Research Article

Formulation and Evaluation of Chitosan based Myrtle oil Microspheres Loaded Rectal Suppositories for the Treatment of Haemorrhoids

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Received: 12-06-2024; **Revised:** 23-08-2024; **Accepted:** 30-08-2024; **Published on:** 15-09-2024.

ABSTRACT

Symptomatic haemorrhoid disease is one of the most prevalent disease associated with significant impact on quality of life. Every year, almost 10 million people in India suffer from the pain of piles. In this article the Myrtle oil (Myrtus communis L.) is used as API which can give relief from the symptoms of haemorrhoids. This oil shows anti-inflammatory, anti-septic, anti-bacterial activities. Due to these beneficial activities we are using myrtle oil for preparation of suppositories. Suppositories are solid medicated dosage form which gives local as well as systemic effect which may increase the bioavailability of drug dosage form from the rectal route. In case of a conventional dosage forms, drugs with short biological half-life require frequent dosing to maintain constant therapeutic levels but Sustained release drug delivery system is able to maintain therapeutic blood or tissue levels of the drug for prolonged time. For sustain release drug delivery microspheres are one of the best choice. The Microspheres are carrier linked drug delivery system in which particle size is ranges from (1-1000 µm) in diameter. Main aim of this project is to give patient a faster relief from the above symptoms of haemorrhoids and also to sustain that effect for a longer period of time. In this article an attempt has been made to formulate and evaluate Microspheres loaded rectal suppositories for the treatment of haemorrhoids. To reduce and to give relief from haemorrhoid symptoms, suppositories will be the best choice of preparation which gives faster relief to the patient.

Keywords: Haemorrhoids, Rectal route, Myrtle oil, Suppositories, Sustain release, Microspheres.

1. INTRODUCTION

The disease haemorrhoid is extremely common in today's industrialised culture. Both men and women often experience haemorrhoids (piles), which typically run in families. Dilated veins at the anus are called haemorrhoids (Greek: haima, which means blood, and rhoos, which means flowing) or piles (Latin: pila, which means a ball). The dilated sections of the anal canal veins are known as haemorrhoids. Shearing of mucosa during the defecation process causes sliding of the structure within the anal canal including the haemorrhoidal and vascular tissues. It is believed that constipation, low-fibre diets, inherited predispositions, and abnormal bowel habits are significant contributors to the pathogenesis of haemorrhoids. Patients with haemorrhoids have to deal with severe pain, bleeding, itching in the perianal area, discomfort in the anus, burning around the anus, and other symptoms.[1]

Haemorrhoids can be treated by a number of medicines and treatment, including creams, lotions, tablets, gels, enemas, suppositories, and more. From the various options mentioned above, suppositories appear to be more economical and effective, and it also has the advantage of both local and systemic action.[2]

A suppository is a solid medication dosage form that is inserted into body cavities. The Latin root of the word "suppositories" means "to place under".[3] The general idea is that the drug is delivered to blood arteries that follow the bigger intestine by inserting the suppository as a solid and allowing it to dissolve or melt inside the body.[4]

Suppositories have a number of benefits, including improved bioavailability, quick absorption, avoiding first-pass metabolism, being appropriate for patients who are asleep, etc. Because suppositories are available in a variety of sizes and forms, it is easier to insert and keep them in the cavity.[3]

Suppositories are made in a variety of shapes and sizes to provide the best possible treatment according on the patient's age, condition, desired release pattern, and the drug type, etc.[1]

Main aim of this project is to give patient a faster relief from the symptoms of haemorrhoids and also to sustain that effect for a longer period of time

The phrase "sustain release" refers to drug delivery systems intended to produce a therapeutic effect that lasts longer than expected by gradually releasing the medicine over a lengthy period of time following a single dose. With a sustained release dosage form, the body receives an initial dosage of the medication to produce the intended therapeutic effect. Over time, the medication is continuously released to maintain its activity over time.[5,6] In this present work, we selected to employ microspheres as a possible drug delivery carrier system in the sector of novel drug delivery in order to produce sustained release from a dosage form.[7] Microspheres are characterised by a free-flowing powder that contains either natural or synthetic polymers. Small spherical particles, measuring between 1 and 1000µm (or 50nm and 2mm) in diameter, are known as microspheres.[8]

In order to increase bioavailability, stability, and the capacity to distribute drugs to particular sites at a predefined rate, multi-particulate drug delivery systems called microspheres are developed to provide extended or controlled drug delivery. Natural, semi-synthetic, and synthetic polymers, as well as other protective ingredients, are used to make them. The variety of methods for the production of microspheres offers opportunities to control the aspects of administration of the pharmaceutical compound. This focus facilitates the precise release of the desired amount of a component at the site of action.[9]

2. MATERIALS AND METHODS

2.1. MATERIALS: Myrtle oil were procured from Shree Venkatesh aromas, Tikamgarh. Other required chemicals were provided by college lab store which were of laboratory and analytical grade.

2.2. METHODS:

2.2.1. Preparation of Microspheres

Microspheres were prepared using single emulsification cross-linking method**.**

Table 1: Composition of different formulation batches of Microspheres

	M1	M2	M3	M4	M5	M6
Myrtle Oil (ml)	5	5	5	5	5	5
Chitosan (mg)	375	375	750	750	1125	1125
Acetic acid (ml)	15	15	15	15	15	15
Liquid paraffin (ml)	150	150	150	150	150	150
Span 80 (ml)	0.4	0.4	0.4	0.4	0.4	0.4
Glutaraldehyde (ml)	1.8	3.7	1.8	3.7	1.8	3.7

The chitosan was weighed and then dispersed in 15 millilitres of acetic acid. In a 150 ml liquid paraffin with 0.4 ml of span 80, the drug-polymer dispersion was introduced. Drug added to the mixture above. Three-blade propellar mechanical stirrers were used to stir this slurry at different revolutions per minute (rpm). After 10 and 40 min, GA (25% aqueous solution) was added batch wise and stirred continuously till 2 h. After that, the suspension of chitosan microspheres in paraffin oil was let to stand for 15 minutes so that gravity could settle the microspheres. Supernatant was decanted and filtered. In order to wash away any remaining oil residue, the microspheres were thoroughly cleaned four times using n-hexane as the solvent. Ultimately, water was used to wash them in order to get rid of extra GA. For a whole day, the microspheres were allowed to dry at room temperature.[7]

2.2.2. Microsphere Characterization

1. Percentage yield:

The weight % of the finished product after drying in relation to the original amount of drug, chitosan, and glutaraldehyde used for preparations was utilised to determine the yield of production.[10]

2. Particle size of Microspheres:

A Horiba SZ 100 particle size analyser was used to measure the microsphere's particle size. The small quantity of microspheres dispersed in a 10 ml distilled water and sonicated for 10 minutes. After that, whatman filter paper was used to filter the sonicated solution. Filtrate is the examined for particle size and zeta potential.[11]

3. Percentage drug encapsulation

The drug content was measured spectrophotometrically at 732 nm after a total of 25 mg microspheres were crushed, dispersed, and sonicated in 100 ml of pH 7.4 phosphate buffer for 20 minutes. The dispersion was then filtered, and the percentage of drug entrapment efficiency was determined using the following equation: [7]

$$\% \text{ Entrapment Efficiency} = \frac{\text{Practical drug content}}{\text{Thereotical drug content}} \times 100$$

4. *In-vitro* release study

Using USP Apparatus II, the dissolution study was carried out in 900 ml of pH 7.4 phosphate buffer at 37 ± 0.5 ºC and 100 ± 2 rpm paddle speed. 80 mg of microspheres were distributed throughout the dissolving media. 5 millilitre aliquots of the dissolving media were sampled at various times, and the sink condition was preserved. With the use of UV light at 732 nm, the absorbance was measured. [12]

2.2.3. Preparation of Microsphere loaded Suppository

Table 2: Formulation table of Glycero-gelatine suppository (all values in %)

Batch	A	B	C
MC oil Microspheres	6	6	6
Glycerine	70	60	50
Gelatine	10	10	10
Water	20	20	20

The melt fusion procedure was used for manufacturing the suppository. The amount of drug required was calculated as well as quantities of base also determined. The first step in this process is to heat the glycerine and water mixture to 45°C on a water bath. After that, in the glycerine-water mixture soaked gelatine was added slowly. Weighed MC Oil-loaded microspheres were added to the above mixture and stirred. The resulting suppository liquid was then taken off the heat supply instantly, placed into a suppository mould, and chilled to solidify. [13]

2.2.4. Characterization of Microsphere loaded suppository

1. Visual examination:

Assessing the suppository's colour and surface features is not too difficult. Verify that the active components are not migrating, fissuring, pitting, fat blooming or sedimenting. Both whole and longitudinally cutted suppository can be examined. [3]

2. Weight Uniformity:

The average weight was calculated using 20 suppositories. Next, the difference from the average was determined by weighing each suppository separately. With the exception of two, which may vary by no more than 7.5%, no suppositories should stray from average weight by more than 5%. [4]

3. Hardness:

The Monsanto hardness tester type was used to test the produced suppositories for hardness. The weight needed for the suppository to collapse used to be a measure for its hardness. The suppositories undergo a hardness or fracture point test to find out their tensile strength and potential resistance to the risks associated with packaging and transportation. [14]

4. Liquefaction Time:

A designed device was used to measure the temperature and liquefaction time. A large pipette was used, one side of which had a wide aperture and the other a tiny one. The pipette was submerged in heated water that was kept at 37 °C. The thin end should be towards the hot water. The sample suppository was gently pushed down the pipette's length until it reached the narrow end, starting at the top and going through the broad end. The liquefaction time is represented by the point at which the suppository begins to dissolve. [4]

5. Dissolution Test:

Studies on the in vitro dissolution of suppositories were conducted in an Electro lab-TDT 08L rotating paddle apparatus (Type II) at 100 rpm, using 900 ml of phosphate buffer (pH 7.4) at 37±0.5 °C. For every test, a single suppository was utilised. 5 ml of sample were taken out using a syringe at prearranged intervals. The same volume of fresh dissolving medium was used to replace the volume removed at each interval, and the temperature was kept at 37±0.5 °C. Using a UV-visible spectrophotometer, the sample's absorbance at 732 nm was measured in order to determine the drug release. [3]

3. RESULTS AND DISCUSSIONS

In the current study, successful attempts were made to develop a Microspheres loaded rectal suppositories for the treatment of haemorrhoids.

The Microsphere were prepared by using single emulsification cross-linking method. The Prepared microspheres were evaluated for Percentage yield, particle size, percent drug encapsulation, In-vitro release study.

1. Percentage yield: Production yield of microspheres was found to be 90.34% shown in Table 3.

2. Particle size: The particle size was found to be 174.1µm shown in figure 1. When the speed of stirring was increased particle size was found to be decreased.

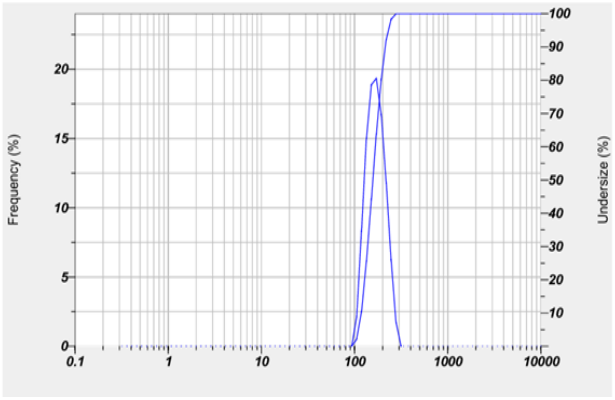

Figure 1: Particle size of optimised microsphere

3. Percentage drug encapsulation: Percent drug entrapment efficiency of the microspheres was found to be 80% shown in Table 3.

4. In-vitro drug release: The optimized batch M3 showed highest drug release of about 81% shown in Figure 2.

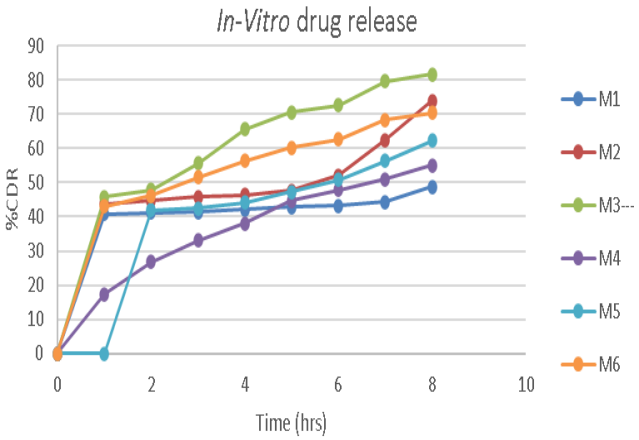

Figure 2: *In-vitro* drug release of all batches of Microspheres

Table 3: Evaluation of all batches of Microspheres

Evaluation parameter	M1	M2	M3	M4	M5	M6
Percentage yield (%)	75.76	74.23	90.34	88.17	80.45	71.24
Percentage encapsulation efficiency (%)	40	57.6	79.8	69.4	76.6	76.2
In-vitro drug release (%) (at the end of 8 hours)	48.68	73.92	81.61	55.11	62.18	70.36

The Microsphere loaded Suppositories were prepared by using melt fusion method. Prepared suppositories were evaluated for Visual examination, weight uniformity, hardness, liquefaction time, *in-vitro* drug release.

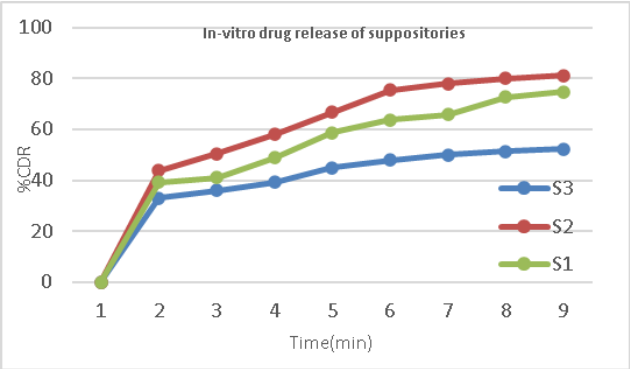

Figure 3: *In-vitro* drug release of Microspheres loaded suppositories

1. visual examination: Physical examination is important for documenting uniformity batches and physical quality assessment tests and observation on suppository. There was absence of pitting, fissuring, fat blooming, cracks, lamination, Capping. All suppositories were appropriate for appearance, and there was no cracking, bubble formation, and sediment accumulation.

2. Weight uniformity: The weight variation studies for all suppositories were found to be within the acceptable range of <5%. The result of all batches were shown in Table 4.

3. Hardness: The suppositories should have good mechanical strength for handling and transportation. Suppository shows good mechanical strength of about 2kg/cm^2 as shown in Table 4.

4. Liquefaction time: The liquefaction time is the time necessary for suppository to liquefy under pressure similar to those found in the rectum. Suppositories showed Liquefaction time 4.9 minutes and temperature 37.4°C which is shown in Table 4.

5. In-vitro drug release: The cumulative percentage of suppositories dissolution is depicted in figure 3. The Optimized batch S2 showed drug release 81.06%.

Table 4: Evaluation parameters of all batches of suppositories

Evaluation parameter	Batches		
	S1	S2	S3
Weight uniformity (mg)	470.7	497.2	495.8
Hardness (kg/cm^2)	1.6	2	1.7
Liquefaction time (min)	6.75	4.95	5.54
Melting range (°C)	39.8	37.4	38.6
In-vitro release (%) (at the end of 8 hours)	52.21	81.06	74.83

6. Drug release kinetics study: The drug release kinetic were studied according to zero order, first order, Higuchi and korsmeyer peppas model given in figure.4 respectively. The regression equation of optimized formulation S2 were found 0.9977 at zero order, 0.7125 at first order, 0.9977 at peppas model, 0.9181 at Higuchi model. The release data were best fitted to zero order and peppas model since it had highest values of R^2. The results were shown in figure. 4

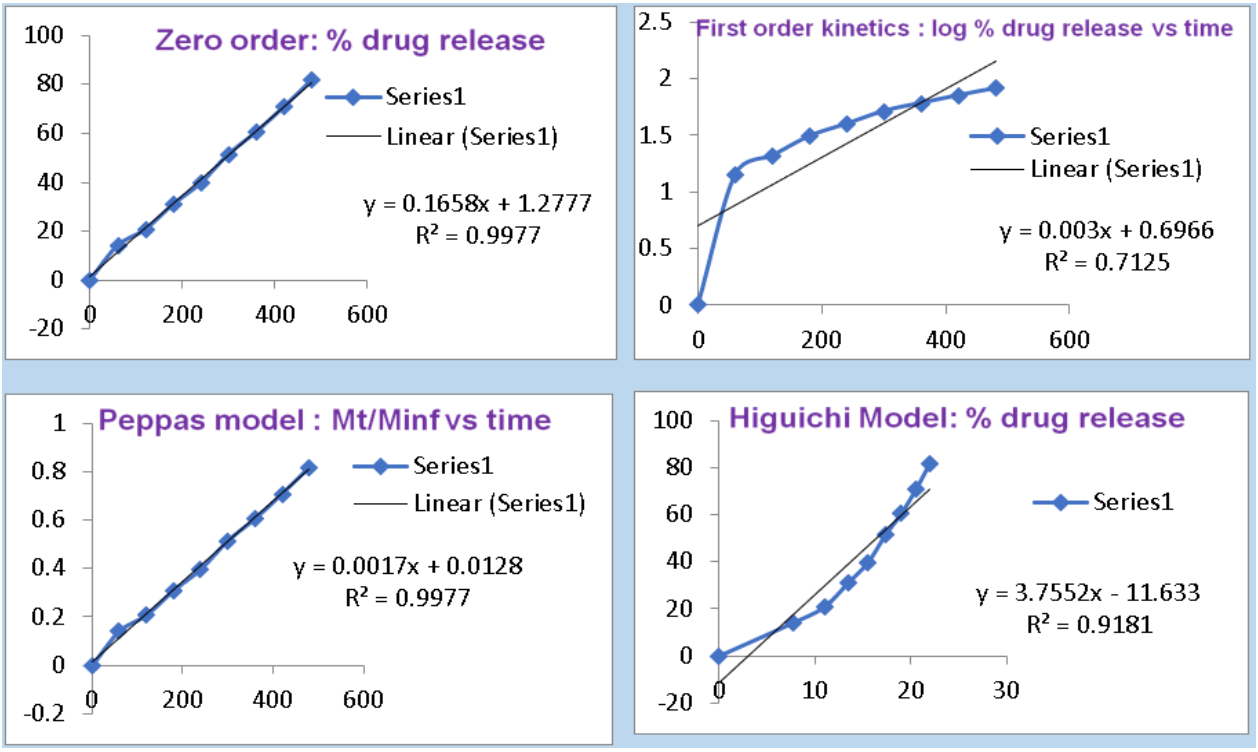

Figure 4: Release kinetic study of optimized Microsphere loaded suppository formulation batch S2

4. CONCLUSION

Present study indicates that it is possible to design microspheres loaded rectal suppositories for the drug Myrtus communis essential oil using melt fusion method. For preparation of Microspheres single emulsification crosslinking method was used. The size of microspheres was influenced by molecular weight of polymers. The microspheres were spherical in shape with a smooth and non-porous surface. The in vitro drug release study from microspheres (M3) proved that the present microspheres have the properties of sustained release formulation. Furthermore, the present microspheres are applicable for rectal use because of their small size, biodegradability, muco-adhesive property. For preparation of suppositories melt fusion method was used. Formulated suppositories (Microspheres loaded suppositories) met the quality requirement for weight measurement, hardness, melting point, liquefaction time, in-vitro drug release. The formulation (S2) exhibited acceptable results in the in-vitro release test, which provides reliable evidence supporting the quality and performance of the formulations. Sustained release suppository has better patient compliance, improve efficiency and safety of patient & improve bioavailability of drug by avoiding first pass metabolism. The prepared microspheres loaded suppositories will be used for treating haemorrhoids.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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