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Research Article

Development and *in vitro* study of Metronidazole loaded cross linked sodium alginate and gellan gum microspheres

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Abstract



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Formulation of metronidazole loaded trivalent ion Al^{+3} cross-linked and gellan gum microspheres was developed. The Metronidazole loaded microspheres were prepared taking sodium alginate, gellan gum as excipients along with maleic anhydride, aluminium chloride as cross-linking agents. The evaluation processes of prepared metronidazole microspheres were done by *in-vitro* release study, microscopic analysis and swelling index. Each of the formulations shows good entrapment efficiency with the maximum entrapment $85.8 \pm 5.63\%$ was governed by the F1 formulation while F2 formulation confirms $82.3 \pm 4.72\%$ drug entrapment. The formulations provided the controlled release delivery pattern of Metronidazole.

Keywords: Microspheres, Metronidazole, Gellan gum, Aluminium chloride, Maleic anhydride.

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INTRODUCTION

Microspheres are solid spherical particles prepared with polymer, wax and some protective materials (like starch, gum, protein, fat and wax) and preferably having particle size of below 200 μm . This is a reliable mean of delivering drug to target specific site & providing maintenance of required concentration at that site ^{1,2}. There occurs a huge difficulty in research and developmental work of new controlled release drug delivery systems through oral route for targeting the drugs to a particular body parts. For this reason the drug has to shift biological membrane *i.e.* other organs, tissues or biological compartment where drug degradation can happen ³. Colon is preferred as the site of treatment for different diseases mainly some inflammatory diseases & colon cancer because it introduces prolonged transit time, decreased proteolytic activity along with neutral pH, among different organs ⁴. Drugs which have permeability and/or stability issues in upper GIT, colon becomes a promising site of release for those drugs ⁵. Now different types of synthetic, semi-synthetic or natural polymers are employed in formulation of controlled drug release systems ⁶⁻⁸.

The goal of the project was to formulate microspheres by utilizing ionotropic gelation process. Here, gellan gum and sodium alginate were utilized as main polymer component whereas aluminium chloride (trivalent Al^{+3} ions) and maleic anhydride were incorporated as cross-linking agents. Metronidazole was chosen for sustained release therapy and this drug is having plasma half life of 6.5 ± 2.9 hours, duration of action of 7 days. The drug is administered in a dose of 500 or 750 milligrams (mg) thrice in a day for 5 to 10 days. Hence

Metronidazole is an ideal candidate for developing sustained pattern release formulation which could result in increased clinical efficacy, decreased dosing frequency and less side effects.

Metronidazole is effective against a large number of protozoa and bacteria. It passes the cell membrane by passive diffusion method in the form of a prodrug. Then it is activated either in bacterial cytoplasm or in particular protozoal organelles, where drug-resistant cells are poor in concentration for drug activation. Later metronidazole drug molecule is transformed by an intracellular reduction reaction, to a nitroso free-radical which is short lived in nature. This reduction reaction helps an electron particle to be transported to the nitro group. Metronidazole is cytotoxic in this form and also capable to interact with DNA molecule. The genuine mechanism of action cannot be entirely elucidated. But it inhibits DNA synthesis and damages DNA by oxidation reaction, leading to both single-strand and double-strand breaks. It causes DNA degradation followed by cell death. In reduced condition activated metronidazole molecule can bind with bacterial DNA non-specifically and inactivate the bacterial DNA and enzymes. It causes rupture of bacterial DNA molecule, through an immediate action but cell lysis does not occur ^{9, 10}.

Gellan gum is a negatively charged exopolysaccharide which is having a high tendency to mix with dissolve in or be wetted by water. It is derived by aerobic method from bacteria called *Sphingomonas elodea*. This polymer is comprised of repeated units of glucose, glucuronic acid and rhamnose in 2:1:1 molecular ratio and two acetyl substituents, acetate and glycerate, linked on glucose residue adjacent of the glucuronic acid ¹¹.

Sodium alginate is derived from Alginic acid which is a linear copolymer having homopolymeric blocks of (1-4)-linked β -D-mannuronate (M) along with its C-5 epimer α -L-guluronate (G) residues, correspondingly. It is prepared by copolymerisation reaction between mannuronic acid and glucuronic acid¹².

MATERIALS AND METHODS

Materials:

Metronidazole and Gellan Gum were purchased from Yarrow Chem Pvt. Ltd. Mumbai. Aluminium chlorides and Maleic anhydride were obtained from Loba chemine, Mumbai. All chemicals and reagents used were of analytical grade.

Methodology

Preparation of Metronidazole Microsphere:

Metronidazole loaded microspheres were formulated by polymer matrix of sodium alginate and gellan gum. Cross linking agent involved in the microsphere formulations were malic anhydride and aluminium chloride. Sodium alginate, 400 mg, was accurately weighed and dissolved in 40 ml of warm water with the help of magnetite stirrer. Gellan gum was also precisely weighed on a digital balance and dissolved in 40 ml of warm water. Temperature of the solution was maintained at

50°C and rotation speed was fixed at 200 rpm. After complete dissolution of two polymers, they were mixed together and become bubble free or uniformly mixed. In separate beaker 400 mg of accurately weighed metronidazole was added in few ml of water and mixed thoroughly. After full dissolution of metronidazole in water, the drug solution was added in the polymer mixture to form a drug-polymer solution. The drug-polymer was rotated at 200 rpm until it became homogenous mixture. On another separate beaker, the counter ion solution was formulated by weighing maleic anhydride or aluminium chloride at weight ratio and mixing in water (50 ml). When mixing was completed, the drug-polymer dispersion was added drop wise into slightly agitated counter ion solution through flat-tipped needle maintaining a minimum distance from the tip of needle and the upper face of counter ion solution. The drug polymer droplet formed microspheres by cross linking with the counter ions. Following an incubation phase of 15 min, finally produced microspheres were separated by proper filtration, washed with double distilled water and allowed for air-drying. Then the formulated metronidazole microspheres were withdrawn from the beaker and cleaned them properly by washing with distilled water again and again to make them free from traces of excess counter ion solution^{13, 14}. Different microsphere formulations with percentage of polymers, drug (metronidazole) and cross-linkers were revealed in table 1.

Table 1: Formulation of Metronidazole Microspheres

Formulation code	Sodium alginate [w/v]	Gellan gum [w/v]	Metronidazole [w/v]	Malic anhydride [%w/v]	Aluminium chloride [%w/v]
F1	400	400	400	1	
F2	400	400	400		1

Evaluation of Microspheres:

Drug entrapment efficiency estimation

Specified amount of dried microspheres were properly weighed, crushed with a mortar-pestle and were taken into buffer solution. It is kept for time period with the purpose that the time should allow the maximum discharge of active agent from the formulation. Then the suspension was filtered & sample was taken to be analyzed with a spectrophotometer¹⁵⁻¹⁸.

Entrapment efficiency (in Percentage) = (Actual drug content/Theoretical drug content) $\times$ 100

Microsphere size analysis:

Randomly select specific number of microspheres and their size was measured using an optical microscope. A standard stage micrometer had to standardize the optical micrometer. The mean diameter of the formulated microsphere was calculated from each batch^{19, 20}.

In vitro drug release study:

The paddle-type dissolution test apparatus is useful for the experiment. First, prepared metronidazole microspheres were properly weighed and taken them in 900 ml buffer solution and temperature of the system was maintained at $37 \pm 0.5^\circ\text{C}$. The paddle rotation was fixed at 50 rpm. After specific time interval specific amount of aliquot was withdrawn from the dissolution media and then that volume was replaced by the fresh buffer media of equal volume. The samples were examined by using a spectrophotometer^{21, 22}.

Swelling study:

This swelling study was carried out in buffer solution. A predetermined known weight sample was taken and dissolved into 50 ml buffer media and kept for specific time. Then they are separated from the media, blotted with tissue paper also reweighed^{23, 24}.

Swelling ratio = (final weight – initial weight)/initial weight

RESULTS AND DISCUSSION

Drug Entrapment Study

Drug entrapment was fairly good in both formulations, the maximum entrapment being exhibited by the formulation [F1] cross-linked with 1% maleic anhydride. The formulation [F2] cross-linked with 1% aluminium chloride also showed good entrapment efficiency. It may be predicted that the lower entrapment of formulation [F2] might be owing to cross-linking effect of aluminium chloride. It is exposed that the entrapment capability declines with the enhancement in Aluminium chloride concentration as it causes greater degree of cross linking.

Table 2: % Entrapment of different batch

Batch Number	% Entrapped
F1	85.8 $\pm$ 5.63
F2	82.3 $\pm$ 4.72

Each value is represented as a mean $\pm$ SEM, n=3

Microsphere Size Analysis

The size distribution range of the microsphere particles was measured by employing an optical microscope while taking 6 particles from both batches. An ocular micrometer was previously attached and the particles were fixed on a slide and their size was measured. From the experiment it is observed that formulation [F1] cross-linked with 1% maleic anhydride is having mean particle size $14.66 \pm 1.72 \mu\text{m}$, formulation [F2] cross-linked with 1% aluminium chloride is having average particle size $16.71 \pm 2.15 \mu\text{m}$. It is found that the mean particle size distribution is less in case of F1 formulation than F2 formulation.

In vitro release studies

The USP type II dissolution test apparatus (paddle type) was engaged for the *in vitro* drug release study of the formulated metronidazole loaded microspheres. These formulations contain gellan gum and sodium alginate as polymer, counterion solutions of maleic anhydride and aluminium chloride in various ratios as cross-linkers. From this release study it is found that 45.290% active agent was discharged from the prepared formulation [F1] cross-linked with 1% maleic anhydride followed by 31.160% release by [F2] cross-linked with 1% Aluminium chloride within a duration of 4 hours. Drug release profile of the microsphere formulations are revealed in figure 1. The quantity of drug release differs for each formulation depending upon the extent of cross linking between the ingredients used in each formulation. It is observed that formulation [F1] had shown greater release than formulation [F2].

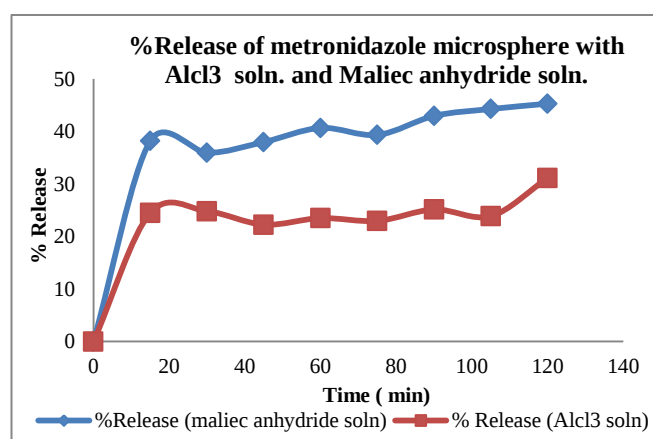


Figure 1: Release Study of Metronidazole Microsphere

Swelling behaviour

From the swelling study, it is evident that more swelling (95.5%) is exhibited by the formulation [F1] cross-linked with 1% maleic anhydride followed by 86.7% swelling by [F2] cross-linked with 1% aluminium chloride. It is also obvious that the percentage of swelling was higher for formulation [F1] than formulation [F2].

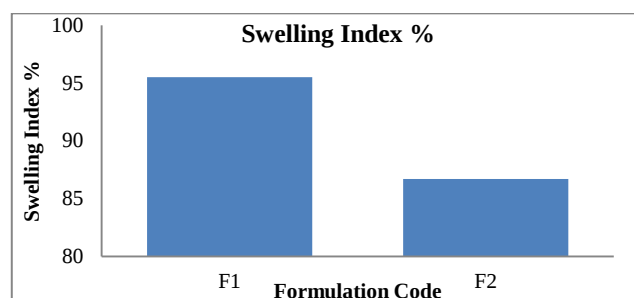


Figure 3: Swelling indices of various formulations of microspheres

CONCLUSION

This project was conducted to attempt the prolong release of Metronidazole microspheres for a prolonged duration of time. To achieve controlled release of delivery pattern of Metronidazole, microsphere formulations were prepared from where rate of release was reduced by incorporating sodium alginate and gellan gum as polymer and maleic anhydride or aluminium chloride as counterion solutions for cross-linking of the drug. Cross linking occurs by formation of covalent bonds among two or additional molecules. The cross linking agents hold the reactive ends to chemically attach with functional groups. This phenomenon is known as cross-linking. After preparing the microspheres, they were evaluated to estimate their entrapment, microscopy, release and swelling. From the experiment, we have come to know that F2 is better controlled release formulation than F1. Swelling of F2 was also lesser while the entrapment percent was well. This study further assures us the chance and possibility of further development and optimisation of the drug taken as a candidate for further investigation.

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CONFLICTS OF INTEREST STATEMENT:

There are no conflicts of interest.

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