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RESEARCH ARTICLE

Development and Comparative Analysis of Metronidazole Microspheres Prepared with Different Combinations of Polymers Using Ionotropic Gelation Technique

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Abstract

Objective: Basis of this experiment was progressive preparation and evaluation of generated metronidazole loaded microsphere by desired ratio of polymers which can improve its therapeutic efficacy and reduce the need for frequent dosing. The use of microspheres can improve the bioavailability of the medication by protecting it from degradation and improving its absorption in the body. Drug release pattern can be altered by swelling Index and diffusion. **Methods:** Metronidazole loaded microsphere was prepared by ionotropic gelation technique using different combination of polymers, such as of Sodium alginate, Hydroxy propyl methylcellulose and Carbopol. Evaluation process for microspheres was performed by size analysis, percentage yield, swelling index, drug entrapment efficiency, drug release study (In-Vitro), kinetics models (Zero order, First order, Higuchi, Hixon-Crowell and Korsmeyers- Peppas) and stability testing. **Findings:** The average sizes of F1, F2 and F3 were observed and F2 had the lowest particle size. Swelling index was observed for all formulations, and F3 had the highest swelling property about 90%. During in-vitro drug release study it was noticed that F2 gives the highest percentage of drug release about 88.44% in comparison to other batches. Drug release kinetic study shows that F2 follows zero order and Higuchi model mostly with R^2 value 0.9987 and 0.9861 respectively. Stability study report for F2 batch was also acceptable for this study. **Novelty:** Previous researches was concentrated on controlled drug release study, bioavailability improvements and for dosage frequency reduction. But novelty of this project is to improve swelling potentiality of formulations by Ionotropic gelation techniques and finding out the association in between swelling property, drug diffusion and release kinetics of drugs that was not detected in prior studies. Also, this study determines better combination of hydrophilic polymer with respect to drug release property and improved release kinetics.

Keywords: Metronidazole; Ionotropic gelation technique; Sodium alginate; Hydroxypropyl methylcellulose; Carbopol; drug release kinetics

1 Introduction

The project is based on Formulation, Evaluation and Comparative Study of Drug release of metronidazole-loaded microspheres prepared with different combinations of Sodium alginate, HPMC & Carbopol using ionotropic gelation technique. Metronidazole is a widely used antibacterial and antiprotozoal drug, which is commonly used in the suppression of bacterial infections, amoebiasis, trichomoniasis, and other parasitic infections.[(1)] However, it's problem related to short half-life and low bioavailability often lead to frequent dosing and increased chances of adverse effects. To address these issues microspheres have been expanded to improve drug efficacy and reduce dosing frequency.[(2)]

One of the most significant advantages of microspheres is their ability to control the extent of drugs release. The release rate can be controlled by modifying the size, shape, and polymer composition of microspheres. Microspheres also protect the drug from degradation[(3)]. In this project, we used ionotropic gelation process to prepare metronidazole-loaded microspheres. The ionotropic gelation process is a simple and widely used technique for preparing microspheres, which involves the cross-linking of negatively charged polymer molecules (such as alginate) with positively charged ions (such as calcium) to form a gel matrix. In this method, Carbopol and HPMC are added to the alginate[(4)] solution for improving the stability and mechanical strength of the microspheres and to control the drug release property. Carbopol is a versatile polymer that is utilized as the coating polymer in the microencapsulation process. In drug transport systems, it is applied to modify release of drugs from microspheres, which are tiny spherical particles used for site specific delivery of drug. When incorporated into metronidazole microspheres, Carbopol provides extended drug release. HPMC is a hydrophilic polymer that expends in water, forming a gel-like matrix that effectively regulates drug diffusion. Sodium alginate was used to increase the gastric retention duration of the microspheres. Alginate based formulations can reduce dosage frequency.[(5)] The microspheres are evaluated for their physical and chemical properties, drug entrapment, and dissolution study. The comparative drug dissolution study will help in identifying the most suitable combination of polymers for controlling the drug release of metronidazole. Most of the research works were done for improvement of dosage frequency and bioavailability but neglected part was the mechanism of diffusion through pores of microspheres that can be altered by improving polymer choice and by increasing swelling property.

2 Methodology

2.1 Materials

Metronidazole, Sodium Alginate, Carbopol and HPMC were purchased from Yarrow Chem Pvt. Ltd. Mumbai. Calcium chlorides obtained from Loba chemine, Mumbai. All chemicals and reagents used were of analytical grade.

2.2 Method of preparation for Metronidazole Microsphere

Metronidazole-loaded microspheres were formulated by Ionotropic gelation method with different combinations of polymers i.e., Sodium alginate, Carbopol and Hydroxypropyl methyl cellulose (HPMC). Three different formulations were prepared with constant amounts of Metronidazole (750 mg), Sodium Alginate (400 mg), and Calcium Chloride (4% w/v). The first batch was prepared with Carbopol and a drug ratio of about

1:30. In the second batch instead of Carbopol, Hydroxy propyl methyl cellulose (HPMC) was used in a 1:1 ratio with the drug. In the Third batch, Carbopol with a drug ratio of 1:30 was added as well and HPMC was added in a 1:1 ratio with the drug. Sodium alginate was dissolved in warm water using magnetite stirrer. Carbopol and alginate polymer combination was prepared in water with 0.06:1 ratio. In case of formulation containing HPMC, Polymer was dissolved individually in water. In another case of formulation containing both Carbopol and HPMC, HPMC was combined with the Carbopol mixture. After complete dissolution of two polymers, it was mixed with sodium alginate mixture which must be free from air bubbles. Precisely weighed Metronidazole was mixed with water in separate beaker and mixed thoroughly. After that the drug solution was added with the polymer mixture to form a drug-polymer solution. The drug-polymer was mixed with agitation until it became homogenous mixture. Preparation of Crosslinking solution was done in another beaker by mixing calcium chloride in distilled water (4% w/v). Then air bubble free mixture was added drop by drop, by using a syringe into the counter-ion solution, maintaining a minimum distance from the tip of syringe and the surface of counter ion solution. The microspheres were kept in cross linking medium for 15 minutes at room temperature with mild agitation. Formulated microspheres were removed using a mesh strainer and cleanse thoroughly (distilled water) to remove the additional residue of cross-linking agents. Finally, microspheres were transfer to a Petri dish and dried with hot air at 60 °C and collected after drying[6].

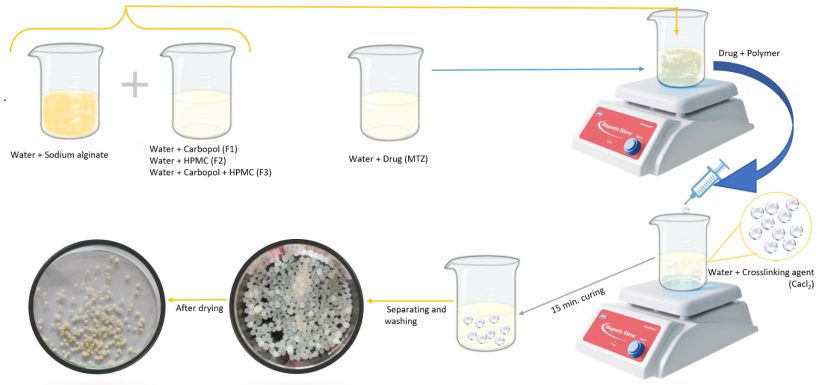

Fig 1. Schematic diagram of preparation of microspheres using ionotropic gelation method

3 Results and Discussion

3.1 Size analysis of microspheres

Size analysis of microspheres was observed using optical microscope. Ten counts microspheres were chosen randomly every time from F1, F2 and F3. Diameters were measured along with observation of other microscopic characters like shape. The average size of microspheres of F1, F2 & F3 was 692 ± 12 μm, 611 ± 22 μm & 814 ± 19 μm (n = 10) respectively with spherical in shape. Size of microspheres can vary from 1 to 1000 μm. But improved drug release can be achieved by small sized microspheres.

3.2 Percentage yield[7]

The formulated microspheres from three formulations were taken and weighed. To measure the percentage (%) yield of microspheres, determined weight was divided by the complete amount of components (all non-volatile) used for the preparation. After study, it was founded that F2 and F3 have the highest percentage yield which was 95.63 % and 91.26 %. Percentage yield may differ in broader range according to compositions and type of preparation.

$$Percentage\ yield = \frac{The\ amount\ of\ microspheres\ obtained}{The\ theoretical\ amount} \times 100$$

3.3 Swelling index

Dried microspheres (W_0) of 10 mg were taken from all three formulations. These were allowed for swelling up to 24 hours with 1 ml of distilled water in three separate test tubes. After 24 hours at 37 ° C, the weight of swelled microspheres was measured

(Ws).[8] Study shows that F2 has the highest swelling index. There is no estimated or fixed value for swelling index available in other studies but from this study it was examined that better swelling can improve bioavailability of drug.

The swelling characteristics (index) were determined (Table 1) by using the formula below:

$$Swelling\ index = \frac{W_S - W_O}{W_O} \times 100$$

Table 1. Result of Swelling index

Batch no.	Swelling index (%)
F1	40
F2	90
F3	83

3.4 Drug entrapment efficiency

Microspheres taken from all three formulations with 10 mg of theoretical drug content were taken separately and crushed using mortar and pestle. Powdered sample was added to 100 ml of distilled water and soaked for one hour.[9] After required dilution this solution was observed under UV- Visible Spectrophotometer at 320 nm. Study represents that F1, F2 and F3 had 61%, 91% and 87% of drug entrapment efficiency.

$$Drug\ entrapment\ efficiency = \frac{Actual\ drug\ content}{Theoretical\ drug\ content} \times 100$$

3.5 *In-vitro* drug release

In-vitro drug release study of Metronidazole microspheres was done using distilled water in type II (USP) dissolution apparatus (Electrolab India). Microspheres with 350 mg of drug content (Metronidazole) were taken in 900 ml of distilled water medium. Procedure was started by rotating paddle at 50 rpm with maintaining temperature at 37±0.5°C. After every 15 minutes interval 5 ml solution was withdrawn and replaced with same amount of fresh distilled water for 4 hours. Recovered samples were diluted and analyzed at 320 nm in UV- Visible Spectrophotometer. In this investigation, we found that F1, F2 and F3 had 73.48 %, 88.44 % and 85.33 % of drug release simultaneously after 4 hours study. Overall F2 formulation was showing the highest release pattern. (Figure 2) According to this study it was recognized that slow release of drug also can give a longer release pattern. There is no drop in movement of graph that represents drug can released even after 4 hours.

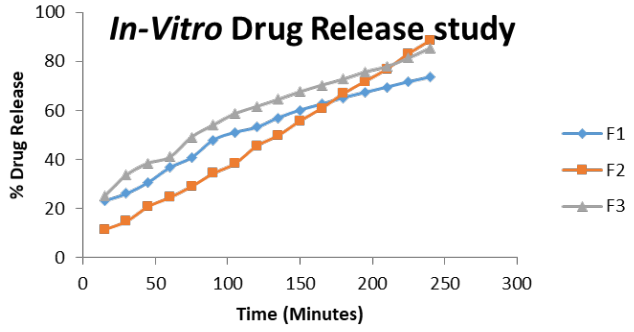

Fig 2. In-vitro drug release study of metronidazole microsphere

3.6 Drug Release kinetics determination

Release kinetics profile for microspheres was rectified by plotting against several kinetics models.[10]

Equation, $F = K_o t$ is designed for kinetics of zero order; where F, t and K_o is drug fraction, release in time and zero order release constant respectively.

Equation, ln (1-F) = - K_1t is for First order kinetics; where F, t and K_1 represents drug fraction, released in time and the first order release constant (first order). [11]

Equation, $F=K_H t^{1/2}$ is for Higuchi model; where F, t and K_H is drug fraction, released in time and the Higuchi dissolution constant respectively.

Equation, $Q^{1/3}=kt+Q_0^{1/3}$ is for Hixson-Crowell model; where drug release in time, the starting value for Q and rate constant is Q, t, Q_0 sequentially.

Equation, $F=K_p t^n$ is for Korsmeyer-Peppas model, where F, t, Kp and n represents the drug fraction, release in time and the Korsmeyer-Peppas rate constant, and exponent for release serially. [12]

Kinetic models study had shown that F2 and F3 mostly follow zero order reaction which means rate of reaction was not depending upon concentrations of loaded drug. Regression co-efficient for F2 and F3 (Zero order model) was 0.9987 and 0.9715 respectively. These formulations also follow higuchi kinetics which promotes diffusion. Regression co-efficient value for Higuchi model of F2 and F3 batch is 0.9861 and 0.9701 respectively (Table 2)

Table 2. Result of curve fitting of metronidazole releasing from microspheres

Kinetic models	Regression co-efficient		
	F1	F2	F3
Zero Order model	0.9694	0.9987	0.9715
First Order model	0.9870	0.9982	0.9715
Higuchi model	0.9634	0.9861	0.9701
Hixon-Crowell model	0.9856	0.9594	0.9911
Korsmeyer-Peppas model	0.9861	0.9149	0.9908

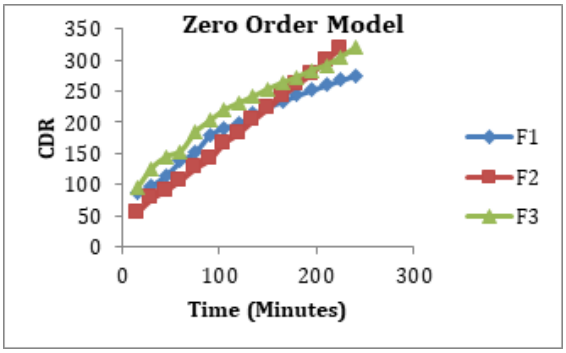

Fig 3. Zero Order Model

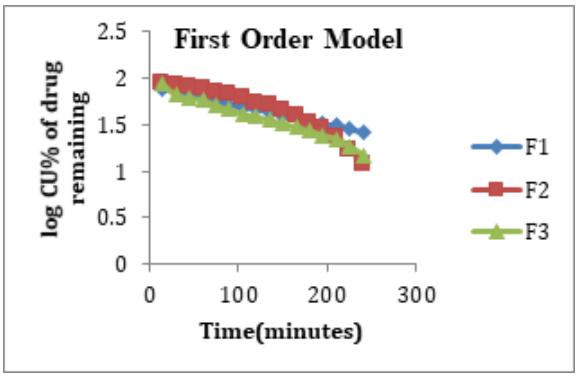

Fig 4. First Order Model

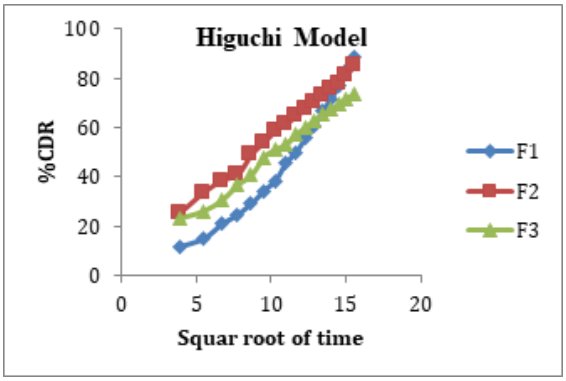

Fig 5. Higuchi Model

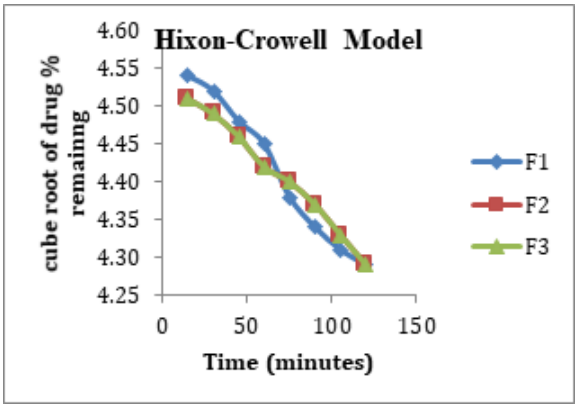

Fig 6. Hixon-Crowell model

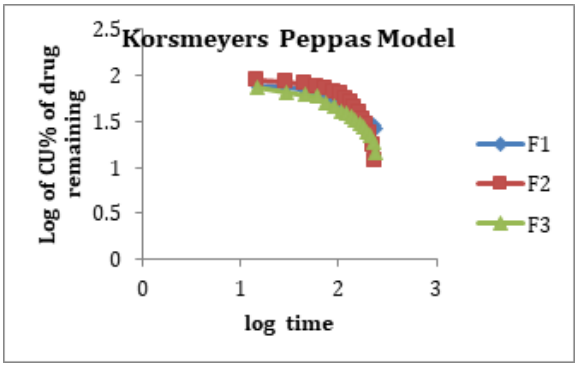

Fig 7. Korsmeyers Peppas model

3.7 Stability testing

After 1 month storage, stability test of F2 was done for Metronidazole microspheres by checking color, odour and *in-vitro* release study of drug.[13] After 1 month completion of storage it was founded that no changes in appearance (color) and odour was noticed there. All of these formulations were whitish yellow with a pleasant odour. After storage, percentage drug release for F2 was 83.48 %, consequently no remarkable change was observed. From other study it was rectified that minimum one-month stability study was required and the formulation must not change its consistency and properties within storage period.

This study was based on preparation and evaluation of three different compositions of metronidazole microsphere by using Ionotropic Gelation Process. Drug delivery through microspheres can achieve prolonged drug delivery system. For

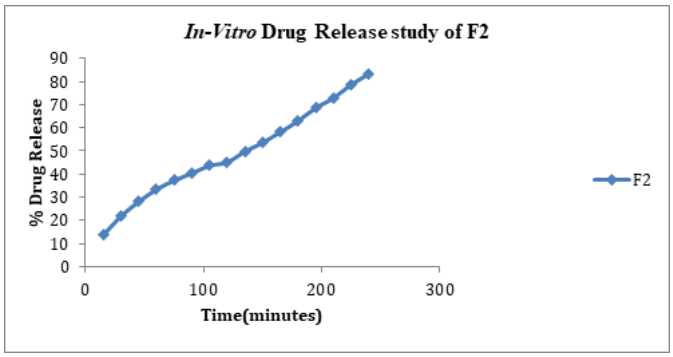

Fig 8. *In-vitro* drug release study of metronidazole microsphere for stability

improvement of bioavailability (Metronidazole), microspheres were formulated by ionotropic gelation method[14]. Polymers preferred for this study were Sodium alginate, Carbopol and Hydroxy propyl methyl cellulose (hydrophilic polymers). Purpose of this study was to investigate on correct composition of polymers that can improve swelling property of microsphere and to prove that swelling index can increase drug release from micro beads. These microspheres also can protect the drug from degradation[15]. Microspheres had already provided sustained drug release and upgrade drug bioavailability in different formulations[16]. The polymeric chains of microspheres had more active calcium-binding sites available, that leads to a higher degree of cross-linking and forms nonporous microspheres that was contributing to the higher drug content and encapsulation efficiency.

A relation between the swelling property (index) and drug release (In-vitro) was also observed in this study. Study shows drug release was increasing with rise in swelling index which was not concentrated in the previous researches. The drug molecules were accommodated inside the polymeric matrix of the formulation. So, the above fact can be described as the system swells, and the pores between the polymeric chains expanded and creating channels from this section the drug regimen can move more freely. This increased mobility of the drug leads to the highest drug release rate. After evaluating several criteria like drug particle size analysis, swelling index, drug entrapment efficiency, and in-vitro drug release and drug release kinetics it was determined that F2 performed most pointedly than others and was selected as the best option for further research. This particular formulation gave best results for swelling index and dissolution study and as well as for percentage yield and drug entrapment efficiency. F2 and F3 both followed zero order model and the Higuchi model. But F2 had the highest regression coefficient for both models. Because F2 followed zero order model that was representing release of drug was not concentration dependent and on the other hand that was following Higuchi model which represented better diffusion through microspheres. Based one month stability study[17] it was determined that F2 was not having any change in Drug content and in vitro drug release profile. Polymer combination of Sodium alginate and HPMC was concluded the best formulation among the formulations prepared.

4 Conclusion

Three different formulations of metronidazole microspheres were synthesized by Ionotropic Gelation Technique with different polymer compositions of Carbopol, Hydroxy propyl methylcellulose and Sodium Alginate. The polymer composition of hydroxy propyl methylcellulose and sodium alginate (F2) gave the most efficacious results in swelling properties (90%) and drug release patterns with the lowest particle size ($611 \pm 22\ \mu$m). In vitro drug release study shows that F2 has the highest drug release (88.44%) compared to other combinations. Drug release kinetic study results denoted that formulations follow the Zero order and Higuchi model so drug release was controlled and drug diffusion was enhanced. According to report bioavailability of dosage form (F2) was also improved and dosage frequency was reduced. For F2 regression coefficient was 0.9987 and 0.9861 for Zero order release kinetics and Higuchi model sequentially. Most important part of this project was to concentrating on swelling index of microspheres. After study, it was proved that formulations with better swelling Index can improved drug diffusion through microspheres and leads to better drug release. Further research work can be done for betterment.

5 Acknowledgement

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