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STUDIES ON FORMULATION AND *IN-VITRO* CHARACTERIZATION OF LANSOPRAZOLE LOADED NANOPONGES

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ABSTRACT

The present study involves the preparation and evaluation of lansoprazole loaded nanosponges by Emulsion solvent diffusion method using ethylcellulose as polymer, Poly vinyl alcohol as a surfactant, dichloromethane as a solvent. Lansoprazole is proton pump inhibitor drug with a short half-life of 1.5 hrs and extentively degraded in acidic pH conditions. The prepared nanosponges(ten formulations F1-F10) were evaluated for percentage yield, incorporation efficiency, particle size, porosity, drug polymer compatibility (IR study,DSC study), scanning electron microscopy and *in-vitro* drug release studies. The particle size and zeta potential of optimised F6 formulation was found to be 313.4 nm and -26.5 mV respectively.SEM analysis revealed that the particles were spherical in shape having smooth surface. The drug-polymer compatibility studies depicted that there was no interaction of between drug and polymer. The % entrapment efficiency of formulations ranges from 22.4% to 69.7 %. *In vitro* dissolution studies showed highest drug release for F6 (88.4%) in 24 hrs showing evidence in enhancement of bioavailability. Kinetic modelling revealed that the *in vitro* drug release follows first order kinetics and non-fickian drug release. The data obtained in this study suggests that nanosponges of Lansoprazole are promising for sustained drug delivery, which can reduce dosing frequency.

INTRODUCTION:

Nanosponges are tiny sponges with a size of about a virus with an average diameter below 1µm. These tiny sponges can circulate around the body until they encounter the specific target site and stick on the surface and began to release the drug in a controlled and predictable manner. Because the drug can be released at the specific target site instead of circulating throughout the body it will be more effective for a particular given dosage [1]. Nanosponges are capable of providing solutions for Several formulation related problems. Owing to their small size and porous nature they can bind poorly- soluble drugs within the matrix and improve their bioavailability[2]. They can be crafted for targeting drugs to specific sites, prevent drug and protein degradation and prolong drug release in a controlled manner. Lansoprazole (LPZ) is a proton pump inhibitor that induces maximum antisecretory activity by inhibiting hydrogen/potassium ATPase pumps in gastric parietal cells to increase intragastric pH. It is effective in treating

acid-reflux-related diseases, including gastric and duodenal ulcers, gastro-esophageal reflux disease (GERD), and erosive esophagitis . LPZ is an acid-labile and poorly water-soluble drug. It is necessary to protect LPZ from degradation in gastric acid when orally administered. The commercial available solid dosage forms of LPZ include enteric-coated granules, tablets, and capsules. The delivery of drugs by nanosponges is one of the approaches used to modify the pharmacokinetics and biodistribution of drugs (19–25). Nanosponges offer numerous advantages as a drug delivery system, including improving the bioavailability of active molecules, extending the half-life of the drug in the body, facilitating permeation of the drug across biological barriers and accumulating at the tumor sites. To date, no nanoparticulate delivery system for LPZ has been developed. It was reported that the particle size played an important role in achieving the efficient uptake of nanoparticles in the ulcerated region of stomach tissue (26,27). The objective of the present work was to develop LPZ-loaded sustained release nanosponges using ethyl cellulose. The emulsion solvent diffusion method was applied to prepare polymeric nanosponges.

EXPERIMENTAL METHODS:

MATERIALS:

Lansoprazole was obtained as a gift sample from Smilax Laboratories, Hyderabad. Eudragit S100 and L 100 were gifted by Mumbai, Enteric capsules were provided as gift sample from Nature Capsules Limited Bengaluru. Ethyl Cellulose, Poly Vinyl Alcohol and Dichloromethane, were purchased from SD fine chemicals .All the other chemicals were of analytical grade purchased from Himedia and Merck. Double distilled water was used throughout the study.

Preparation of Lansoprazole Nanosponges:

Lansoprazole nanosponges were prepared by Emulsion solvent evaporation technique[1,4,7]. Ethyl cellulose (EC) was used for the preparation of nanosponges. Nanosponges were prepared using different proportions of polymers and polyvinyl alcohol. The dispersed phase containing Lansoprazole and polymer in 20ml of dichloromethane was added slowly to a definite amount of PVA in 100mL of aqueous continuous phase with 1000 rpm stirring speed using magnetic stirrer for 2 hrs. The selected nanosponges were collected by filtration and dried in oven at 40ºC for 24hrs and packed in vials. The prepared nanosponge formulations with different Drug:polymer ratio are listed in table 1

Characterization of nanosponges [1,4,7,8,10,13,15]:

Particle size distribution:

The particle size of the nanosponges was measured by Horiba scientific instruments(particle size analyser).Samples were prepared by diluting nanosponges with sufficient amount of water in concentration of 0.0001% to 0.1%.The average particle size was determined by Laser Diffractometer .

Zeta potential:

The zeta potential was measured using Laser Doppler Microelectrophoresis (Horiba Scientific)

Scanning electron microscopy: (SEM):

The morphology and surface of nanosponges were observed using SEM(S-3700) The samples of freeze dried nanosponges were dispersed on a glass slide, and kept under a vaccum. The samples were coated with a thin gold/palladium layer using a sputter coat unit.

Percentage yield:

The percentage yield can be determined by calculating initial weight of raw materials and final weight of nanosponges.

Percentage yield= Practical yield/ Theoretical yield x100

In case of E1 to E8, Theoretical yield =Total solids weight = Weight of Lansoprazole (drug)+ Weight of ethyl cellulose (polymer) +Weight of polyvinyl alcohol (cross-linker)

Drug content:

The drug content in each formulation was determined by weighing nanosponges equivalent to 30 mg of Lansoprazole and dissolving in 100 ml of phosphate buffer followed by stirring .The solution was filtered through 0.45µ membrane filter diluted suitably and absorbance of resultant solution was measured spectrophotometrically at 284nm using methanol as blank. The drug content of prepared NS's was determined by the formula. Drug content%= Weight of drug in nanosponges / Weight of nanospongesx100

Drug entrapment efficiency:

Also known as association efficiency. The drug loaded nanosponges were centrifuged at a speed of 15000 rpm and -4ºC for about 30 min. The supernatant was assayed for non bound drug concentration by UV spectrophotometer.

Entrapment efficiency%= Bound drug-unbound drug/ Bound drugx100

Drug-polymer compatibility studies:

i) Differential scanning calorimetry (DSC):

The differential scanning calorimetry thermograms were recorded using differential scanning calorimeter (DSC; Mettler Toledo,Japan).Indium standard was used to calibrate the DSC temperature and enthalpy scale.Approximately 2-5 mg of each sample was heated in a pierced aluminium pan from 30ºC to 300ºC at a heating rate of 10ºC/min under a stream of nitrogen at a flow rate of 50 ml/min. Thermal data analyses of the DSC thermogram were conducted using STARe software(version 5.21).

ii) Fourier transform infrared spectroscopy (FT-IR):

The FT-IR spectra of Lansoprazole, ethyl cellulose and optimised formulation were recorded using Fourier transform infrared spectrophotometer (Bruker Alpha-T ,Switzerland) to investigate any interaction between Lansoprazole and polymer in formulated nanosponges. The samples were grounded with KBr and pressed into a disk shape for measurement. The prepared pellets were scanned over a frequency range of 4000-400 cm^{-1}

***In-vitro* drug release studies:** The release of drug from the optimized nanosponges was studied by using dialysis bag. Nanosponge formulation (equivalent to 30 mg of Lansoprazole) was taken in a dialysis bag (cut –off 12,000) and placed in a beaker containing 100 mL of dissolution medium. In order to simulate the pH changes along the GI tract, pH 1.2, 7.4 and 6.8 were sequentially used as dissolution media (Cheng G et al., 2004). When performing the experiments the pH 1.2 medium was first used for 2 hrs then removed and the fresh pH 7.4 phosphate buffer was added .After 4 hrs the medium was removed and the colonic fluid pH 6.8 buffer was added for subsequent hours(18hrs). Rotation speed was 100 rpm, temperature was maintained at 37±0.5ºC. At specified time intervals (1, 2, 3,4,6,8,10,12,18 and 24 hrs) 2 mL aliquot of each sample was withdrawn and replaced by an equal volume of the release medium. Samples were filtered and amounts of drug released were determined spectrophotometrically at wavelength of 284nm.The data were presented as mean ± SD of at least triplicates.

RESULTS AND DISCUSSIONS:

Effect of variables on the preparation of nanosponges:

The effect of various variables like drug/polymer ratio, stirring rate,

stirring speed, volume of internal phase, surfactant concentration and sonication time on the particle size and drug entrapment efficiency of nanosponges was studied. The effect of drug: polymer on the physical characteristics of the formulated nanosponges was examined for various drug: polymer ratios nanosponges at stirring speed of 1000rpm for 2hrs. The mean particle size of nanosponges can be influenced by drug: polymer ratio. It was observed that as drug: polymer ratio increases, the particle size is decreased. This is probably due to the fact that at higher relative drug content, the amount of polymer available per nanosponge to encapsulate the drug becomes less, thus reducing the thickness of the polymer wall and hence smaller nanosponges. The effect of stirring rate on the physical characteristics of the formulated nanosponges was examined for 1:2 drug: polymer ratio nanosponges. The stirring rate was varied in the range of 500 to 2000 rpm. The dispersion of the drug and polymer into the aqueous phase was found to be dependent on the agitation speed. As the speed was increased, the size of nanosponges was found to be reduced.

The nanosponges obtained were spherical and uniform. When the rate of stirring was increased up to 1000 rpm the spherical nanosponges were formed with mean particle size of about 300nm. It was noted that at higher stirring rate the production yield was decreased. Possibly, at the higher stirring rates the polymer adhered to paddle due to the turbulence created within the external phase, and hence production yield decreased. The stirring time also plays a crucial role in the formation of nanosponges. In our study we found that the stirring time of 2hrs yielded the nanosponges with optimum particle size and potential. The impact of the volume of internal phase i.e., dichloromethane on particle size and %drug entrapment efficiency was studied. It was found that 20ml of dichloromethane yielded a required particle size and % drug entrapment efficiency. The volume of dichloromethane is important in the formation of emulsion droplet at the initial stage and also solidification of the drug and the polymer in the droplet. The concentration of the surfactant need to be minimised to avoid foaming, particle aggregation and decrease in entrapment efficiency. In the present study 0.2% w/v surfactant concentration was found to be optimum.

Particle size Measurement:

The particle size distribution of nanosponges is shown in table 2.The mean particle size was found to increase with decrease in polymer amount or large increase in polymer amount. This may be due to less zeta potential leading to particle aggregation. However formulation F10 containing very large amount of polymer also showed the increase in mean particle size. This may be due to availability of large quantity of polymer.

Fourier transform infrared (FTIR) analysis:

Lansoprazole pure drug and nanosponge F6 formulation were subjected for FTIR analysis and results are shown in Fig 5, Fig 6 and Table 3. No significant shifts are observed in the positions of wave numbers when compared to that of pure drug. These results showed that there was no chemical interaction or changes during nanosponge preparation and drug was stable in all nanosponge formulations.

DSC studies:

DSC thermogram of pure Lansoprazole showed sharp peak at 194.5°C corresponding to its melting point.The thermogram of Lansoprazole nanosponges showed a similar endothermic peak at 197.8°C which

confirms that there was no polymer drug interaction.

Table 1: Formulation of ethyl cellulose nanosponges

S.no	Formulation code	Ratio of drug: ethyl cellulose(mg)	Dichloromethane (ml)	Distilled water (ml)
1	F1	50:250	20	100
2	F2	100:50	20	100
3	F3	100:100	20	100
4	F4	100:150	20	100
5	F5	100:200	20	100
6	F6	100:250	20	100
7	F7	100:300	20	100
8	F8	100:350	20	100
9	F9	100:400	20	100
10	F10	150:450	20	100

Table 2: Determination of Particle size and Percentage of Entrapment efficiency

S.NO	Formulation code	Particle size (nm)	%Yield	%Entrapment efficiency
1	FI 50:250	Irregular	56.4	61.8
2	F2 100:50	Large irregular	60.2	57.3
3	F3 100:100	Aggregates	58	59
4	F4 100:150	>1000	67.2	58.4
5	F5 100:200	462	69.6	70.6
6	F6 100:250	313.4	70.8	78.9
7	F7 100:300	594	61.5	71.2
8	F8 100:350	680	64.6	69.6
9	F9 100:400	734	68.3	61.3
10	F10 100:450	>1000	62.8	60.5

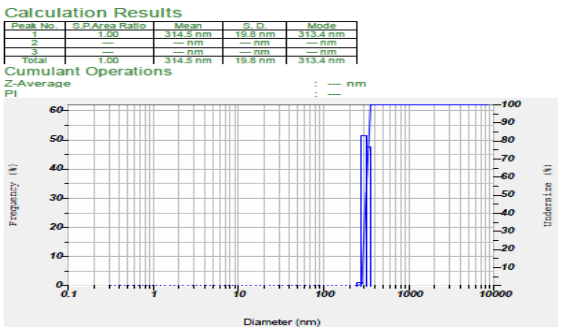


Fig1 :Particle size of F6 - 313.4nm

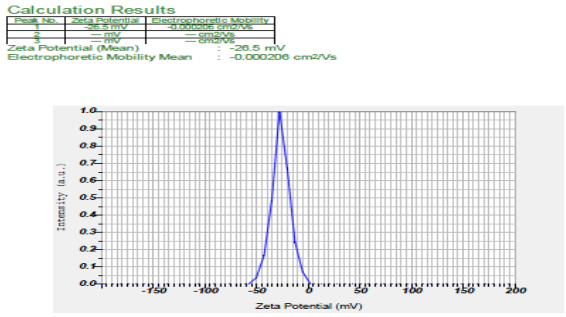
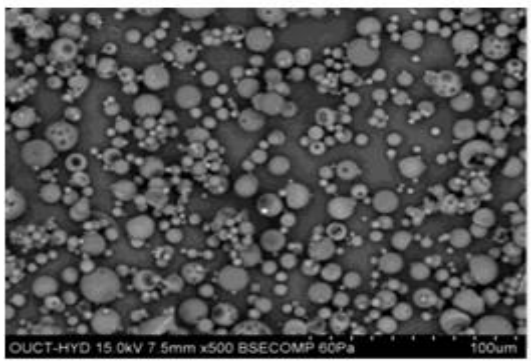


Fig 2: Zeta potential of F6 -26.5mv

Formulation F6 showed a particle size of 313.4±6.1 nm (Fig 1),zeta potential of -26.5mv (Fig 2), 78.9±4.3 % entrapment efficiency which are acceptable and considered as optimised.

Particle shape and morphology: The shape and morphology was examined using Scanning electron microscopy (SEM) (Zeiss EVOMa 15) for the formulation F6 (Fig 3& 4)



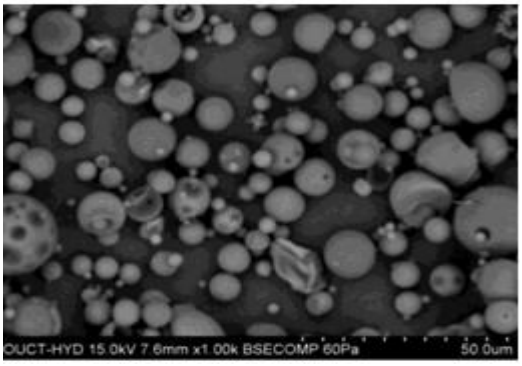


Fig 3: SEM image with Mag = 2.50 KX **Fig 4: SEM image with Mag = 7.50 KX**

It was found that the nanosponges obtained were spherical in shape with smooth surface.

Fig 5 FTIR spectra of Lansoprazole

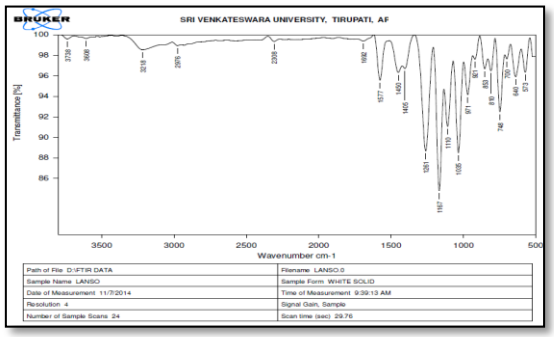


Fig 6: FTIR spectra of formulation

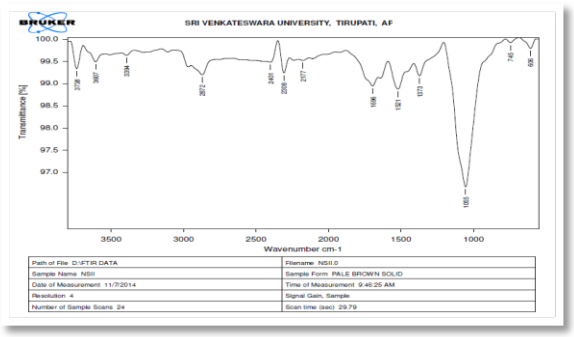


Table 3: FT-IR data of Lansoprazole, Ethyl cellulose and Lansoprazole loaded nanosponges

Group frequency (in cm^{-1})	Frequency of lansoprazole(in cm^{-1})	Frequency lansoprazole loaded nanosponges (in cm^{-1})	Frequency of ethylcellulose (in cm^{-1})
Aromatic N-H	3608	3607	---
Aromatic C-N	2308	2308	---
Aromatic C-H	2976	2872	2874
C=C streching	1577	1571	---
S=O streching	1261	1373	1055
O-H streching	---	3394	3458

Fig 7: DSC thermogram of Lansoprazole

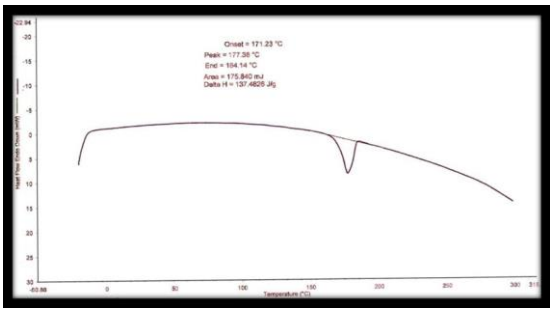
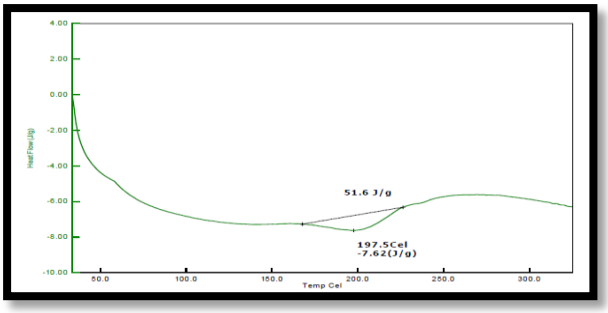


Fig 8:DSC Thermogram of formulation



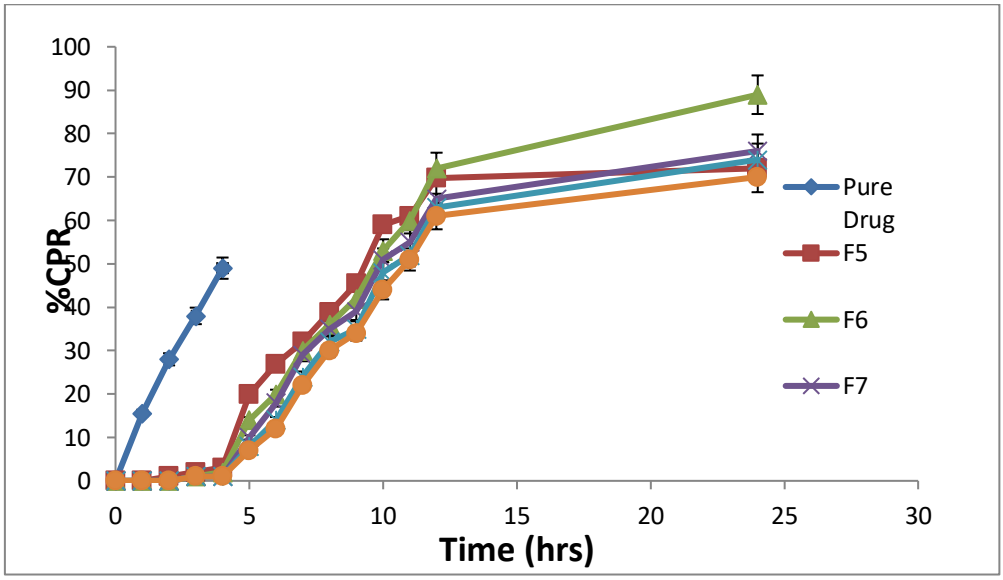


Fig 9: *In-vitro* drug release profile of pure Lansoprazole, F5, F6 ,F7, F8 and F9 formulations

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

In-vitro drug release studies were carried out for pure drug and various formulations prepared (F5-F9).For formulations F1-F4, F10 dissolution studies were not carried out because of their irregular shape and large particle size. Formulations F5 to F9 showed extended release up to 24 hrs. The drug release was found to be controlled as these formulations contain higher concentration of ethyl cellulose sufficient for encapsulating the drug. Lansoprazole is acid liable drug, as ethyl cellulose is used as polymer it hindered its drug release in stomach and protected it from degradation. All these formulations have depicted 23 to 40 percent improvement in cumulative drug release when compared to pure drug. This is attributed to nanoparticular range of drug in nanosponges which is directly related to its absorption and there by dissolution is increased in nanosponge formulations. All these factors synergised the bioavailability of lansoprazole through nanosponge formulation.

CONCLUSION:

In the light of these findings, it can be concluded that Lansoprazole loaded nanosponges are of appropriate particle size obtained by emulsion solvent diffusion method. All the results of characterization tests indicated that the drug is encapsulated within the nanocore. This nanosponge based formulation possesses optimum particle size and zeta potential, %entrapment efficiency as well

as desired drug release profile. *In-vitro* drug release studies proved that the formulation F6 has shown improved bioavailabilty by nearly two times when compared to pure drug. The nanosponge systems have been found to have good potential for bioavailability enhancement,prolonged drug release and therefore can be beneficial such as dose reduction, reduced frequency of administration and thereby avoiding related systemic side effects. However to justify its utility pharmacokinetic and pharmacodynamic studies are required

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REFERENCES:

1. Raja Ch NV, Kumar GK, Anusha K. Fabrication And Evaluation Of Ciprofloxacin Loaded Nanosponges For Sustained Release; International Journal Of Research In Pharmaceutical And Nano Sciences. 2(1); 1-9: 2013.
2. D'souza JI. The Nanosponge Drug Delivery System: For Delivering an Active Ingredient by Controlled Time Release. Pharmainfo.net; 6(3): 62:2008
3. Davankov, Ilyin MM, Tsyurupa MP, Timofeeva GI, Dubrovina LV. From a Dissolved Polystyrene Coil to Intramolecularly- Hyper –Cross-Linked"Nanosponge". Macromolecules. 29(26): 8398-403;2006
4. Prathima , Sreeja K. Formulation and Evaluation of Voriconazole Loaded Nanosponges for Oral and Topical Delivery: International Journal of Drug Development & Research.5(1); 1-13: 2013.
5. Eki, Jingquan, Zhongfan J, Cyrille B,Thomas PD. Biodegradable Star Polymers Functionalized With-Cyclodextrin Inclusion Complexes. Bio macromolecules; 10 (9); 2699–707: 2009.
6. Freitas RA What is Nanomedicine? : Nanomedicine. Nanotech. Biol. Med. 1:2–9; 2005.
7. GarveyJ. Nanosponge drug delivery claimed to work better than direct injection on tumors, Gizmag emerging technology magazine, posted on June 17, 2010
8. Gilardi G, Trota F, Cavalli R, Ferruti P, Ranuccie, Nardo G, Roggero C, Tumiatti V.Cyclodextrin nanosponges as carrier forbiocatalysts , and in the delivery and release of enzymes,proteins, vaccines and antibodies.4(2); 1-13:2009.
9. Jilsha G, Vidya V. Nanosponges: A Novel Approach of Drug Delivery System.International Journal of Pharmaceutical Sciences. Rev. Res:19(2); 119-123:2013