ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 02, pp.26846-26852 February, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

FORMULATION AND EVALUATION OF SUSTAINED RELEASE MUCOADHESIVE MICROSPHERES OF LANSOPRAZOLE SODIUM

***Abhishek Mishra, Dr. Shamim Ahmad, Yogendra Kumar, Ankul Kumar**

Translam Institute of Pharmaceutical Education and Research, Meerut, 250001

ARTICLE INFO

Article History:
Received 24th November, 2015
Received in revised form
15th December, 2015
Accepted 07th January, 2016
Published online 27th February, 2016

Key words:

Mucoadhesive microsphere,
Sustain release,
Lansoprazole,
Bioavailability.

ABSTRACT

The Drug Lansoprazole has short half-life and hence requires frequent administration. Therefore the possible way for formulating a sustained release formulation of Mucoadhesive Microsphere. These formulation are prepared by Ionic gelation techniques by using polymer Sodium Alginate and Tragacanth. Various evaluation parameters are assessed, with a view to obtain sustained release of Lansoprazole. In the present study six formulations are formulated by using Sodium Alginate and Tragacanth various proportions. The prepared Lansoprazole microspheres are then subjected to IR, SEM, particle size and size distribution, % yield, Swelling Index, Micrometric, in vitro dissolution studies. The IR Spectra revealed that, there is no interaction between the polymer and Lansoprazole. Lansoprazole microspheres is spherical in nature, which was confirmed by SEM. Lansoprazole microspheres with normal frequency distribution are obtained. The in vitro release profile of Lansoprazole microspheres showed that sustained release is dependent upon the polymer concentration.

Citation: **Abhishek Mishra, Dr. Shamim Ahmad, Yogendra Kumar, Ankul Kumar, 2016.** "Formulation and evaluation of sustained release mucoadhesive microspheres of lansoprazole sodium", *International Journal of Current Research,* 8, (02), 26846-26852.

INTRODUCTION

Lansooprazole is a Proton pump inhibitors used in the treatment of gastroesophageal reflux disease (GERD), reflux esophagitis, gastric ulcers, duodenal ulcers and Zollinger-Ellison Syndrome, etc. Microspheres are the carrier linked drug delivery system in which particle size is ranges from (1-1000 μm) range in diameter having a core of drug and entirely outer layers of polymers as coating material. However, the success of these microspheres is limited due to their short residence time at site of absorption. It would, therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membrane. This can be achieved by coupling mucoadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive microspheres have advantages like efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site (Ikeda *et al.,* 1992). The basic goal of therapy is to achieve a steady state blood level that is therapeutically effective and nontoxic for an extended period of time. The design of proper dosage regimen is an important element in accomplishing this goal. Sustained release, sustained action, controlled release, extended action, timed release, depot and repository dosage forms are terms used to identify drug therapy systems that are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose. The term controlled release has become associated with those systems from which therapeutic agents may be automatically delivered at predetermined rates over a long period of time (Jain, 1997).

****Corresponding author: Abhishek Mishra***
Translam Institute of Pharmaceutical Education and Research, Meerut, 250001

MATERIALS AND METHODS

Formulation of Sustained release mucoadhesive microsphere

Materials

Lansoprazole Sodium was given by Jubilent life science Company as a gift sample. Sodium alginate and Tragacanth was purchased from market of Khari Bauli, Delhi. All chemical and reagents were used of laboratory grade.

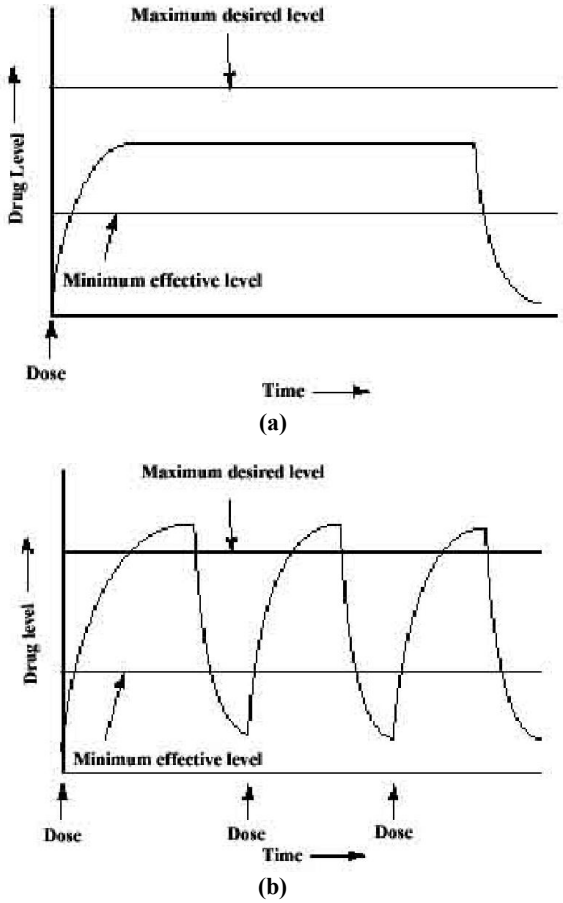

Figure 1. Drug concentration levels for (a) conventional system; (b) controlled release system

Preparation of Mucoadhesive microspheres

Mucoadhesive microspheres were prepared by Ionotropic gelation method. Firstly sodium alginate was dissolved in sufficient amount of water with maintaining the temperature between 40-50ºC. Then required amount of polymer was added into it. When the polymer dissolved, drug was added into it and dispersed them. We were prepared a 10% Calcium chloride solution as a continuous phase and placed on the magnetic stirrer. The drug and polymers solution were filled into the syringe and drop wise added into the calcium chloride solution by using needle.

Table 1. Different Concentration of Drug and Polymer

Batch No.	Conc. of Drug (Lansoprazole sodium) (mg)	Conc.of Polymer (Sodium-Alginate) (mg)	Conc. of Polymer (Tragacanth gum) (mg)
F1	50	300	300
F2	50	300	400
F3	50	300	500
F4	50	400	300
F5	50	400	400
F6	50	400	500

The prepared microsphere was allowed to stand in the calcium chloride solution for 30 minute. After that it was filtered by using whattman filter paper. Dried it in the hot air oven at 50ºC temperature and stored it. Composition of prepared floating microsphere was showed in following table

Evaluation of mucoadhesive microspheres Surface morphology

Surface characteristics of mucoadhesive microspheres will be analysed by using scanning electron microscopy. Samples were coated with gold dust under vacuum prior to observation. Cross sections were made in order to observe the core and internal structure of the microspheres. These studies were useful in the examination of internal and external morphology of mucoadhesive microspheres (Tiwari, 2010).

Particle Size

Particle size analysis of drug-loaded microspheres was performed by optical microscopy using a Compound microscope. A small amount of dry microspheres was suspended in n-hexane (10 mL). The suspension was ultrasonicated for 5 seconds. A small drop of suspension thus obtained was placed on a clean glass slide. The slide containing microspheres was mounted on the stage of the microscope and 50 particles are measured using a calibrated ocular micrometer. The average particle size was determined by using the Edmondson's Equation (Thanoo *et al.,* 1992).

$$(\text{Stage reading})/(\text{Occular reading}) \times 100$$

Micromeritics: Microspheres can be characterized for their micromeritics properties such as angle of repose, compressibility index and Hausner's ratio. Standard values of angle of repose, Carr's index and Hausner's ratio are represented in table (Garg and Gupta, 2009).

Angle of Repose: Angle of repose (θ) of the mucoadhesive microspheres measures the resistance to particles flow, and is calculated according to fixed funnel standing cone method. In this method 30 mL of microspheres were poured into a conical flask having a 0.9 cm diameter and placed 10 cm above the surface. After letting the microspheres flow freely from the height of 10 cm to the surface, the tangent of angle of repose was determined by using the formula mention in equation.

$$\tan\theta = \frac{2H}{D}$$

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

$$\text{Carr}\,\% = \frac{\text{Tapped density} - \text{Fluff density}}{\text{Tapped density}} \times 100$$

Hausner's ratio: Hausner's ratio of mucoadhesive microspheres is determined by comparing the tapped density to the fluff density using the equation.

Standard value of various flow parameters

Angle of repose	Carr's index	Hausner's ratio	Type of flow
>20 °	5-15%	--	Excellent
20-30	12-16%	<1.25	Good
30-40	18-21%	--	Fair to passable
--	23-35%	>1.25	Poor
--	33-38%	1.25-1.5	Very poor
>40 °	>40	--	Extremely poor

Percentage yield

Percentage yield of mucoadhesive microspheres is calculated by dividing actual weight of product to total amount of all non-volatile components that are used in the preparation of mucoadhesive microspheres and was represented by following formula (Jain *et al.,* 2005).

$$\%Yield = \frac{Actual\ weight\ of\ Microsphere}{Total\ weight\ of\ Microsphere} \times 100$$

Swelling index

This technique was used for Characterization of sodium alginate microspheres. Different solution (100ml) was taken such as (distilled water, buffer solution of pH (1.2, 7.4) are taken and alginate microspheres (100mg) are placed in a Petridis and kept on the above solution and swelling is allowed at 37 0C for different time interval and changes in weight variation between initial weight of microspheres and weight due to swelling is measured by taking weight periodically and soaking with filter paper (Gopferich, 1994). The swelling index of the microsphere is calculated by using the formula:

$$\text{Swelling index} = \frac{\text{mass of swollen microspheres} - \text{mass of dry microspheres}}{\text{mass of dry microsphere}} \times 100$$

Drug entrapment efficiency

Estimation of drug content in mucoadhesive microspheres is carried out by dissolving the 10 mg of crushed microspheres in 10 mL of 0.1 N HCl. 1 mL of this extract was transferred to a 10 mL of volumetric flask and the volume was made up using 0.1 N HCl. The solution was filtered and the absorbance was measusered spectrophotometrically Systronics (2202), at 233 nm using calibration curve. Each batch was examined for drug content in a triplicate manner. The entrapment efficiency of mucoadhesive microspheres is calculated by dividing the actual drug content by the theoretical drug content of microspheres (Patel *et al.,* 2006).

$$\text{DEE} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

In vitro drug release studies

Release rate of drug from mucoadhesive microspheres was determined using USP dissolution apparatus type II (paddle type) at 37 ± 0.5 ^{o}C. The dissolution test was carried out using 900 ml of 0.1 N HCl dissolution medium at 50 rpm for the required period of time upto 12 hrs. Microspheres equivalent to 60 mg of Reloxifene were used for the test. At an appropriate interval, specific volume of aliquots were withdrawn and replaced with an equivalent volume of fresh dissolution medium to maintain the constant volume of dissolution medium. The sample solutions were filtered through whatman filter paper and solutions are analysed at 233 nm using UV spectrophotometer (Rao *et al.,* 2009).

In vitro drug release kinetics

Mathematical modelling of the release process plays a significant role as it establishes and demonstrates the mechanism of drug release and also provides more general guidelines for the development of systems. The profile and kinetics of drug release are important because they correlate *in vitro* and *in vivo* drug responses by comparing results of pharmacokinetics and dissolution profile patterns. Different mathematical models may be applied for describing the kinetics of drug release process from microspheres; the most being the one which best fit the experimental results. These models best explain the drug release from various pharmaceutical systems resulting from a simple phenomenon, or when this phenomenon by being the rate limiting step, condition all other processes occurring in the system. In present study, *in vitro* dissolution data was fitted to the PCP Disso v3 software which provides the best fitted model and describes the kinetics of drug release from fabricated mucoadhesive microspheres.

RESULTS AND DISCUSSION

Preformulation Studies

Organoleptic properties of Lansoprazole

The given sample of the drug was observed for its organoleptic properties:

Table 2. Organoleptic properties of Lansoprazole drug

S.No	Type	Description
1	Taste	Bitter
2	Odour	Odorless
3	Colour	White and off white
4	State	Amorphous

which is in accordance the US phamacopoeial monograph**.**

Melting point determination

The capillary tube method is used for determination of melting point in the present study. Then one side of the tube was sealed with the help of flame. The drug was filled into the capillary from one open side. The capillary tube was placed into the melting point apparatus (Nitin Scientific). The melting point range was noted from where the drug starts melting to where it melts completely. Melting point of Lansoprazole was found to be in a range of 160-180 ^{0}C which is in accordance with the standard melting point of Lansoprazole. The standard value and observed value are shown below,

Table 3. Melting Point of Lansoprazole Drug

Melting point	Observed value	Mean value
1.	170 ^{0}C	
2.	178 ^{0}C	176^{0}C
3.	180 ^{0}C	

The observed value is indicates the presence of Lansoprazole pure drug.

Solubility

The solubility of a substance is the amount of that substance that will dissolve in a given amount of solvent. 1 gm of Lansoprazole is taken and dissolved in 10 ml of various solvents (Saltiel and Fask, 1999).

Table 4. Solubility of Lansoprazole Drug

Methanol	Freely Soluble
Water	Soluble
Ethanol	Soluble
Liquid paraffin	Not Soluble
Chloroform	Not Soluble

The solubility of drug was found to be highest in methanol and lowest in liquid paraffin and chloroform. The solubility studies depict that the drug is soluble in polar solvents as it is soluble in water as well as ethanol and insoluble in liquid paraffin and chloform.

Compatibility study of drug and polymer

IR study was performed to study compatibility between drug and polymer. Drug and polymers was mixed in 1:1 ratio after that mixer was placed on FTIR spectroscopy. Take the spectra of it and interpret it.

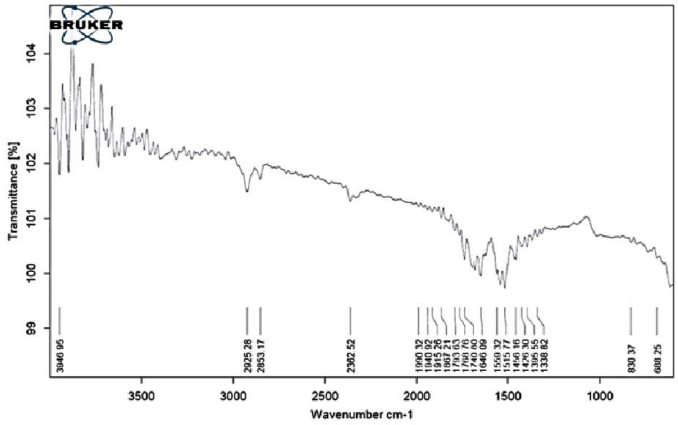

Fig. 2. IR Spectrum of physical mixture of Lansoprazole sodium, Sodium Alginate, and Tragacanth

Compatibility studies are performed using IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and various excipients are studied. The characteristic absorption peaks of Lansoprazole obtained at 3863.28cm^{-1}, 2984.74 cm^{-1}, 2362.37cm^{-1}, and 1678.60 cm^{-}.As shown in above figures. The peaks obtained in the spectrum of each physical mixture are correlated with the peaks of pure drug of Lansoprazole Sodium. The parent peaks of drug did not show any deviation which indicates that the drug is compatible with the formulation components (Bhople *et al.,* 2012).

Evaluation of Sustained release mucoadhesive microsphere

Particle Size Determination

The average particle size of the different microsphere formulations can be depicted graphically as in Figure 6.2 and Figure 6.3. The trend depicted was that the formulation F1 has the biggest particle size and F6 has smallest particle size with others having little difference in their size ranges.

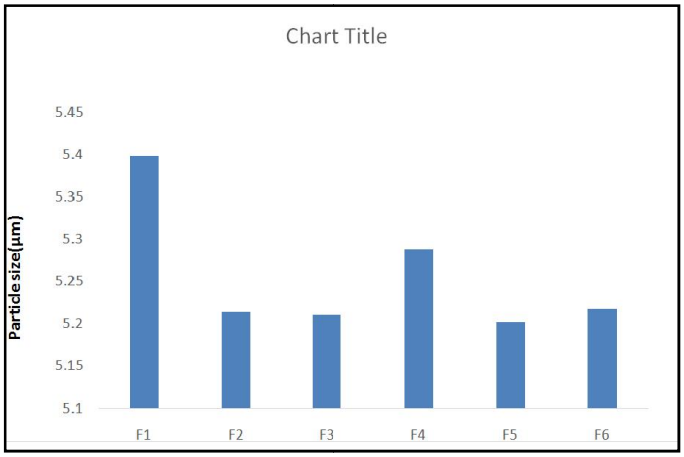

Fig. 3. Particle sizes of different formulations

Table 5. Observation table for particle size of different formulations

S.No.	Formulation1	Formulation2	Formulation3	Formulatin4	Formulation5	Formulation6
1.	0.0052	0.0056	0.0045	0.0053	0.0066	0.0041
2.	0.0044	0.0051	0.0058	0.0055	0.0038	0.0067
3.	0.0056	0.0047	0.0067	0.0054	0.0064	0.0044
4.	0.0064	0.0046	0.0037	0.0053	0.0053	0.0036
5.	0.0063	0.0037	0.0056	0.0057	0.0057	0.0058
6.	0.0055	0.0051	0.0048	0.0064	0.0035	0.0064
7.	0.0064	0.0063	0.0063	0.0057	0.0064	0.0056
8.	0.0052	0.0042	0.0051	0.0064	0.0057	0.0037
9.	0.0069	0.0043	0.0046	0.0067	0.0056	0.0043
10.	0.0058	0.0047	0.0044	0.0045	0.0046	0.0036
Avg.Particle Size	0.00577	0.00483	0.00515	0.00569	0.00469	0.00562

Table 6. Micrometric parameters of the different formulations

Formulation	Bulk density gm/cm^3	Tapped Density gm/cm^3	Carr's Index	Hausner ratio	Angle of repose
F1	0.190	0.196	0.06	0.193	27
F2	0.160	0.176	0.18	0.180	32.3
F3	0.74	0.84	0.28	0.170	31
F4	0.78	0.102	0.64	0.140	24
F5	0.152	0.164	0.14	0.185	19.3
F6	0.104	0.122	0.34	0.170	37.8

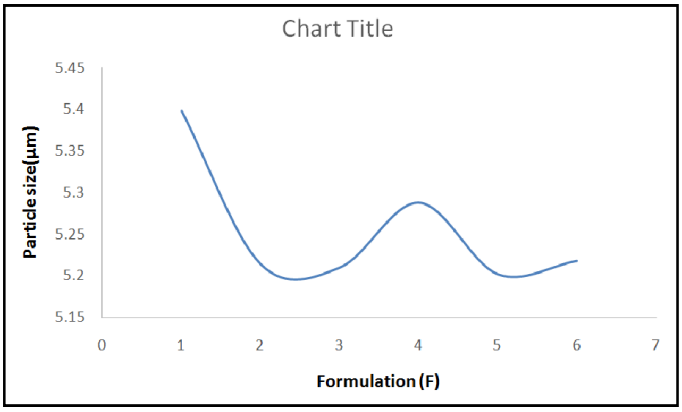

Fig. 4. Particle size of different formulation

Surface morphology of Microsphere of Lansoprazole Drug (SEM)

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture, and to examine the morphology of fractured or sectioned surface. SEM is probably the most commonly used method for characterizing drug delivery systems, owing in large to simplicity of sample preparation and ease of operation. SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan). Dry Lansoprazole microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of Lansoprazole microspheres were taken by random scanning of the stub (Magharla *et al.,* 2010).

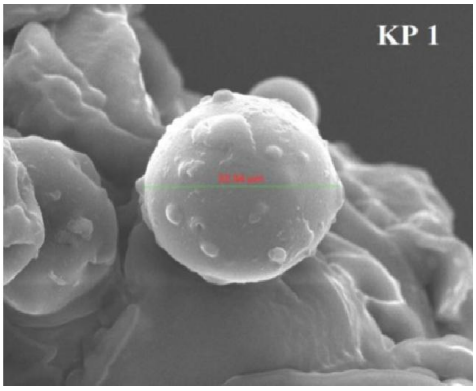

Fig. 5. SEM photographs of Lansoprazole microspheres

Surface morphology of Lansoprazole microspheres (SEM) The surface morphology of the Lansoprazole microspheres was studied by SEM. Surface smoothness of Lansoprazole microspheres was increased by increasing the polymer concentration, which was confirmed by SEM. At higher polymer concentration (1:6) the Lansoprazole microspheres with smooth surface was obtained.

Micrometric study: The results of micrometric study of Mucoadhesive Microspheres are given below:

During the micromeritic study, it was found that formulation F1 has higher bulk density in comparison to the other formulations. Tapped density was found for F1 to F6 in the range of 0.74-0.190. The Carr's compressibility index was found for F1 to F6 in the range of 0.06- 0.64.The Carr index is an indication of the compressibility of a powder. The smaller the Carr's Index the better the flow properties. In a free-flowing powder, the bulk density and tapped density would be close in value, therefore, the Carr index would be small. On the other hand, in a poor-flowing powder where there are greater interparticle interactions, the difference between the bulk and tapped density observed would be greater, therefore, the Carr index would be bigger. A Carr index greater than 25 is considered to be an indication of poor flowability, and below 15, of good flowability. Thus the value of Carr's index for all the formulations depicts that they have good flow properties. Hausner's ratio was found for F1 to F6 in the range of 0.140 – 0.192. As the value of Hausner ratio greater than 1.25 is considered to be an indication of poor flowability.

Percentage yield

The maximum percentage yield was found of F5 formulation and was noted to be 97.47%%. It was found that average percentage yield was greater than 50 % for all the batches. The percentage yield of the various formulations is tabulated in Table 6

Table 7. % yield of defferent formulation

S. NO	Formulation code	Percentage yield
1	F1	91.57%
2	F2	82.71%
3	F3	58.24%
4	F4	95.38%
5	F5	96.41%
6	F6	96.43%

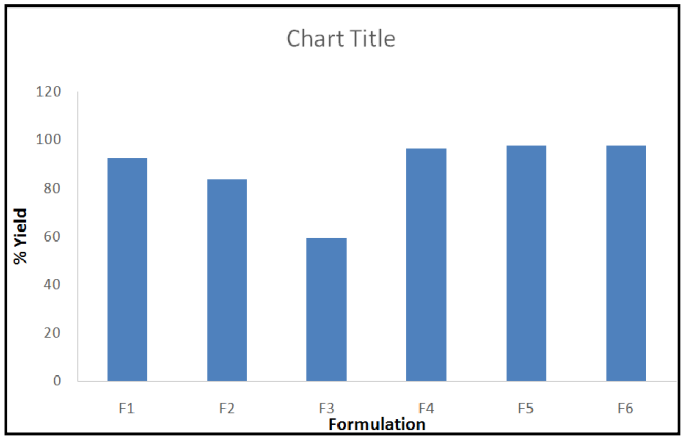

Fig. 6. % yield of different formulation

Swelling index

From the above data we concluded that the formulations shows swelling index in respective order: F5< F1<F3<F6<F2<F4. Hence we said that the formulation F5 possess higher swelling index and F4 shows low swelling index.

Drug entrapment efficiency

With the increase in polymer concentration, increased entrapment efficiency was seen probably because with increasing polymer content, more particles of Lansoprazole would be coated leading to higher encapsulation efficiency.

Table 8. Swelling index of the formulations

Time (min)	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)
0	0	0	0	0	0	0
30	153	176	185	176	207	228
60	158	178	186	182	206	226
120	163	184	188	187	213	237
150	933	918	910	612	938	924
210	942	931	934	620	947	938
330	974	963	970	636	977	968

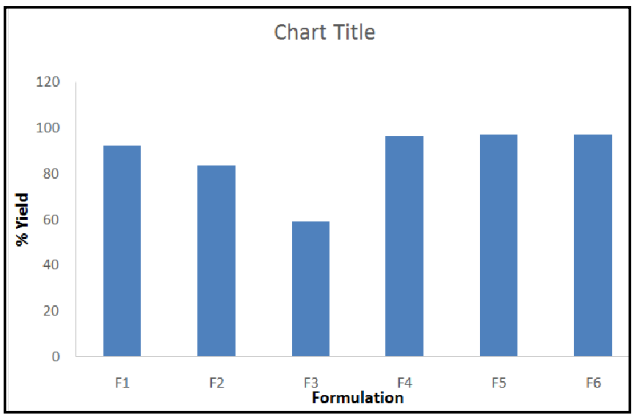

Fig. 7. Swelling index of different formulation

Table 9. Drug entrapment efficiency of various formulations

Formulation	% Entrapment Efficiency
F1	70.28
F2	72.19
F3	74.86
F4	73.34
F5	76.93
F6	79.28

In-vitro studies of drug release study

In-vitro release profile of Lansoprazole from the microspheres is examined in 0.1N HCl (pH 1.2) and 7.4 Phosphate buffer using USP (XXI) one stage dissolution rate test apparatus. Microspheres equivalent to 100 mg of drug was taken and packed in capsule and is suspended in dissolution medium at 50 rpm and 37 ± 0.5°C. An aliquot of 5 ml is withdrawn periodically at different intervals of half hour, one hour and 2 hour, same volume of fresh medium is replaced. The samples are filtered through Whatman filter paper and analysed spectrophotometry at 285 nm for amount of drug released (Parmar *et al.*, 2012).

Table 10. Absorbance value of different formulation

Time	Absorbance values at 285 nm in 0.1N HCL					
	F1	F2	F3	F4	F5	F6
30min	0.323	0.321	0.375	0.326	0.345	0.296
60	0.328	0.372	0.303	0.400	0.353	0.344
120	0.471	0.437	0.445	0.402	0.443	0.403

	Absorbance values at 285 nm in 7.4 Phosphate Buffer					
	F1	F2	F3	F4	F5	F6
180	0.316	0.314	0.345	0.364	0.301	0.312
240	0.411	0.403	0.363	0.393	0.362	0.367
360	0.552	0.541	0.581	0.521	0.503	0.473
480	0.664	0.545	0.657	0.646	0.557	0.624

Table 11. % Release of different formulation

Time	% Release values at 285 nm in 0.1N HCL					
	F1	F2	F3	F4	F5	F6
30min	12.29	11.65	13.63	11.84	12.51	10.75
60	12.33	13.32	11.06	14.44	12.84	12.41
120	17.12	15.67	16.21	14.23	16.12	14.53

Time	% Release values at 285 nm in 7.4 Phosphate Buffer					
	F1	F2	F3	F4	F5	F6
180	20.52	20.51	22.63	23.68	19.54	20.36
240	26.38	26.42	23.62	25.63	23.71	23.35
360	36.17	35.17	37.85	33.84	32.84	30.67
480	43.21	41.21	42.76	41.76	39.21	40.32

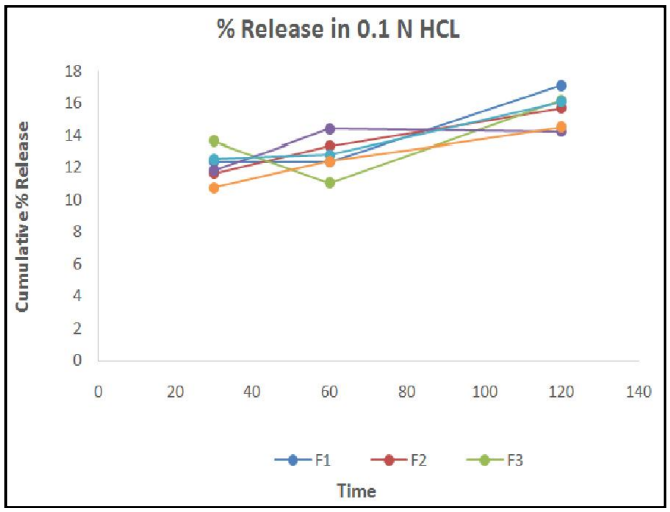

Fig. 8. Comparative in vitro release profile of Lansoprazole microspheres

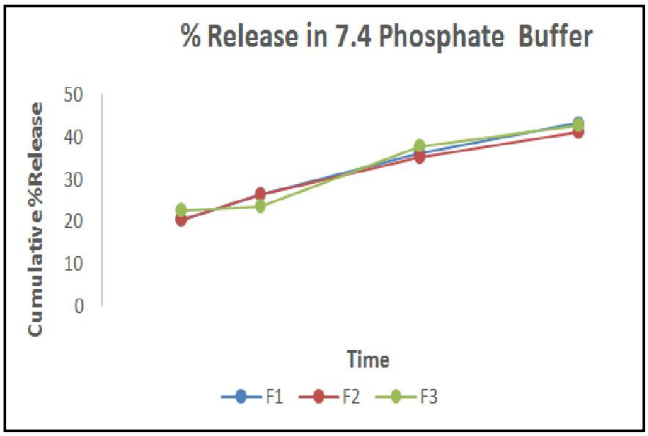

Fig. 9. Comparative in vitro release profile of Lansoprazole microspheres

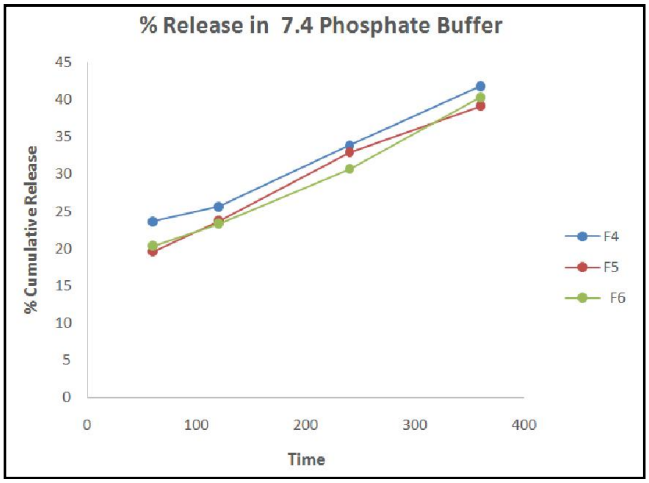

Fig. 10. Comparative in vitro release profile of Lansoprazole microspheres

Conclusion

In the present study six formulations are formulated by using Sodium Alginate and tragacanth in various proportions. All the formulations are subjected for evaluation. Results of preformulation studies, IR, Swelling index, particle size and size distribution, % yield, *in vitro* dissolution and, Flow property had shown satisfactory results. Preformulation studies like melting point, Solubility and UV analysis of Lansoprazole are complied with IP standards. The IR Spectra's revealed that, there is no interaction between polymer and Lansoprazole. The polymer used is compatible with the Lansoprazole. As the drug to polymer ratio is increased, the mean particle size of Lansoprazole microspheres was also increased. Lansoprazole microspheres with normal frequency distribution are obtained. From the results it can be inferred that there is a proper distribution of Lansoprazole in the microspheres and the deviation is within the acceptable limits. On the basis of release data and graphical analysis formulation F6 showed a good sustained release profile with maximum entrapment efficiency because of high polymer concentration. Hence, from all the above obtained data it can be summarized that it is possible to formulate a promising sustained release mucoadhecive microspheres of Lansoprazole by ionic gelation techniques using an ideal polymer like Sodium Alginate and Tregacanth.

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