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Design and Evaluation of Sodium Alginate Based Microspheres Loaded With Misoprostal

K. Ranjith Kumar[1], D.V. R. N. Bhikshapathi[2*]

1.Research Scholar, Mewar University, Chittorgarh, Rajasthan, India

2.Research Supervisor, Mewar University, Chittorgarh, Rajasthan, India

ABSTRACT

In the present investigation efforts were made to develop Misoprostal loaded microspheres to obtain a desirable drug release profile by Ionic gelation method using hydrophilic polymers and cross linking agent to decrease the gastric irritation and to enhance the drug penetration. Microspheres were prepared by using sodium alginate and calcium chloride in different ratios. All the microspheres were evaluated for particle size, percentage yield, drug entrapment efficiency, stability studies and for *in vitro* release kinetics and found to be within the limits. Among all the formulations S7 was selected as optimized formulation based on the physico chemical properties and drug release studies. *In vitro* drug release study of formulation S7 showed 97.17% drug release up to 12h in a controlled manner, which is essential for an anti ulcer therapy. The innovator Misoprostal marketed product shows the drug release of 95.23 in 1 h. The drug release of optimized formulation S7 followed zero order release and Higuchi kinetics indicating diffusion controlled drug release. FT- IR study showed no drug excipient interaction takes place.

Keywords: Misoprostol, sodium alginate, microspheres, scanning electron microscopy, release order kinetics.

*Corresponding Author Email: dbpathi71@gmail.com

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INTRODUCTION

Oral drug administration is by far the most preferable route for taking medications. However, their short circulating half life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect. This results in pill burden and consequently, patient complains. Rational approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamic profile is to release the drug in a controlled manner and site specific manner [1].

Microspheric drug delivery has advantage over various other dosage forms like we know for lungs disease now a days aerolised drugs are used for local delivery of drugs but it has disadvantage of shorter duration of action so for sustained release and reducing side effects and hence to achieve better patient compliance microspheres can be used. It also has advantage over liposomes as it is physicochemically more stable. Moreover the microspheres are of micron size so they can easily fit into various capillary beds which are also having micron size [2].

For the treatment of chronic diseases it is important to take medication several times, this may lead to fluctuating drug level in body. In order to avoid frequent drug administration and maintenance of therapeutic drug level in body it is essential to administer drug by a sustained release system. Drugs with short elimination half life are most suitable for sustained release formulations. Sustained delivery of drugs can be achieved by microspheres formulation [3].

The microsphere requires a polymeric substance as a carrier and a core material [4,5]. Microspheres have been widely accepted as a mean to achieve oral and parenteral controlled release [6,7,8].

Peptic ulcer disease, also known as a peptic ulcer or stomach ulcer, is a break in the lining of the stomach, first part of the small intestine, or occasionally the lower esophagus. Common causes include the bacteria, *Helicobacter pylori* and non-steroidal anti-inflammatory drugs [9].

Misoprostol, a histamine H_2-receptor antagonist and proton pump inhibitor, which is used to reduce the risk of acute peptic ulcers, stomach ulcers in patients treated with nonsteroidal anti-inflammatory drugs, after oral administration, misoprostol is rapidly and almost completely absorbed from the gastrointestinal tract. However, it undergoes extensive first pass metabolism to form misoprostol acid. After a single dose of 0.4 mg oral misoprostol, the plasma misoprostol level increases rapidly and peaks at about 30 minutes, declines rapidly by

120 minutes. The aim of present work is to design and in vitro evaluation of Misoprostal microspheres to enhance its bioavailability and prolonged drug release [10].

MATERIALS AND METHOD

Materials:

Misoprostol pure drug was generous gift from Splendid Labs, Pune, India. Sodium alginate was obtained from Pruthvi Chemicals, Mumbai; Calcium chloride was purchased from SD fine chemicals, Mumbai. All other chemicals used were of analytical grade.

Methods

Formulation of Misoprostol microspheres:

Misoprostol microspheres were prepared using polymers such as sodium alginate and calcium chloride by Ionotropic gelation method. Different formulation trials of Misoprostal were prepared using different concentration of polymer and cross linking agent. Total 14 formulations were developed using sodium alginate and calcium chloride in different concentrations. In this method weighed quantity of Misoprostal was added to 100 ml sodium alginate solution and thoroughly mixed at 500 rpm. Resultant solution was extruded drop wise with the help of syringe and needle into 100 ml aqueous calcium chloride solution and stirred at 100 rpm. After stirring for 10 minutes the obtained microspheres were washed with water and dried at 60^{o} C for 2 hrs in a hot air oven and stored in a dessicater [11].

Table 1: Formulation trials of Misoprostal normal microspheres:

Formulation code	Misoprostol (mg)	Sodium alginate	Calcium chloride
S1	2	1%	7%
S2	2	1.2 %	7%
S3	2	1.4%	7%
S4	2	1.6%	7%
S5	2	1.8%	7%
S6	2	2%	7%
S7	2	2.2%	7%
S8	2	1%	10%
S9	2	1.2%	10%
S10	2	1.4%	10%
S11	2	1.6%	10%
S12	2	1.8%	10%
S13	2	2%	10%
S14	2	2.2%	10%

Evaluation of Misoprostal microspheres:

Particle size:

The 100 microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle diameters of more than 100 microspheres were measured randomly by optical microscope.[12]

Angle of repose:

Angle of repose (Θ) of microspheres measures the resistance to particles flow, and is calculated according to fixed funnel standing cone method. Where (Θ) is angle of repose, H/D is surface area of the free standing height of the microspheres heap that is formed on a graph paper after making the microspheres flow from glass funnel (13).

$$\theta = \tan^{-1}(h/r)$$

Bulk density: Volume of the microspheres in the measuring cylinder was noted as bulk density.

$$\text{Bulk density} = \frac{\text{Wt of powder}}{\text{Bulk volume of powder}}$$

Tapped density: Change in the microspheres volume was observed in mechanical tapping apparatus.

$$\text{Tapped density} = \frac{\text{Wt of microspheres}}{\text{Tapped volume of microspheres}}$$

Compressibility index:

Also called as Carr's index and is computed according to the following equation.

$$\text{Carr's compressibility index} = \frac{\text{Tapped density - Bulk density}}{\text{Tapped density}} \times 100$$

Hausner's ratio:

Hausner's ratio of microspheres is determined by comparing the tapped density to the fluff density using the equation [14].

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Swelling index:

Swelling index was determined by measuring the extent of swelling of microspheres in the given medium. Exactly weighed amount of microspheres were allowed to swell in given

medium. The excess surface adhered liquid drops were removed by blotting and the swollen microspheres were weighed by using microbalance. The hydro gel microspheres then dried in an oven at 60 degrees for 5h until there was no change in the dried mass of sample. The swelling index of the microsphere was calculated by using the formula [15]

Swelling index= (Mass of swollen microspheres - Mass of dry microspheres/mass of dried microspheres) X 100.

Drug entrapment efficiency and % yield:

In order to determine the entrapment efficiency, 10 mg of formulated microspheres were thoroughly crushed by triturating and suspended in required quantity of methanol followed by agitation to dissolve the polymer and extract the drug. After filtration, suitable dilutions were made and drug content assayed spectrophotometrically at particular wavelength using calibration curve. Each batch should be examined for drug content in a triplicate manner [16].

% Drug entrapment = Calculated drug concentration /Theoretical drug concentration x 100

% yield = Total weight of microspheres / Total weight of drug and polymer x 100

***In vitro* drug release studies:**

In vitro drug release studies for developed Misoprostal microspheres were carried out by using dissolution apparatus II paddle type (Electrolab TDL-08L). The drug release profile was studied in 900 ml of 0.1 N HCl at 37± 0.5^0C temperature at 100 rpm. The amount of drug release was determined at different time intervals of 0, 1, 2, 3, 4, 6, 8, 10 & 12 hours by UV visible spectrophotometer (Shimadzu UV 1800) at 218nm [17].

Kinetic modeling of drug release:

In order to understand the mechanism and kinetics of drug release, the result of the *in vitro* dissolution study of microspheres were fitted with various kinetic equations, like zero order (percentage release vs. time), first order. (Log percentage of drug remaining to be released vs time) and Higuchi's model (Percentage drug release vs square root of time), Correlation coefficient (r^2) values were calculated for the linear curves obtained by regression analysis of the above plots.

Drug excipient compatibility studies

The drug excipient compatibility studies were carried out by Fourier transmission infrared spectroscopy (FTIR) method, Differential Scanning Calorimetry (DSC).

Fourier transforms infrared spectroscopy (FTIR)

FTIR spectra for pure drug, physical mixture and optimized formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR

Affinity 1 Spectrophotometer. The samples were dispersed in KBr and compressed into disc/pellet by application of pressure. The pellets were placed in the light path for recording the IR spectra. The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

SEM studies

The surface and shape characteristics of pellets were determined by scanning electron microscopy (SEM) (HITACHI, S-3700N). Photographs were taken and recorded at suitable magnification.

Stability studies

The stability study of the optimized formulation was carried out under different conditions according to ICH guidelines. The optimized microspheres were stored in a stability chamber for stability studies (REMI make). Accelerated Stability studies were carried out at 40 ^{0}C / 75 % RH for the best formulations for 6 months. The microspheres were characterized for the percentage yield, entrapment efficiency & cumulative % drug released during the stability study period.

RESULTS AND DISCUSSION

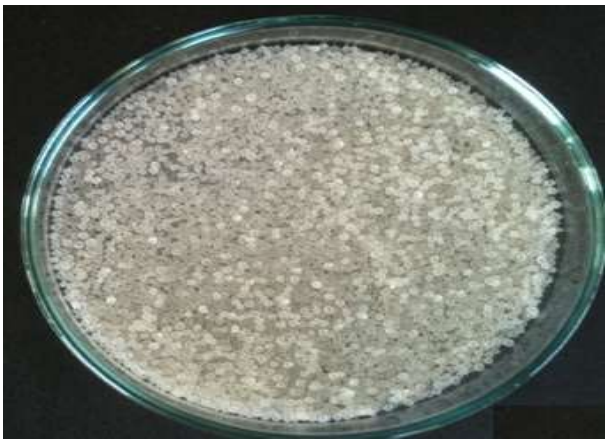

Figure 1: **Misoprostal Microspheres**

Table 2: Micromeritic properties of Misoprostal microspheres:

Formulation code	Particle size (µm)	Bulk density(g/cc³)	Tapped density(g/cc³)	Angle of repose	Carr's index	Swelling index
S1	61.12±0.08	0.66	0.69	28˚.74	9.34%	64%
S2	65.29±0.13	0.74	0.72	29˚.67	8.34%	69%
S3	67.43±0.04	0.76	0.73	30˚.54	9.12%	60%
S4	69.67±0.09	0.79	0.73	31˚.15	9.23%	61%
S5	73.45±0.04	0.89	0.75	27˚.93	14.56%	69%
S6	92.45±0.09	0.92	0.72	26˚.21	13.95%	87%
S7	82.45±0.09	0.94	0.73	25˚.54	8.32%	95%
S8	67.45±0.04	0.69	0.65	27˚.93	14.56%	69%

S9	78.45±0.09	0.67	0.62	28˚.54	13.95%	70%
S10	81.23±0.14	0.69	0.56	27˚.91	10.32%	75%
S11	85.12±0.08	0.66	0.59	26˚.74	9.34%	84%
S12	87.29±0.13	0.74	0.62	28˚.67	10.34%	93%
S13	91.43±0.04	0.76	0.73	27˚.54	11.12%	92%
S14	94.13±0.09	0.87	0.78	29˚.15	9.23%	89.2%

All fourteen formulations were evaluated for various micromeretic and physico chemical parameters and the results are tabulated in Table 2. Among all the formulations S7 shown best results of particle size, bulk density, tapped density, angle of repose, carr's index and swelling index of 82.45±0.09, 0.94, 0.73, 25˚.54, 10.32% and 95% respectively.

Table 3: Percentage drug yield, entrapment efficiency, In vitro cumulative % drug release of Misoprostal microspheres

Formulation code	Percentage yield	Entrapment efficiency	In vitro cumulative % drug release
S1	60.00%	63.00%	83.34%
S2	71.00%	72.00%	82.30%
S3	73.00%	80.00%	80.20%
S4	83.87%	83.30%	83.50%
S5	78.30%	73.20%	82.30%
S6	91.30%	91.30%	92.12%
S7	96.30%	94.10%	97.17%
S8	76.00%	74.03%	82.30%
S9	71.00%	72.00%	80.20%
S10	76.00%	83.00%	83.50%
S11	89.09%	85.00%	82.42%
S12	82.50%	86.66%	86.41%
S13	93.30%	91.03%	94.36%
S14	85.30%	84.88%	85.00%

The percentage yield and entrapment efficiency of all the formulations were measured by assay method and found to be within the limits. The formulation S7 shows good percentage yield, entrapment efficiency, In vitro cumulative % drug release of 96.30%, 94.10% and 97.17% respectively and the results were depicted in Table 3.

***In vitro* drug release studies**:

Misoprostal microspheres were evaluated for in vitro drug release studies in 0.1N HCL and the results are depicted in Table 4 and 5. The formulation S7 shows best drug release of 97.17% within 12h. The drug release was in controlled manner when compared with innovator product Misoprostal i.e 95.23% within 1h.

Table 4: *In vitro* cumulative % drug release of Misoprostal microspheres formulations S1 to S7:

Time (h)	S1	S2	S3	S4	S5	S6	S7	Marketed product
0	0%	0%	0%	0%	0%	0%	0%	0%
1	21.09%	22.07%	15.23%	20.23%	22.34%	14.31%	16.10%	95.23%
2	26.08%	25.06%	23.12%	24.12%	25.34%	21.15%	24.30%	--
3	28.23%	30.05%	30.05%	31.04%	30.12%	28.19%	30.20%	---
4	39.11%	38.20%	38.90%	44.40%	38.20%	37.23%	39.40%	---
6	50.39%	51.30%	49.90%	51.70%	51.30%	51.73%	53.80%	---
8	66.23%	63.30%	61.21%	60.30%	63.30%	66.46%	68.60%	---
10	70.20%	69.90%	71.22%	70.30%	73.30%	81.45%	83.90%	---
12	83.34%	82.30%	80.20%	83.50%	82.30%	92.12%	97.17%	---

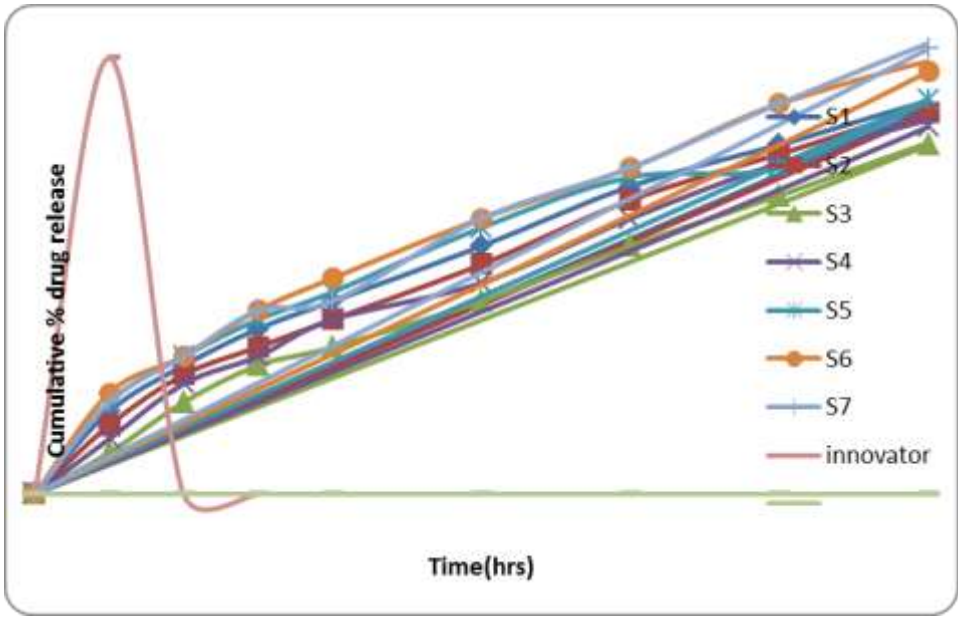

Figure 2: in vitro cumulative % drug release of Misoprostal sodium alginate microspheres formulations S1 to S7

Table 5: In vitro cumulative % drug Misoprostal sodium alginate release of microspheres formulations S8 to S14

Time (h)	S8	S9	S10	S11	S12	S13	S14
0	0%	0%	0%	0%	0%	0%	0%
1	22.05%	15.23%	20.23%	22.00%	15.22%	15.62%	21.63%
2	25.40%	23.34%	24.80%	25.40%	24.23%	23.01%	32.01%
3	30.01%	30.08%	31.38%	30.55%	31.96%	29.11%	37.11%
4	38.20%	38.90%	44.40%	38.20%	40.10%	38.24%	44.83%
6	51.30%	49.91%	51.60%	51.30%	54.20%	52.83%	57.76%
8	63.35%	61.20%	60.30%	63.30%	68.24%	67.03%	64.60%
10	69.90%	70.10%	70.60%	69.92%	72.32%	82.22%	75.56%
12	82.30%	80.20%	83.50%	82.42%	86.41%	94.36%	85.00%

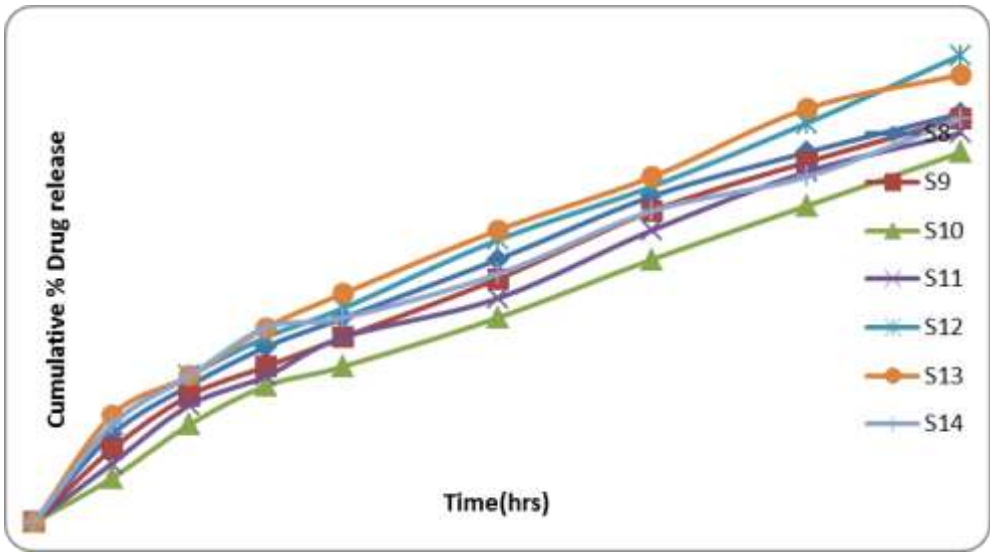

Figure 3: In vitro cumulative % drug release of Misoprostal sodium alginate microspheres S8 to S14:

Mathematical modeling of Misoprostal optimized microspheres (S7):

Table 6: Release order kinetics of optimized microspheres (S7)

Formula Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
S7	0.995	7.853	0.858	0.111	0.979	33.5	0.644	2.126

The *in vitro* release profiles from optimized formulations were applied on various kinetic models. The best fit with the highest correlation coefficient was observed in zero order and Higuchi model, indicating diffusion controlled principle. Further the n value obtained from the Korsmeyer plots i.e. 2.126 suggest that the drug release from microspheres was anomalous Non fickian diffusion.

Drug excipient compatibility studies

Fourier Transform Infrared Spectroscopy (FTIR)

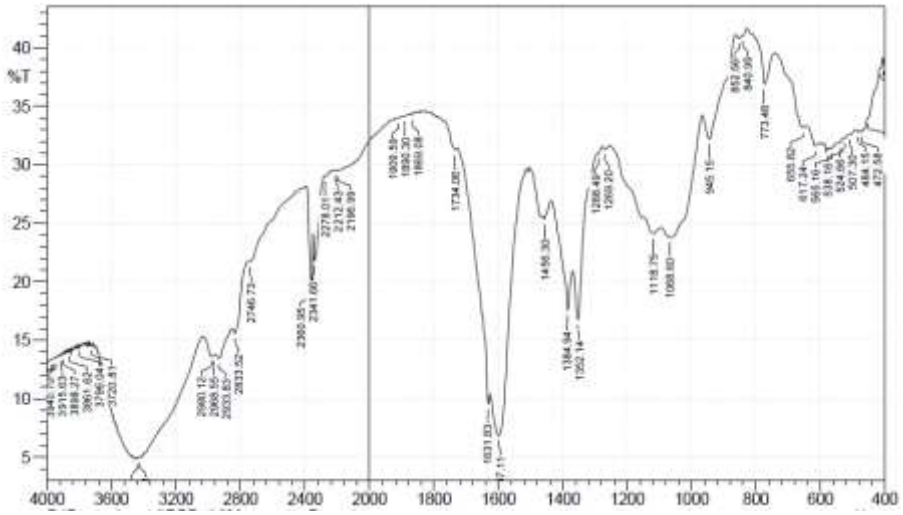

Figure 4: FT-IR spectrum of pure drug misoprostol

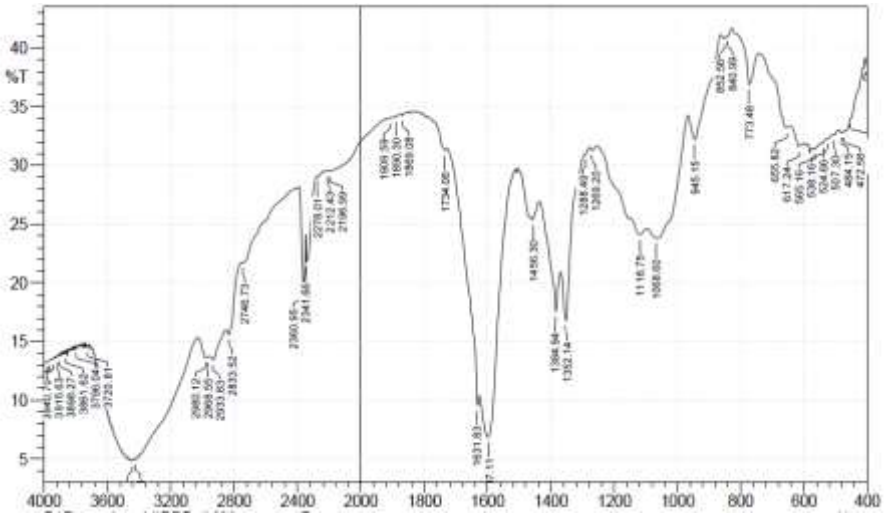

Figure 5: FT-IR spectrum of Misoprostal+ Sodium alginate

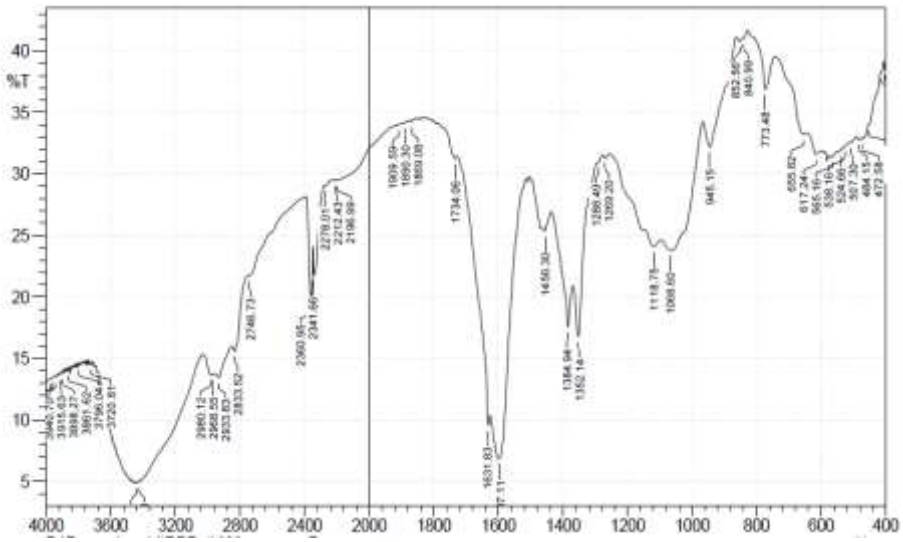

Figure 6: FT-IR spectrum of optimized formulation of normal microspheres

The IR spectrum of Misoprostol pure drug showed peaks at 2930 cm-^{1}, 1735 cm-^{1}, 1381 cm-^{1}, and 1050 cm-^{1} which represented various bending and stretching vibrations of the different groups present in the drug molecule. Overall there was no alteration in peaks of Misoprostol pure drug and optimized formulation, suggesting that there was no interaction between drug & excipients. There is additional peaks appeared or disappeared hence no significant changes in peaks of optimized formulation was observed when compared to pure drug, indicating absence of any interaction.

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SEM of Misoprostal microspheres

The external and internal morphology of controlled release microspheres were studied by Scanning Electron Microscopy.

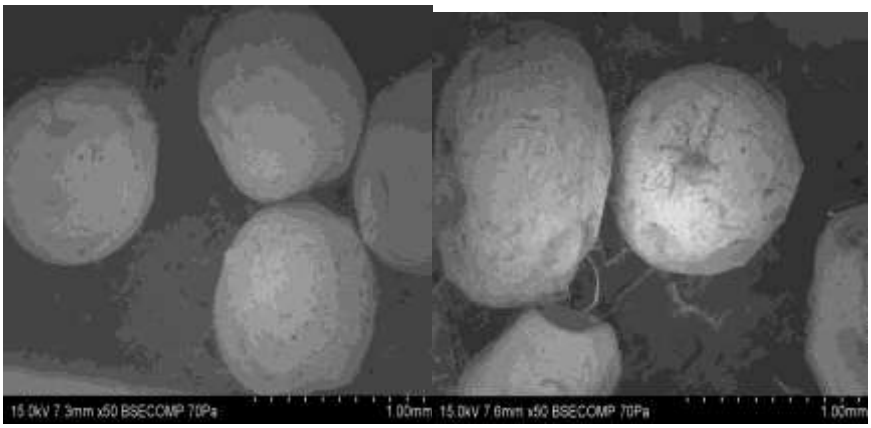

Figure 7: Scanning electron micrographs of Misoprostal microspheres

Morphology of the various formulations of alginate microspheres prepared was found to be discrete and spherical in shape (Figure 7). The surface of the alginate microspheres was rough due to higher concentration of drug uniformly dispersed at the molecular level in the alginate matrices. There are no crystals on surface which states that is drug is uniformly distributed.

Table 7: Stability studies of Misoprostal optimized formulation:

Retest Time For Optimized formulation	Percentage yield	Entrapment efficiency	*In-vitro* drug release profile (%)
0 days	96.30	94.10	97.17
30 days	94.40	93.4	95.20
60 days	93.22	93.13	94.33

120 days	92.13	92.55	93.68
180 days	91.34	92.15	93.26

Optimized formulation (S7) was selected for stability studies on the basis of high cumulative % drug release. Stability studies were conducted by performing Percentage yield, %Entrapment efficiency and *In-vitro* drug release profile for 6 months according to ICH guidelines. From these results it was concluded that, optimized formulation is stable and retained their original properties with minor differences.

CONCLUSION

From the above data, it could be concluded that Misoprostal microspheres exhibited prolonged and controlled release effect compared to Innovator product. Prepared Misoprostal microspheres were characterized for particle size, scanning electron microscopy, FT-IR study, percentage yield, drug entrapment, stability studies and found to be within the limits. Among all the formulations S7 was selected as optimized Misoprostal formulations based on the physico chemical and release studies. In the *in vitro* release study of formulation S7 showed 97.17% in 12 h in a controlled manner, which is essential for disease like peptic ulcer. The marketed product shows the drug release of 95.23% within 1 h.

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