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FORMULATION AND EVALUATION OF CHITOSAN NANOPARTICLES OF ESOMEPRAZOLE FOR THE TREATMENT OF GASTROESOPHAGEAL REFLUX DISEASE

Shashwat Singh*, Vinod Kumar Dhote, Kanika Dhote and Surendra Jain

Truba institute of Pharmacy Bhopal.

***Corresponding Author: Shashwat Singh**
Truba institute of Pharmacy Bhopal.

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ABSTRACT
Gastroesophageal reflux disease (GERD) is a condition in which the digestive acid in the stomach comes in contact with the esophagus. The irritation caused by this disorder is known as heartburn. Long-term contact between gastric acids and the esophagus can cause permanent damage to the esophagus. Esomeprazole reduces the production of digestive acids, thus reducing their effect on the esophagus. Esomeprazole is a proton pump inhibitor which reduces acid secretion through inhibition of the H+/ K+ ATPase in gastric parietal cells. Esomeprazole having a shorter biological half life (1-1.5 hours) hence we are selected as a chitosan nanoparticles, So it should be improve the bioavailability, reduce the number of doses, maintain constant therapeutic levels of the drug over 24 hours and to increase patient compliance for the treatment of gastroesophageal reflux disease, zollinger ellison syndrome and peptic ulcer disease. The purpose of the present study was to prepare and evaluate the chitosan nanoparticles of esomeprazole for the treatment of GERD by ionic gelation method. Nanoparticles were subjected to various characterization techniques such as FTIR, particle size, scanning electron microscopy (SEM), drug entrapment efficiency, in-vitro release studies and zeta potential are also determined for the developed formulations. The entrapment efficiencies were found to be minimum and maximum of 40.65±1.22% and 64.85±1.50%, the optimum entrapment efficiency was found to be 64.85±1.50%, particle size varied from 215.57nm to 257.74nm, the zeta potential of the best chitosan preparation (F2) was found to be -30.5mV, which confirms the stability of prepared nanosuspension. In-vitro release of drug follows first order and showed sustained release behavior for a period of 24 hr. The study demonstrated the successful preparation of sustained release polymeric nanoparticles of esomeprazole. KEYWORDS: Gastroesophageal reflux disease, Esomeprazole, Chitosan nanoparticles, Ionic gelation method.

INTRODUCTION
Gastro-esophageal reflux (GER) refers to the involuntary passage of gastric contents into the esophagus. In children, it often represents a physiological phenomenon, especially in infants with innocent regurgitation. Conversely, GER disease (GERD) occurs when the reflux of gastric contents causes troublesome symptoms and/or complications. It is one of the most common causes of foregut symptoms across all pediatric age groups. In addition to heartburn, regurgitation and difficulty swallowing are common GERD symptoms. GERD also includes subcategories of diagnosis: non-erosive esophageal reflux disease (NERD) and the additional pathologies that result as GERD progresses, including esophageal ulcer, esophageal stricture, Barrett's esophagus, and Barrett's carcinoma (esophageal adeno-carcinoma).[1] GERD, is a very common disorder. Gastroesophageal refers to the stomach and the esophagus. Reflux refers to the back-flow of acidic or non-acidic stomach contents into the esophagus. There is no known single cause of GERD. It occurs when the esophageal defenses are overwhelmed by stomach contents that reflux into the esophagus. A band of muscles at the junction of the stomach and esophagus called the lower esophageal sphincter (LES) normally acts, in conjunction with the diaphragm, as a barrier to prevent reflux of stomach contents into the esophagus. If that barrier is relaxed at inappropriate times or is otherwise compromised, reflux occurs.[2] GERD is characterized by symptoms and/or tissue damage that results from repeated or prolonged exposure of the lining of the esophagus to contents from the stomach. If tissue damage is present, the individual is said to have esophagitis or erosive GERD. The presence of symptoms with no evident tissue damage is referred to as non-erosive GERD.[3] GERD symptoms are often persistent, such as chronic heartburn and regurgitation of acid. But sometimes there are no apparent symptoms, and the presence of GERD is revealed when complications become evident.[3] During last two

decades, considerable attention has been given to the development of novel drug delivery system (NDDS).[4] The rational for control drug delivery is to alter the pharmacokinetics and pharmacodynamics of drug substance in order to improve the therapeutic efficacy and safety through the use of novel drug delivery system. Besides more traditional matrix or reservoir drug delivery system, colloidal drug delivery system has gained in popularity. The major colloidal drug delivery system includes liposome and polymeric nanoparticles. These systems have been investigated primarily for site specific drug delivery, for controlled drug delivery, and also for the enhancement of dissolution rate/bioavailability of poorly water-soluble drugs. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. Nanoparticles are sub-nano sized colloidal structures composed of synthetic or semi synthetic polymers. They offer advantages like increased bioavailability, site specific drug delivery, sustained release of drug over longer period of time, retention of dosage form in entire length of gastrointestinal tract and convenient to patient due to reduction in frequent dosing.[5] Chitosan nanoparticles have gained significant importance since chitosan is the natural colon targeted polymer.[6] The formation of chitosan nanoparticles include simple and non toxic method called as ionic gelation method. This method includes cations formation of chitosan dissolved in the aqueous acid solution.[7] This formed solution is then added to the aqueous TPP solution under stirring conditions. Esomeprazole[8], bis(5-methoxy-2-[(S)- [(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulfinyl]-1-H-benzimidazole-1-yl) is a compound that inhibits gastric acid secretion. Esomeprazole is cost-effective in the treatment of gastric oesophageal reflux diseases. Esomeprazole is the S-isomer of omeprazole, the first single optical isomer proton pump inhibitor, generally provides better acid control than current racemic proton pump inhibitors and has a favorable pharmacokinetic profile relative to omeprazole.[9] In this research work, esomeprazole nanoparticles were prepared by high result oriented ionic gelation method. Chitosan was used along with sodium triphosphate. The formulations are developed with a scope of better therapeutic efficacy, solubility and penetration at the inflamed site.

MATERIALS AND METHODS

Materials

Esomeprazole was obtained as a gift sample from Mylan Pharmaceuticals Pvt Ltd in Jubilee Hills, Hyderabad, (India). Chitosan was obtained from HiMedia Laboratories Ltd, Mumbai, India. Sodium triphosphate, acetic acid, ethanol, acetone, dichloromethane and light liquid paraffin were purchased from Central Drug House Pvt Ltd, Mumbai, India. All other reagents and chemicals used were of analytical grade.

Preformulation studies

Physical characteristics

By visual examination, the drug was identified for physical characters like colour, texture, odour etc.

Solubility

Solubility of the drug was determined by taking some quantity of drug (about 10 mg) in the 10 ml volumetric flasks separately and added the 10 ml of the solvent (water, ethanol, methanol, 0.1N HCL, 0.1N NaOH, chloroform and 7.4 pH buffer) Shake vigorously and kept for some time. Note the solubility of the drug in various solvents (at room temperature).

Melting point

A small quantity of powder was placed into a fusion tube. That tube was placed in the melting point determining apparatus (Chemline) containing castor oil. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

FTIR spectroscopy

The concentration of the sample in KBr should be in the range of 0.2% to 1 %. The pellet is a lot thicker than a liquid film, consequently a decrease concentration in the sample is required (Beer's Law). For the die set that you'll be the usage of, about 80 mg of the mixture is wanted. Too excessive of an attention causes typically difficulties to obtain clean pellets. FTIR spectra of the samples were recorded over a spectral region from 4700 to 400 cm-1 using 20 scans with 4 cm-1 resolution.

Determination of λ_{max} of esomeprazole

Esomeprazole, 100 mg, was accurately weighted into a 100 ml volumetric flask, dissolved in 0.1N HCl, and the volume was made up with 0.1N HCl. Pipette 1 ml of this solution into a 10 ml volumetric flask with 0.1N HCl as the volume and marks it as stock. Prepare an appropriate dilution to bring the concentration down to 2-12µg/ml. The resulting solution is scanned with a UV spectrophotometer (UV-1700 Shimadzu corporation, Japan) in the range of (200-400 nm) to determine the absorption maximum (λ max). Concentration vs. absorbance was shown on a graph.

Fabrication of esomeprazole loaded nanoparticles by ionic gelation technique

Blank chitosan nanoparticles were prepared by ionic gelation method. Different concentrations of polymer, ranging from 0.15 to 0.65 % w/v, were dissolved in 1.5 %v/v acetic acid solution. Sodium tripolyphosphate solution was also prepared in distilled water in concentrations ranging from 0.10 to 0.60 %w/v. Sodium tripolyphosphate solution was added drop wise with a syringe to chitosan solution while stirring, followed by sonication for 20 min. The resulting suspension was subsequently centrifuged at 15000 rpm for 10 min. The pellets obtained were re-suspended in de ionized water

by sonication, centrifuged and dried at room temperature (about 25°C). Drug-loaded chitosan nanoparticles were formed spontaneously upon drop wise addition of 12 ml of 0.4 % aqueous sodium tripolyphosphate solution to 20 ml of 0.35 %w/v chitosan solution containing 2- 5 mg/ml of the drug under magnetic stirring, followed by sonication. The resulting nanoparticle suspensions were centrifuged 4 times (15 min each) at 15000 rpm, washed with distilled water and dried.[10]

Table 1: Composition of esomeprazole chitosan nanoparticles.

Ingredients	Formulations					
	F1	F2	F3	F4	F5	F6
Esomeprazole (mg)	40	40	40	4	40	40
Chitosan %	0.15	0.25	0.35	0.45	0.55	0.65
Acetic acid (ml)	1.5	1.5	1.5	1.5	1.5	1.5
Sodium tri phosphate (mg)	0.10	0.20	0.30	0.40	0.50	0.60
Distilled water (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Evaluation of nanoparticles

Drug entrapment efficiency

For the determination of encapsulation efficiency accurately weighed NPs (10 mg) were added to 10 ml of distilled water and after the equilibrium solubility was attained, clear supernatant after centrifugation was filtered and 1 ml of the filtrate was mixed with 4 ml of methanolic HCl. Resulting sample was analyzed on UV visible spectrophotometer at 279 nm.[11] Percentage entrapment was calculated as follows:

$$\% \text{ Entrapment efficiency} = \frac{\text{Actual drug content in nanoparticles}}{\text{Theoritical drug content}} \times 100$$

Particle size determination

The mean size of the nanoparticle preparations were measured by laser diffraction analyzer (Malvern). Each sample was diluted with water until the appropriate concentration of particles was achieved and measured. All measurements were performed at 25°C.[11]

Scanning electron microscopy

Scanning electron microscopy (SEM) was also conducted to characterize the surface morphology of the nanoparticles for which a drop of nanoparticle system was mounted on clear glass stub, air dried and coated with Polaron E 5100 Sputter coater (Polaron,) and visualized under Scanning Electron Microscope (SEM Leo 430,).[12]

Zeta potential

Zeta potential is a measure of surface charge. The surface charge of nanoparticle can be determined by using Zeta sizer. Zeta potential is a measurement of the overall charge of the particles in media and it indicates the stability of the particles in the sense that the higher the zeta potential the more stable the particles.[12]

In vitro drug release from the formulation

In vitro drug release studies were performed by using a Franz diffusion cell with a receptor compartment capacity of 60 ml. The cellulose acetate membrane was used for the determination of drug from the prepared esomeprazole nanoparticle. The cellulose acetate membrane having a pore size 0.45 μ was mounted between the donor and receptor compartment of the diffusion cell. The prepared nanoparticle was placed on the cellulose acetate membrane and covered with aluminum foil. The receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a hot plate magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads, and the temperature was maintained at 32 ± 0.5°C, because the normal temperature of human is 32°C. The samples were withdrawn at different time intervals and analyzed for drug content spectrophotometrically.[11] The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal.

Mathematical treatment of in-vitro release data

The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage forms characteristics are used.

Zero-order kinetics

The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. The following relation can, in a simple way, express this model:

$$Q_t = Q_o + K_o t$$

Where $Q_t$is the amount of drug dissolved in time t, $Q_o$is the initialamount of drug in the solution (most times, Q_o=0) and $K_o$is the zero order release constant.[13]

First-order kinetics

The following relation expresses this model:

$$\log Q_t = \log Q_o + \frac{K_1 t}{2.303}$$

Where $Q_t$is the amount of drug dissolved in time t, $Q_o$is the initialamount of drug in the solution and $K_1$is the zero order release constant.

In this way a graphic of the decimal logarithm of the released amount of drug versus time will be linear. The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release drug in a way that is

proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminish.

Korsmeyer-Peppas model

Korsmeyer*et al.* used a simple empirical equation to describe general solute release behaviour from controlled release polymer matrices:

$$\frac{M_t}{M_\infty} = a\, t^n$$

Where $M_t/M_\infty$is fraction of drug released, a is kinetic constant, t is release time and n is the diffusional exponent for drug release. 'n' is the slope value of log M_t/M_∞ versus log time curve. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. Peppas used this n value in order to characterize different release mechanisms, concluding for values for a slab, of *n* =0.5 for fickian diffusion and higher values of *n*, between 0.5 and 1.0, or *n* =1.0, for mass transfer following a non-fickian model. In case of a cylinder *n* =0.45 instead of 0.5, and 0.89 instead of 1.0. This equation can only be used in systems with a drug diffusion coefficient fairly concentration independent. To the determination of the exponent *n* the portion of the release curve where M_t/M_∞< 0.6should only be used. To use this equation it is also necessary that release occurs in a one-dimensional way and that the system width-thickness or length-thickness relation be at least 10. A modified form of this equation was developed to accommodate the lag time (*l*) in the beginning of the drug release from the pharmaceutical dosage form:

$$\frac{M_{t-l}}{M_\infty} = a\,(t-l)^n$$

When there is the possibility of a burst effect, b, this equation becomes:

$$\frac{M_t}{M_\infty} = at^n + b$$

In the absence of lag time or burst effect, l and bvalue would be zero and only $at^n$is used. This mathematical model, also known as *Power Law*, has been used very frequently to describe release from several different pharmaceutical modified release dosage forms.[14,15]

Stability studies for optimized formulation

Stability of a formulation on storage is of great concern as it is the major restraint in their development as marketed preparation. Optimized nanoparticle formulation (F2) were stored in amber colored bottles and subjected to exhaustive stability testing at 4±1°C and room temperature for 3 month period. Samples were withdrawn periodically and formulation was observed on the basis of % EE, average particle size and physical appearance.

RESULTS AND DISCUSSIONS

The melting point of esomeprazole (pure drug) was found to be 173-175°C. Esomeprazole was soluble in chloroform, DMSO and methanol, freely soluble in 0.1 N HCl and slightly soluble in water. Identification of esomeprazole was done by FTIR spectroscopy with respect to marker compound. It was identified from the result of IR spectrum as per specification fig. 1. The broad peak at 3897.44cm^{-1} in the spectra of the pure drug corresponds to N-H (stretching). The peak at 1002.05 cm^{-1} corresponds to S=0 (stretching), the peak at 1155.47 cm^{-1} for C-O-C (stretching).The calibration curve of esomeprazole was found to be linear in the concentration range of 2-12 µg/ml at 279 nm fig. 2. Partition coefficient and moisture content of esomeprazole was found to be 1.7 K and 0.0711 respectively. Percentage yield of different formulation was determined by weighing the nanoparticles after drying. The percentage yield of different formulation was in range of 63.23±0.41-73.23±0.45%. The drug entrapment of different formulations was in range of 40.65±1.22-64.85±1.50% w/w. This is due to the mucoadhesion characteristics of chitosan that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of esomeprazole nanoparticles. The maximum percentage yield and entrapment efficiency was found formulation F2 table 2. The mean size of the nanoparticle preparations were measured by laser diffraction analyzer (Malvern). Average particle size of chitosan nanoparticle was calculated by sum of all particles size/number of particles. From the particle size determination of esomeprazole nanoparticles, it was observed that the formulations showed the particle size from 215.57-257.74 nm, table 3. Zeta potential of optimized formulation F2 nanoparticles was found to be -30.5mV fig. 3. The SEM photomicrographs of the chitosan nanoparticles were taken and characterized in terms of sphericity and particles clumping. As observed in photomicrograph the nanoparticles having smooth surface and perfectly spherical fig. 4. The *In-vitro* diffusion study was taken by using franz diffusion cell which shows cumulative % drug release of esomeprazole nanoparticle formulation. Among all the formulations, F2 was selected for in-vitro drug release study because it showed highest drug content table 4 & fig. 5. Zero order kinetic models refer to the process of constant drug release from a drug delivery device independent of the concentration. The zero order graph of F2 formulation showed the constant drug release from the chitosan nanoparticle, the results of the zero order model was found to be y = 3.595x + 19.94, R^2= 0.889. The first order kinetic model describes the release from system where release rate is concentration dependent. The results of first order kinetic model was found to be y =-0.054x + 2.081, R^2= 0.988. The Higuchi model is used to describe the limits for transport and drug release. The

Higuchi model of patches was found to be 23.96x - 15.75, R^2= 0.966. From the above results, it is concluded that the prepared chitosan nanoparticles are following first order kinetic model which is dose dependent table 5 & fig. 6-8. There were no significant changes in % EE and physical appearance in nanoparticle formulation was observed after 3 month of storage at 4ºC.

Table 2: Percentage yield and entrapment efficiency for different formulation.

Formulation	% Percentage Yield	% EE of Nanoparticles
F1	65.56±0.32	60.23±1.25
F2	73.23±0.45	64.85±1.50
F3	70.12±0.45	40.65±1.22
F4	68.85±0.65	42.45±1.25
F5	65.45±0.56	49.46±1.25
F6	63.23±0.41	52.28±1.25

Table 3: Particle size of esomeprazole chitosan nanoparticle.

S. No	Formulation	Particle size (nm)
1.	F1	253.46
2.	F2	215.57
3.	F3	246.57
4.	F4	236.87
5.	F5	257.74
6.	F6	230.47

Table 4: *In vitro* drug release of all formulations of esomeprazole nanoparticles.

S. No.	Time in hours	% cumulative drug release					
		F1	F2	F3	F4	F5	F6
1	2	12.73±0.28	16.84±0.42	13.78±0.12	17.46±0.33	14.73±0.48	11.73±0.26
2	4	22.73±0.30	25.63±0.48	20.63±0.17	19.03±0.28	21.75±0.37	18.74±0.23
3	6	35.93±0.46	41.63±0.32	31.65±0.50	40.67±0.45	38.85±0.42	32.74±0.52
4	8	49.63±0.49	55.85±0.33	46.83±0.48	42.70±0.32	48.84±0.32	41.47±0.43
5	10	61.83±0.52	65.93±0.40	58.62±0.28	63.45±0.30	60.85±0.32	56.47±0.30
6	12	68.27±0.35	72.84±0.20	69.63±0.20	65.47±0.23	69.64±0.23	64.38±0.31
7	16	77.93±0.23	80.84±0.15	75.6±0,23	74.46±0.15	76.73±0.18	72.84±0.22
8	24	90.73±0.15	94.85±0.05	88.67±0.12	90.56±0.10	91.74±0.10	87.57±0.12

Table 5: Regression analysis data of nanoparticle formulation.

Formulation	Model	Kinetic parameter values
F2	Zero Order	y= 3.595x + 19.94 R^2=0.889
	First Order	y= -0.054x + 2.081 R^2=0.988
	Higuchi	y= 23.96x - 15.75 R^2= 0.966

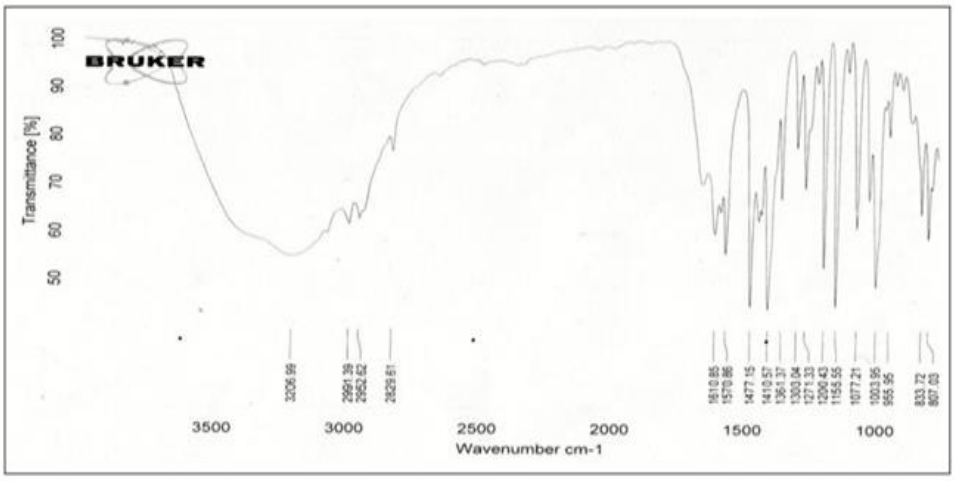

Fig. 1: FT-IR spectrum of pure drug (Esomeprazole).

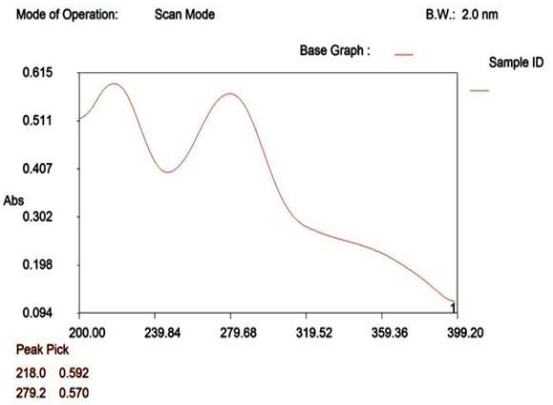

Fig. 2: Wavelength maxima of esomeprazole in 0.1N HCl.

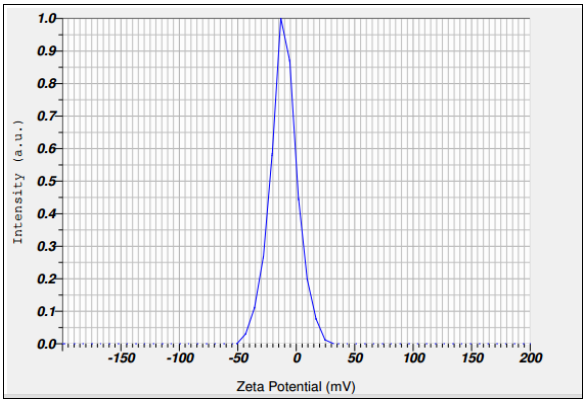

Fig. 3: Zeta potential of chitosan nanoparticle (F2).

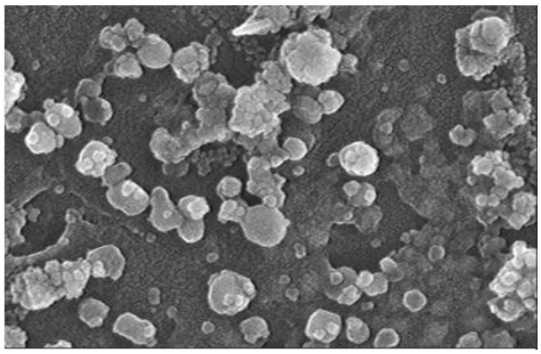

Fig. 4: Scanning electronic microscopy of optimized formulation (F2).

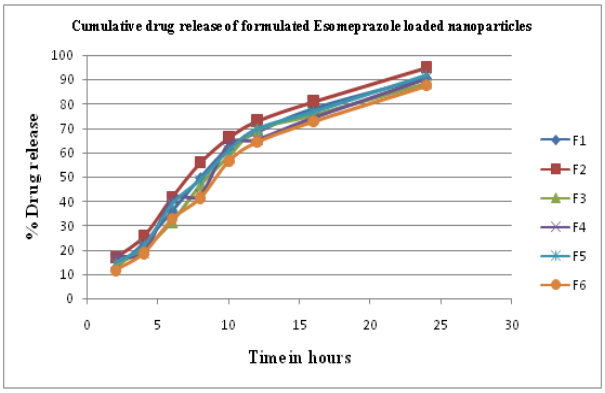

Fig. 5: Cumulative % drug release of all the formulations.

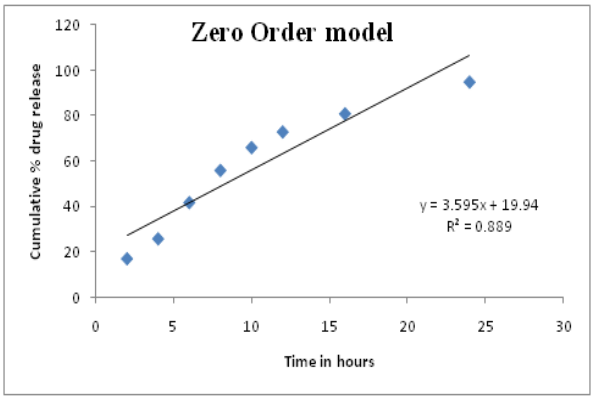

Fig. 6: Zero order model of F2 formulation.

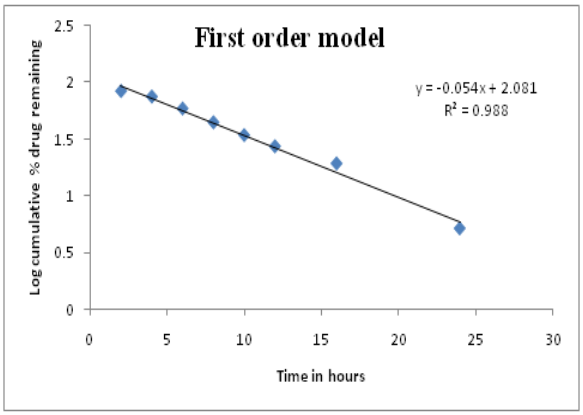

Fig. 7: First order model of F2 formulation.

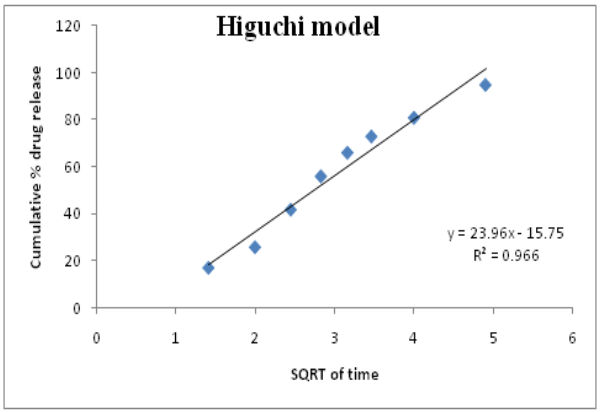

Fig. 8: Higuchi model of F2 formulation.

CONCLUSION

The nanoparticles containing esomeprazole (F2) exhibited most of the ideal characters required for an oral controlled release dosage forms. The nanoparticles (F2) of lower particle size (215.57nm) aided with negatively charged surface charge (-30.5mV) has been achieved. The release profile indicated continuous controlled release up to 24 hr. Hence it can be concluded that the newly developed oral controlled drug delivery system - nanoparticles of esomeprazole is considered to be ideal and effective in the management of gastroesophageal reflux disease and related conditions.

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