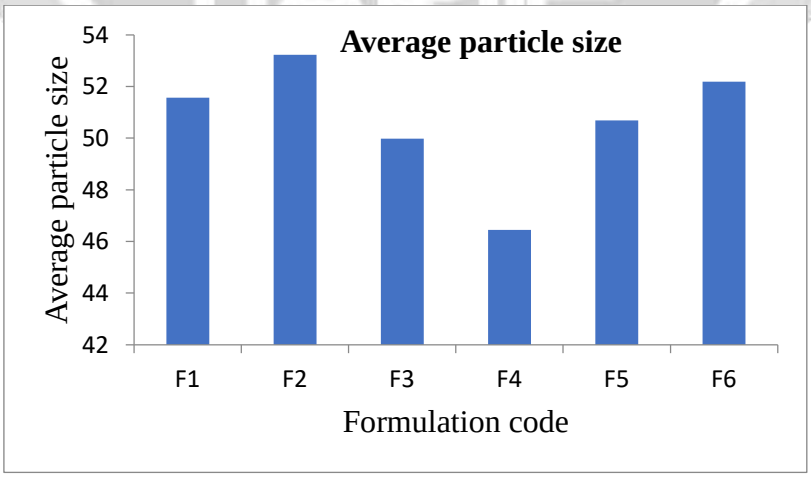

Fig. 5 Average particle size of Tinidazole loaded microsponge

3.2.2. Determination of morphology: The morphology of produced microsponge containing tinidazole and HPMC were spherical, porous, non-aggregated as observed in SEM. Following picture represent the morphology of prepared Tinidazole loaded microsponge.

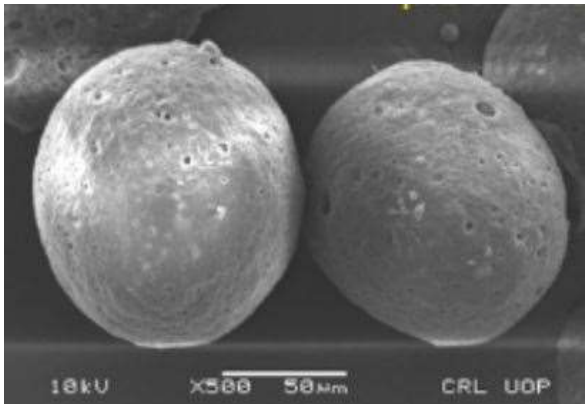

Fig. 6 Morphology of Tinidazole loaded microsponge

3.2.3. In-vitro dissolution study:

Tinidazole loaded microsponges were allowed to *in-vitro* dissolution test and the microspongs were releases the drug up-to 12 hrs. The cumulative amount of drug release (%CDR) was increased gradually with increase in time. **Table no-4** represents the cumulative amount of drug release of every formulation with time.

Table no-4: In-vitro drug release of Tinidazole loaded microsponge.

Time (hrs)	F1 (%CDR)	F2 (%CDR)	F3 (%CDR)	F4 (%CDR)	F5 (%CDR)	F6 (%CDR)
1	7.21	8.23	7.61	9.47	8.98	8.96
2	14.69	16.87	18.20	17.69	14.9	15.60
3	22.87	22.69	36.48	29.85	20.52	39.52
4	39.11	34.29	49.55	49.61	26.82	46.11
5	58.77	39.52	65.21	58.25	35.78	59.55
6	61.55	49.58	75.23	65.97	46.65	68.47
7	73.69	54.31	82.69	78.36	58.87	78.38
8	75.27	67.36	84.52	82.36	67.12	82.79
9	77.85	78.69	86.94	84.49	75.08	83.65
10	78.63	79.58	87.11	86.58	82.54	84.57
11	79.45	81.69	88.92	87.01	90.48	91.52
12	79.58	83.26	89.35	88.85	98.86	93.65

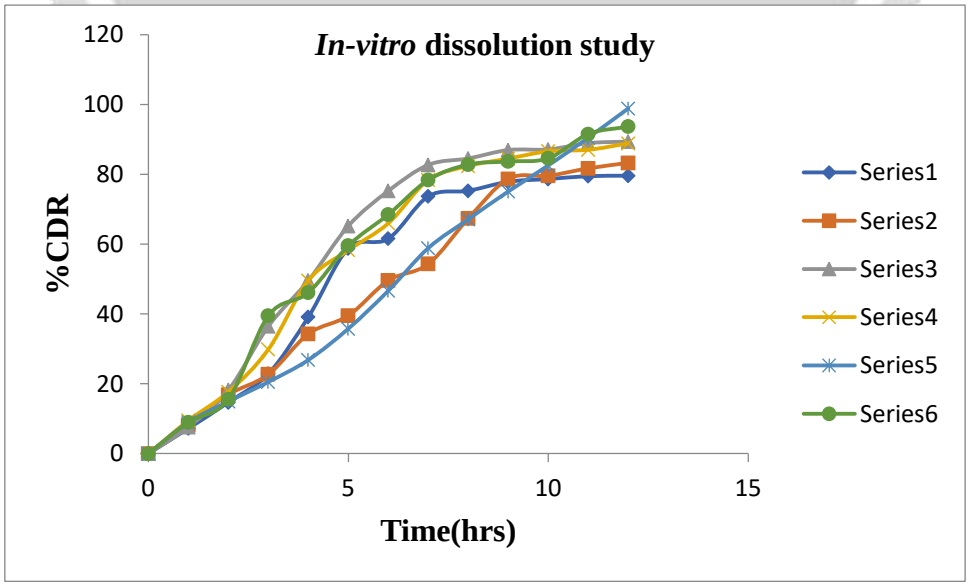

Fig. 7 In-vitro dissolution test

4. DISCUSSION: Tinidazole loaded microsponge were prepared by quasi emulsion solvent diffusion method by using DCM as an internal phase and PVA with water as an external phase. After successful preparation of microsponges they are allowed to different evaluation test like entrapment efficiency, % drug content, % yield, particle size, morphology and *in-vitro* dissolution test. The range of entrapment efficiency of 6 formulations

between 72.52-93.58% and the percentage yield range was found to be 80.18-89.95% and the drug loading was between 45.26-65.89% and particle size observed under optical microscope between 46.45-53%. The maximum %CDR was observed in F5 formulation that is 98.86% in acid buffer.

5. CONCLUSION

Tinidazole loaded microsponge were prepared by using quasi emulsion method, the formulations developed were found to be spherical in shape & porous in nature. All the formulations evaluation parameters were within an acceptable range from all these formulation batches among them F5 was found to be best in terms of entrapment efficiency, % yield, particle size, % drug release and %CDR.

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