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FORMULATION AND DEVELOPMENT OF METRONIDAZOLE MUCOADHESIVE MICROSPHERES BY IONOTROPIC GELATION COMPLEXATION TECHNIQUE

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ABSTRACT
The aim of present work is to prolong the gastric residence time of metronidazole by formulating into mucoadhesive control release microspheres. Metronidazole is a Antibacterial (systemic); antiprotozoal and antiamoebic drug. The mucoadhesive microspheres were prepared by ionotropic gelation method of polyelectrolyte complexation technique using sodium alginate as control release polymer and carbopol934 as mucoadhesive polymer along with drug in different proportions and calcium chloride as agent of multivalent cations. The prepared microspheres were characterized by micrometric properties, percentage entrapment efficiency, mean particle size, invitro wash-off test, invitro release studies. The data obtained in this study suggests that a micro particulate mucoadhesive dosage form of metronidazole can be successfully formulated to give prolonged residence time of drug and hence improved bioavailability.

KEYWORDS: gastric residence time, metronidazole, sodium alginate, carbopol 934, inotropic gelation method.

INTRODUCTION
Microspheres are defined as solid colloidal approximately spherical shape particles size ranging from 1µm to 1000 µm in diameter. However, the success of these microspheres is limited due to their short residence time at site of absorption. To overcome this limitation the microspheres are coupled with mucoadhesion character by incorporating mucoadhesive polymers into their formulation to develop mucoadhesive microspheres. Mucoadhesion is the attachment of the drug along with a suitable carrier to the mucosal layer. Mucoadhesion is a complex phenomenon which involves wetting, adsorption and interpenetration of polymer chains.

Metronidazole is a Antibacterial (systemic); antiprotozoal and antiamoebic drug of Nitroimidazoles group. Metronidazole is effective in the treatment of systemic and enteric obligate anaerobic bacterial infections, including *Clostridium* species, *Fusobacterium* species, and penicillinase-producing strains of *Bacteroides.* Surgical therapy may be necessary to completely resolve isolated infections. Metronidazole is not clinically effective against facultative anaerobes or obligate aerobes. However, it is often combined with another antibiotic or antibiotics effective against aerobes to treat mixed bacterial infections. Metronidazole is considered effective in the treatment of some protozoal infections in animals. Metronidazole is reduced as it enters the target cell where it interacts with bacterial or protozoal DNA, causing a loss of helical structure and strand breakage in the DNA; these effects inhibit nucleic acid synthesis and cause death of the cell.

The mucoadhesive microspheres are formulated by using mucoadhesive polymers, these mucoadhesive polymers have the property of adhering to mucin epithelial surface. A short list of Mucoadhesive polymers is given in Table no. 1.

MATERIALS
Metronidazole received from Hetero drugs Ltd Hyderabad, White to pale yellow, odorless crystals or crystalline powder. Is stable in air, but darkens on exposure to light, Sparingly soluble in water and in alcohol; slightly soluble in ether and in chloroform. Sodium alginate, carbopol 934, and calcium chloride received from Himedia laboratories.

METHOD
Metronidazole mucoadhesive microspheres were prepared by ionotropic gelation method using polyelectrolyte complexation technique. In this method the required amount of metronidazole, sodium alginate, carbopol 934 and calcium chloride were weighed and passed through sieve no ≠ 60. Sodium alginate and corbopol were dissolved in water to form a homogenous polymer solution by continuous stirring to which

metronidazole was added. The resultant drug dispersion was loaded into dry disposable syringe with needle size no 20 and added drop wise into 2% w/v solution of calcium chloride under constant stirring. The added droplets were retained in the calcium chloride solution for 15 min to complete the curing reaction and to produce the spherical rigid microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with water and dried at room temperature for 24 hours. Total 6 formulations were prepared by using different drug and polymer ratios.

Total 6 formulations were by using different drug and polymer ratios, are listed in table 2.

Table no. 1: list of Mucoadhesive polymers

Synthetic polymers	Natural polymers
Cellulose derivatives	Tragacanth
polycarbophil	Sodium alginate
Poly (ethylene oxide).	Karaya gum
Poly (vinyl pyrrolidone).	Guar gum
Poly (vinyl alcohol).	Gelatin
Poly(hydroxyethyl methylacrylate)	Chitosan
Hydroxyl propyl cellulose	Soluble starch
Poly ethylene oxide	Xantham gum
Methyl cellulose (MC)	Lecithin

Table 2: composition of different formulations

Sl.no	Formulation code	composition	Ratio
1.	F1	Drug : sod.alginate	1 : 2
2.	F2	Drug : sod.alginate : carbopol	1 : 1.8 : 0.2
3.	F3	Drug : sod.alginate	1 : 4
4.	F4	Drug : sod.alginate : carbopol	1 : 3 : 1
5.	F5	Drug : sod.alginate : carbopol	1 : 2 : 2
6.	F6	Drug : sod.alginate : carbopol	1 : 1 : 3

EVALUATION METHODS

Micromeritic properties: The prepared mucoadhsive microspheres were characterized by their micromeritic properties such as particle size, bulk density, tapped density, compressibility index Hausner's ratio and angle of repose.

A) Tapped density: The prepared mucoadhesive microspheres were transferred to a graduated cylinders of bulk density apparatus and the tapping was set for 100 times. After 100 tappings, volume of microspheres was visually examined. The ratio of weight of microspheres to volume of microspheres after 100 tappings gives tapped density.

Tapped density= Mass of microspheres in grams / Voiume of microspheres after 100 tapping.

B) Bulk density: The prepared mucoadhesive microspheres were transferred to a graduated cylinder of bulk density apparatus and the volume occupied by the microspheres was noted. This volume is bulk volume and it includes true volume of the power and the void space among the microspheres.

Bulk density = Weight of microspheres in grams/ Bulk volume of microspheres in cm^3

C) Carr's compressibility index: The compressibility index is a measure of flow of a powder to be compressed. It was determined by using the bulk and tapped densities.

Carr's compressibility index = Tapped density- Bulk density x100/Tapped density

D) Hausner's ratio: Tapped density and bulk density were measured and the Hausner's ratio was calculated using the following formula:

Hausner's Ratio = Tapped density/Bulk density

The values less than 1.25 indicate good flow where as greater than 1.25 indicates poor flow.

E) Angle of repose: Angle of repose is defined as the maximum angle possible between the surface of the pile and the horizontal plane, which can be deterined by using fixed funnel method. In fixed funnel method a funnel was set with its bottom tip at a given height (h) above a graph paper which is placed on a flat horizontal platform.

Accurately weighed microspheres were carefully poured though the funnel onto the graph paper until the apex of the conical pile just touches the bottom tip of the funnel. The radius(r) of the base of the conical pile was measured by drawing a circle around the pile. The angle of repose (θ) was calculated by using the following formula.

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

Where, θ is Angle of repose

h is height in cm

r is radius

F) Partical size: The particle size of prepared mucoadhsive microspheres was measured by microscopic technique by using standard electron microscope for about 100 particles were measured and their average size was determined. The mucoadhsive microspheres and settled portions of microspheres were recovered separately and were dired and weighed.

Invitro wash off test: The mucoadhesive properties of the microspheres were evaluated by the invitro wash off test. A2 X 3 cm piece of goat intestine mucosa was tied on to a glass slide using thread. Microspheres were spread (~50) onto the wet, rinsed, tissue specimen and the prepared slide was hung onto to the one of the grooves of the USP tablet dissintegrating test apparatus. The dissintegrating test apparatus was operated such that

the tissue specimen was given regular up and down movements in the beaker containing the simulated gastric fluid USP pH 1.2 buffer. At the end of 30 minutes, 1 hr and at hourly intervals up to 8 hrs the number of microspheres still adhering onto the tissue was counted. The results of invitro wash off test of batches B1 to B7 were shown in table no 5 and 6.

Entrapment Efficiency: The washed microspheres are allowed to lyse to determine the entrapment efficiency or the percent entrapment of the microspheres. The active constituents are determined from the lysate as per monograph requirement. The percent encapsulation efficiency of all formulations were shown in table no 9 and is calculated using following equation.

% Entrapment = Actual content/Theoretical content x 100.

I*n Vitro* drug release: To carry out *In Vitro* drug release, accurately weighed 50 mg of loaded microspheres were dispersed in dissolution fluid in a beaker and maintained at 37±2° C under continuous stirring at 100 rpm. At selected time intervals 5 ml samples were withdrawn through a hypodermic syringe fitted with a 0.4 µm Millipore filter and replaced with the same volume of pre-warmed fresh buffer solution to maintain a constant volume of the receptor compartment. The samples were analyzed spectrophotometrically. The released drug content was determined from the standard calibration curve of given drug.

Drug polymer interaction (FTIR) study

IR spectroscopy can be performed by Fourier transformed infrared spectrophotometer. The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000- 600 cm^{-1}. FTIR study was carried on pure drug, physical mixture, formulations and empty microspheres.

RESULTS AND DISCUSSION

The results have shown that the micrometric studies of mucoadhesive microspheres have concluded that, the micrometrics found to be shown in table no 3 and 4 excellent flow properties, where as invitro comparision studies found to be shown that formulation F5 has shown good, mucoadhesion properties and entrapment efficiency in table no 5, in vitro drug release.

Table 3: Flow properties of all formulations

BATCH CODE	ANGLE OF REPOSE (θ)	BULK DENSITY (g/cm2)	TAPPED DENSITY (g/cm2)	HAUSNERS RATIO	CARRS INDEX
F1	16	0.71	0.81	1.14	12.34
F2	12	0.79	0.85	1.07	7.05
F3	14	0.68	0.81	1.19	16.04
F4	11	0.80	0.87	1.09	8.04
F5	13	0.70	0.76	1.08	7.89
F6	15	0.61	0.68	1.09	10.29

Table 4: Mean particle size of all formulations

BATCH CODE	PARTICLE SIZE µm	ENTRAPMENT EFFICIENCY
F1	170	70%
F2	140	78%
F3	190	75%
F4	285	82%
F5	200	91%
F6	225	87%

Table 5: percent mucoadhesive property of all formulations in pH 1.2 buffer

Time (hr)	F1	F2	F3	F4	F5	F6
0.5	35%	70%	45%	78%	82%	75%
1	20%	65%	27%	78%	78%	69%
2	02%	53%	05%	71%	78%	67%
3	---	40%	---	71%	72%	65%
4	---	30%	---	68%	72%	65%
5	---	25%	---	68%	72%	60%
6	---	17%	---	68%	70%	58%
7	---	05%	---	65%	70%	50%
8	---	---	---	60%	68%	40%

Figure 12: SEM of optimized formula F5.

Table 1: invitro drug release results of all formulations in pH 6.8 buffer.

TIME	F1	F2	F3	F4	F5	F6
01hr	05%	05%	07%	07%	18%	11%
02hr	12%	11%	11%	14%	25%	19%
03hr	17%	18%	18%	19%	38%	27%
04hr	22%	24%	22%	27%	44%	35%
05hr	30%	31%	30%	35%	49%	41%
06hr	34%	36%	37%	41%	55%	48%
07hr	39%	41%	42%	49%	62%	55%
08hr	44%	47%	49%	57%	69%	61%
09hr	52%	54%	56%	66%	76%	68%
10hr	59%	60%	61%	72%	81%	75%
11hr	63%	66%	69%	79%	86%	80%
12hr	68%	72%	75%	85%	91%	87%

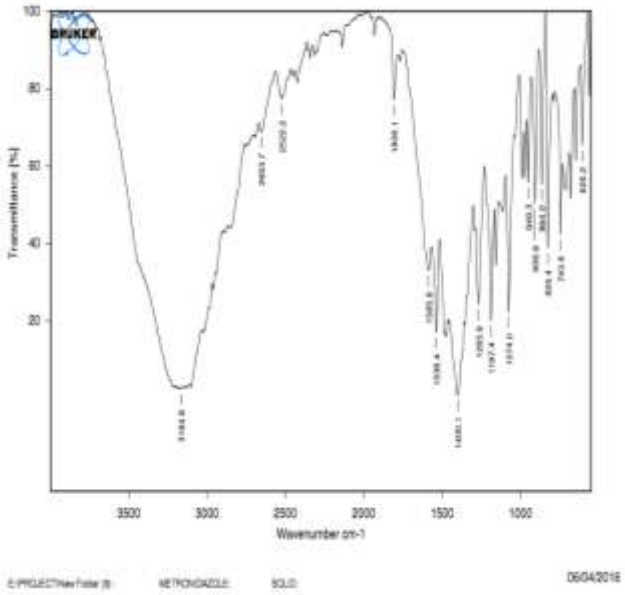

Figure 2: FTIR of metronidazole.

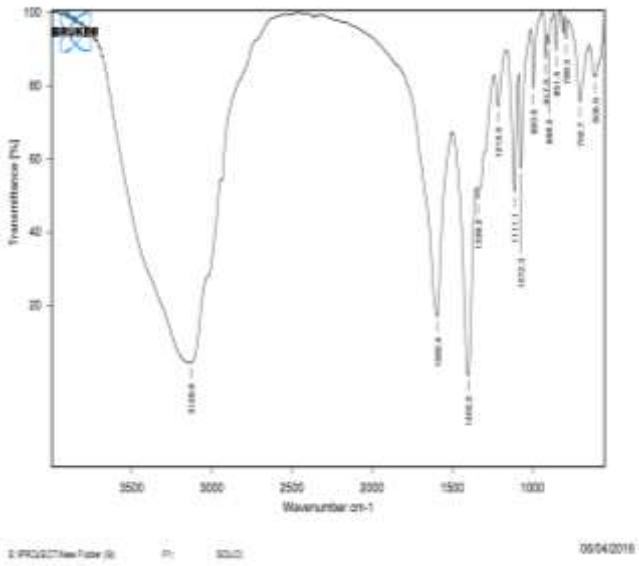

Figure 3: FTIR of metronidazole and sodium alginate.

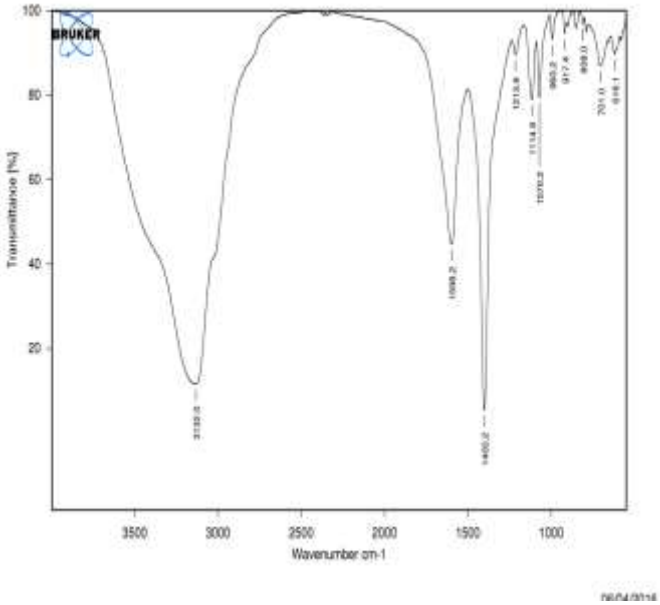

Figure 4: FTIR of metronidazole, sodium alginate and carbopol.

CONCLUSION

The mucoadhesive microspheres Metronidazole using carbopol934 and sodium alginate in different ratios were sucessfully formulated by ionotropic gelation method. All the formulations have shown good flow properties and good particle size.

The invitro wash off comparision test, ivitro drug release were carried out in 1.2 pH and 6.8pH buffers and F5 formulation has shown good mucoadhesion property and ivitro relase amongst all the 6 formulations. Formulation F5 has shown good drug and excipient compatibility.

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