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Formulation and evaluation of lansoprazole loaded enteric coated microspheres

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ABSTRACT

The research focuses on the development of multiparticulate delivery system for acid-labile Lansoprazole to prevent its degradation in the acidic environment of the stomach and enhance its bioavailability via intestinal absorption. This problem can be solved by enteric coating. In this project, cellulose acetate phthalate a polymer usually utilized for gastrointestinal film coating of tablets, was used to prepare enteric microspheres of lansoprazole with solvent evaporation technique in various formulations such as F1, F2, F3, F4, F5 with drug: polymer ratios of 1:1, 1:2, 1:3, 1:4, 1:5 respectively. FTIR study indicated compatibility between drug and polymer. Increase in concentration of polymer increased spheriocity and mean diameter of the microspheres. The drug entrapment efficiency was in the range of 72.23% to 88.64%. SEM revealed that microspheres were found spherical and porous. In-vitro study proves that drug release slowly increases as the pH of the medium increased and prevents degradation of drug in acidic pH. In-vitro drug release was found to be 92.80%, 94.55%, 92.72%, 96.34%, 98.65% in all 5 formulations. All 5 formulations showed gastric resistance around 80-90%. So it is concluded that the developed enteric coated microspheres of Lansoprazole prevented drug release in the stomach which would lead to significant improvement in its bioavailability through enhanced intestinal absorption.

Keywords: Microencapsulation, Solvent evaporation, Microspheres, CAP, Lansoprazole.

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INTRODUCTION

Oral drug delivery route is perhaps the preferred way to take medicines. Though, their short circulation half- life and limited absorption through a specified intestine segment restricts several other drugs' therapeutic effects[1]. Currently, no drug delivery system is ideally conductive to achieving all the ambitious goals, but genuine efforts have been made to accomplish them via novel drug delivery approaches. A number of new drug delivery technologies have developed that include different paths of implementation to ensure managed and targeted delivery of drugs[3]. The focused drug delivery method was designed to try to concentrate the drug in the tissues of interest while increasing the overall concentration of the drug in the other tissues. The drug is therefore placed on the target site. Therefore, the drug does not affect the surrounding tissues[2]. Microspheres were tiny spherical bodies with just a diameter of 1 to 1000 in the µm range[4]. Micro particles from different natural and synthetic components can be produced. It is possible to alter the drug behaviour *in vivo* by mixing the drug with a carrier molecule. The carrier's activity drastically alters clearance kinetics, tissue distribution, metabolism i.e., kinetics and cellular interaction of the drug. Using these improvements in the action of pharmacodynamics will result in increased therapeutic output. A wide range of materials namely immunoglobulin serum proteins, liposomes, microspheres, microcapsules, nanoparticles and even cells like erythrocytes were also used as drug carriers[5]. Oral microspheres were used to support the release of drugs and to reducing or eliminating discomfort of the gastrointestinal tract. Moreover, multiparticulate delivery systems in the gastrointestinal tract distributed more evenly. This tends to result in a much more reproducible uptake of the drug and lessens local irritation better than single unit dosage forms along with no disintegrating polymer tablets. It is also possible to avoid excessive intestinal accumulation of the

polymeric content which can arise with matrix tablets during chronic dosing[6]. In 1997, microspheres were first designed for the drug's continuous action. Micro particles have since proven to also be good fits for continued and controlled release of drugs and became an option to conventional or immediate release compositions[7]. Such particles also provide the active drug ingredients that are pharmacologically active but are hard to deliver due to the low water solubility. In such kinds of drugs, obtaining the necessary therapeutic concentrations of the medication throughout the blood stream is difficult, allowing higher levels of C_{max}, t_{max} and area under curve to be achieved[8]. Microspheres based formulations are capable of releasing a steady amount of drug throughout the blood or targeting drugs to specific body sites. While establishing the drug delivery system, some of the key points to be taken into account are the type of carrier used, the route of administration, the drug delivery target and the strategy to improve the therapeutic effectiveness of the drug. These were all the factors that can be minimizing the adverse effects of the active pharmaceutical entity[9].

An enteric coating is a polymer barrier applied on oral medication that prevents its dissolution or disintegration in the gastric environment[10]. This helps by either protecting drugs from the acidity of the stomach, the stomach from the detrimental effects of the drug, or to release the drug after the stomach (usually in the upper tract of the intestine). Some drugs are unstable at the pH of gastric acid, and need to be protected from degradation. Enteric coating is also an effective method to obtain drug targeting (such as gastro-resistant drugs)[11]. Other drugs such as some anti helminthic may need to reach a high concentration in a specific part of the intestine. Enteric coating may also be used during studies as a research tool to determine drug absorption. Enteric coated medications pertain to the "delayed action" dosage form category[12]. From a pharmacological point of view the term "enteric coating" is not entirely correct, as gastric resistance can be also obtained by adding enteric polymeric systems to the matrix of the dosage form. Tablets, mini-tablets, pellets and granules (usually filled into capsule shells) are the most common enteric-coated dosage forms[13].

Some of the enteric coating polymers are[14]:

- Methyl acrylate-methacrylic acid copolymers
- Cellulose acetate phthalate (CAP)
- Cellulose acetate succinate
- Hydroxypropyl methyl cellulose phthalate
- Hydroxypropyl methyl cellulose acetate succinate (hypromellose acetate succinate)
- Polyvinyl acetate phthalate (PVAP)[22]
- Methyl methacrylate-methacrylic acid copolymers
- Shellac
- Cellulose acetate trimellitate
- Sodium alginate[28]
- Enteric coating aqueous solution (ethyl cellulose, medium chain triglycerides (coconut), oleic acid, sodium alginate, stearic acid) (coated soft gels)

MATERIALS AND METHODS

Materials

The materials used in this research are Lansoprazole procured as gift sample from Hetero drugs Pvt. Ltd, Hyderabad, Cellulose acetate phthalate from FMC Biopolymers, Acetone FMC Biopolymers, Span 80, Liquid Paraffin from Ferro industries.

Method

Drug excipient compatibility studies: IR spectra of drug, drug and polymers and excipients were obtained by using FTIR spectrophotometer. FTIR spectra of pure drug, and its physical mixture were obtained by using KBr pellets methods. About 2% (w/w) of samples was mixed with potassium bromide (KBr) disc. Each disc was scanned at a resolution of 4 cm^{-1} over a wave number region of 400–4000 cm^{-1} by a FTIR spectrometer[15].

Standard plot for Lansoprazole

Acid buffer (pH 1.2)

Accurately weighed 100mg of Lansoprazole was taken in 100ml of volumetric flask. Dissolve and makeup the volume with pH 1.2 buffer solution. Take 10ml of this solution in a 100ml of volumetric flask and makeup the volume with pH 1.2 buffer solution to get working with stock solution.

Table 1: Standard calibration table of Lansoprazole

S.no	Concentration (µg/ml)	Absorbance at 284 nm	
		pH 1.2	pH 6.8
1	0	0	0
2	0.2	0138±0.02	0.085±0.03
3	0.4	0.266±0.01	0.149±0.05
4	0.6	0.407±0.03	0.243±0.01
5	0.8	0.535±0.04	0.305±0.02
6	1	0.661±0.02	0.468±0.03

From this stock solution aliquots 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml were pipetted out into a series of 10ml volumetric flasks and makeup to mark with pH 1.2 buffer solution in order to get concentration. The absorbance of the resulting solution was then measured at 284nm using UV spectrophotometer against respective parent solvent as a blank. The standard curve was obtained by plotting absorbance vs. concentration in µg/ml[16].

Phosphate buffer (pH 6.8)

Accurately weighed 100mg of Lansoprazole was dissolved in 100ml of pH 6.8 phosphate buffer solution. Take 10ml of this solution in a 100ml of volumetric flask and makeup the volume with phosphate buffer (pH 6.8) solution to get working with stock-solution[16]. From this stock solution aliquots of 0.2ml, 0.4ml, 0.6ml, 0.8ml, and 1ml were pipetted out into a

Table 2: Formulation design of Microspheres

S.no	Formulation code	Lansoprazole (mg)	Cellulose acetate phthalate (mg)	Liquid paraffin (ml)	Span 80 (%)	Drug: polymer Ratio
1.	F1	30	30	100	1	1:1
2.	F2	30	60	100	1	1:2
3.	F3	30	90	100	1	1:3
4.	F4	30	120	100	1	1:4
5.	F5	30	150	100	1	1:5

series of 10ml volumetric flasks and makeup to the mark with pH 6.8 phosphate buffer solution in order to get concentration.

The absorbance of the resulting solution was then measured at 284nm using UV spectrophotometer against respective parent solvent as a blank (i.e. pH 6.8 buffer solution). The standard curve was obtained by plotting absorbance vs. concentration in µg/ml.

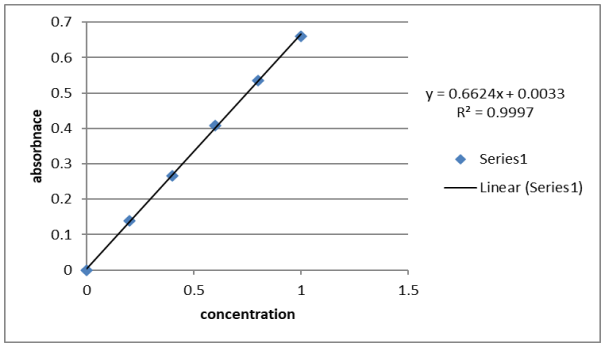

Figure 1: Standard calibration curve of Lansoprazole at pH 1.2

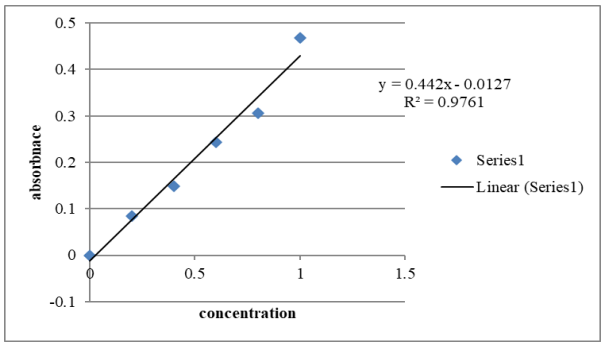

Figure 2: Standard calibration curve of Lansoprazole at pH 6.8

Formulation and development of Lansoprazole microspheres

Solvent evaporation method

Microspheres were prepared by using solvent evaporation method. Cellulose acetate phthalate was dissolved in acetone at different concentrations. Light mineral oil with 1%(w/v) of span 80 was placed in a beaker and mixed at 400 rpm using two-bladed propeller stirrer. Drug was dispersed in polymer solution at different ratios of 1:1, 1:2, 1:3, 1:4 and 1:5. Subsequently, this mixture was poured into the oil phase and mixed until all solvent was evaporated at room temperature. Microspheres were filtered, washed with ether and collected. The detailed composition of the various formulations prepared is mentioned in (Table 2).

Evaluation parameters of microspheres

Particle size analysis

Particle size analysis of drug-loaded microspheres was performed by optical microscopy. A small amount of microspheres were suspended in purified water (10ml). Mount the sample on a clean glass slide and placed it on mechanical stage of the microscope. The eye piece of the microscope fitted with a micrometer by which the size of the spheres could be appeared. This process was repeated for each batch of prepared microspheres[20].

Surface morphology

The surface morphological details of the microspheres were determined by using a scanning electron microscope (SEM) model JSM, 35CF JEOL, Japan. The samples were dried thoroughly in vacuum desiccator before mounting on brass specimen studies. The samples were mounted on a specimen studies using double sided adhesive tape and gold-palladium alloy of 120Å Knees was coated on the sample using spatter coating unit in argon ambient of 8-10 Pascal with plasma voltage about 2Kv and discharge current about 20mA. The sputtering was done for nearly 3 minutes to obtain uniform coating on the samples to enable good quality SEM images. The SEM operated at low accelerating voltage of about 15Kv with load current of about 80mA. The condenser lens position was maintained between 4.4-5.1. The objective lens aperture has a diameter of 240 µ and working distance WD = 39mm[19].

In-vitro dissolution studies[16]

The method is specified in USP for drug release study was followed:

Apparatus: - USP XIII dissolution rate test apparatus employing the round bottom dissolution vessel and rotating basket assembly.

Acid stage: - 500ml of acid buffer pH 1.2. (Simulated gastric fluid without enzymes).

Buffer stage: - 500ml of pH 6.8 phosphate buffer (duodenal fluid).

Hard gelatin capsules were filled with microspheres equivalent to 30mg of Lansoprazole and were evaluated for *in-vitro* dissolution studies. The study was carried out in a USP rotating basket apparatus. Dissolution fluid consists of 500ml of simulated gastro intestinal fluid of increasing pH namely pH 1.2, pH 6.8 maintained temperature at $37^0C \pm 5^0C$ and the rpm should be at a constant speed of 75. Aliquots of samples were withdrawn after predetermined periods of time and the same volume of fresh medium was added immediately to the test medium. The with drawl samples were filtered through a 0.45µm membrane filter. The drug content was determined in the filtrate after appropriate dilution and analyzed at 284nm spectrophotometrically using Shimadzu 1201 UV- visible spectrophotometer. Corresponding concentrations in the samples were calculated from standard plot and calculate cumulative percentage of drug release from each formulation.

Drug entrapment efficiency (DEE):

Drug entrapment efficiency of microspheres was performed by accurately weighed 30mg of microspheres were suspended in 100ml of phosphate buffer pH6.8. The resulting solution was kept for 24hrs. Next day it was stirred for 15min and subjected for filtration. After suitable dilution, Lansoprazole content in the filtrate was analyzed spectrophotometrically at 284nm using Shimadzu 1201 UV- visible spectrophotometer[18].

The obtained absorbance was plotted on the standard curve to get the exact concentration of the entrapped drug.

The drug entrapment efficiency was determined using following relationship:-

$$\%\ DEE = \left(\frac{\text{Actual drug content}}{\text{Theoretical drug content}}\right) X100$$

Kinetic treatment:

The data obtained from the *in-vitro* dissolution studies subjected for kinetic treatment to obtain the order of release and best fit model for the formulation as follows:

Zero-order model: The data obtained was plotted as Cumulative amount of drug released versus time.

First order model: The data obtained was plotted as log cumulative percentage of drug un release versus time.

Higuchi model: The data obtained was plotted as cumulative percentage drug release versus square root of time.

Korsmeyer - Peppas model: The data obtained was plotted as log cumulative percentage of drug un release versus log time.

RESULTS AND DISCUSSION

Drug excipient compatibility studies:

FTIR spectrum of Lansoprasole and physical mixture of Lansoprasole and excipient were obtained according to procedure given in the previous section and results were given in (Figure 3, Figure 4, Figure 5, Figure 6) and characteristic peak values were given in (**Error! Reference source not found.**).

Table 3: FTIR spectra of physical mixture

S.no	Functional groups	Theoretical value in cm^{-1}	Observed value in cm^{-1}
1	N-H	1306-1275	1281.47
2	N=N & C=C	1600-1430	1455.99
3	C-F	1400-1000	1404.03
4	Pyridine ring	1600-1430	1579.47

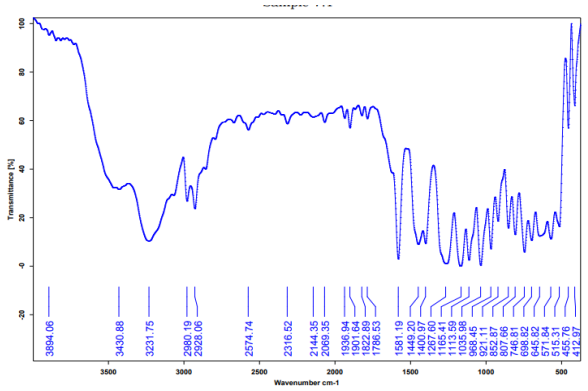

Figure 3: FTIR spectra of Lansoprazole

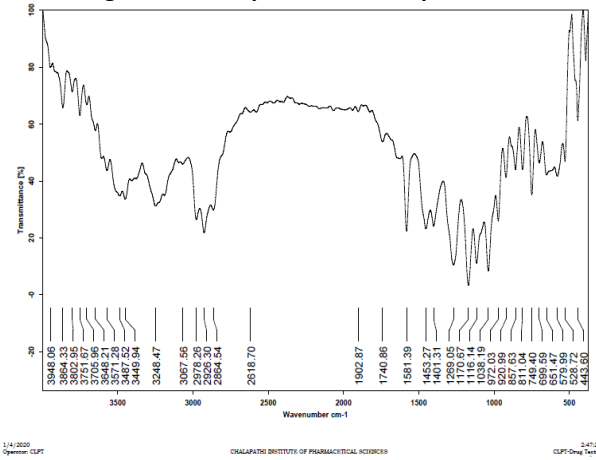

Figure 4: FTIR spectra of Lansoprazole with span 80

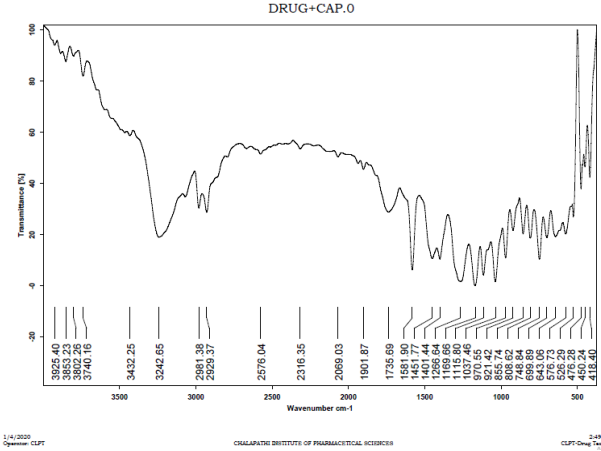

Figure 5: FTIR spectra of Lansoprazole with CAP

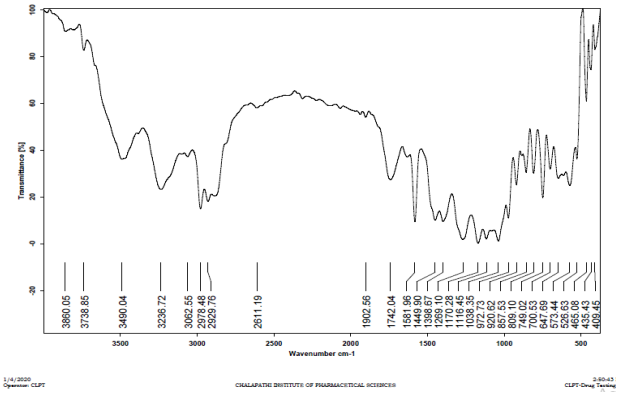

Figure 6: FTIR spectra of physical mixture

Evaluation of microspheres

Particle size analysis

By keeping all the variables constant except polymer concentration, slightly increased particle size was observed with the increase in polymer concentration. A higher concentration of polymer used in formulation F5 produced a more viscous dispersion, which formed larger droplets and consequently larger microspheres.

Table 4: Average particle sizes of microspheres from F1 to F5

Formulation code	Average size (µm)
F1	74.11±1.17
F2	80.03±1.09
F3	82.29±0.99
F4	86.07±1.11
F5	88.25±0.49

In-vitro drug release studies

The *in-vitro* dissolution studies carried out at various pH values (pH 1.2 and pH 6.8). Significant effect of pH on the release rate of Lansoprazole was observed. The rate of dissolution for microspheres was negligible at pH 1.2. This may be due to the enteric coating formed with CAP around the drug particles by means of microspherization. The amount of drug released was significantly high at higher pH values since CAP becomes soluble above pH 6.0. Results for *in-vitro* dissolution studies for formulation F1 to F5 were given in (Table 5) and the cumulative percent drug release graph for formulation F1 to F5 were shown in (Figure 7) respectively.

Table 5: Cumulative percentage of Lansoprazole release in 0.1N HCL and pH 6.8

S.no	Time (hrs)	Cumulative percent drug release				
		F1	F2	F3	F4	F5
1.	0	0	0	0	0	0
2	0.25	5.41	6.52	7.77	10.61	9.44
3.	0.5	10.02	14.93	16.54	21.83	17.97
4.	0.75	16.52	17.83	22.18	26.35	23.73
5.	1	20.43	22.86	26.27	32.04	28.82
6.	2	30.24	34.65	37.74	49.22	39.91
7.	3	33.55	38.09	40.43	56.86	47.09
8.	4	36.83	40.55	46.15	64.31	53.16
9.	5	39.98	45.58	52.36	71.79	61.18
10.	6	45.41	53.52	59.28	78.96	72.15
11.	7	52.25	61.15	66.04	84.42	77.84
12.	8	57.08	69.53	73.38	86.21	82.18
13.	9	64.22	74.69	79.19	90.81	88.86
14.	10	73.93	80.48	86.75	94.27	92.23
15.	11	87.04	85.69	89.68	94.82	93.68
16.	12	92.8	94.55	92.72	96.34	98.65

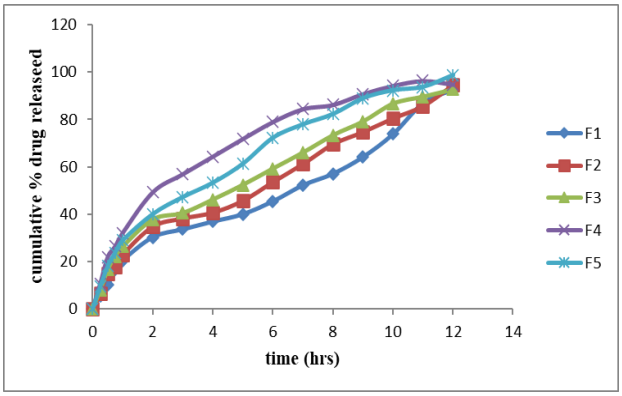

Figure 7: *In vitro* drug release profile plot of Lansoprazole

Surface morphology by SEM

The scanning electron microscopy of formulation F5 showed that prepared microspheres have good coating and it is helpful in controlling the release of drug in acidic medium. Surface smoothness of Lansoprazole microspheres increased with increase in the polymer concentration and was confirmed by SEM.

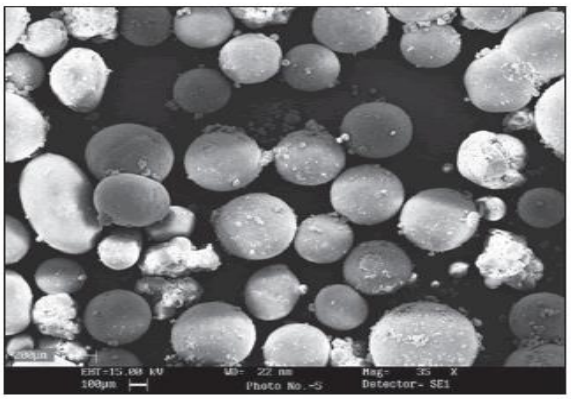

Figure 8: Scanning electron micrograph of Lansoprazole microspheres of formulation F5 prepared from Cellulose acetate phthalate

Drug entrapment efficiency

The entrapment efficiency of Lansoprazole microsphere formulations F1 to F5 were 72.23%, 74.93%, 79.62%, 81.37%, 88.64% respectively. It was further observed that the drug entrapment was proportional to the durg: polymer ratio and size of the Lansoprazole microspheres. The entrapment efficiency increased with an increase in polymer concentration owing to the increase in the viscosity of the polymer solution.

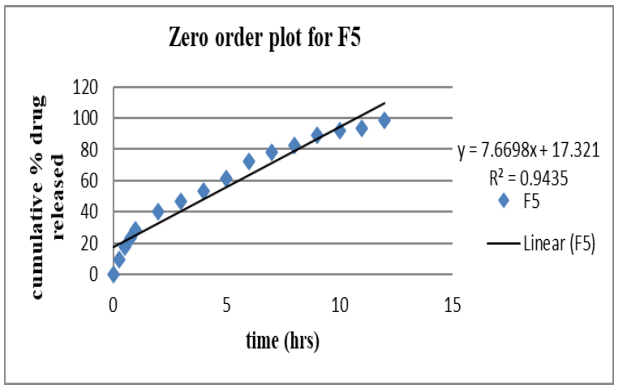

Figure 9: Zero order plot for formulation F5

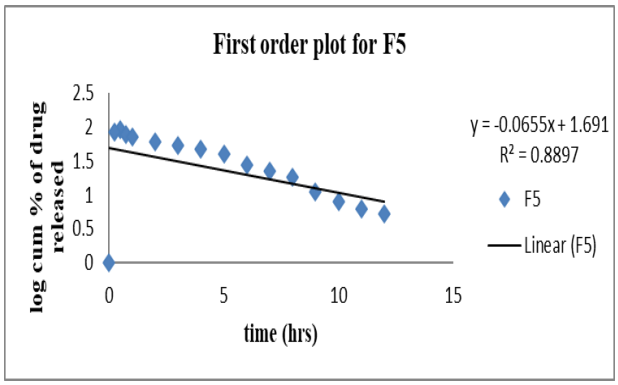

Figure 10: First order plot for formulation F5

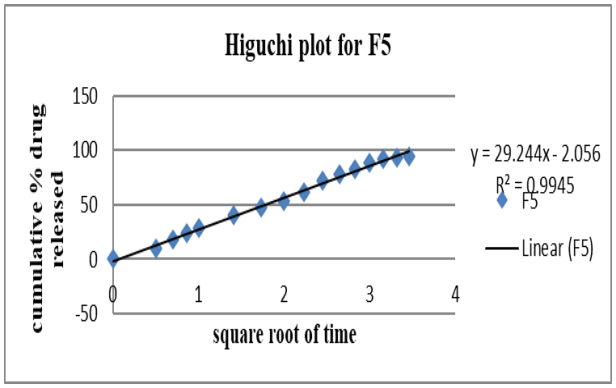

Figure 11: Higuchi plot for formulation F5

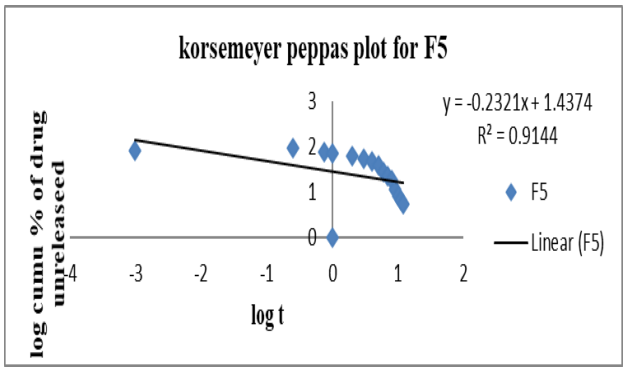

Figure 12: Korsemeyer-peppas plot for formulation F5

Table 6: Kinetic results (r2 values) of formulation F5

Formulation code	Zero order	1st order	Higuchi model	Korsemeyer-peppas model
F5	0.9435	0.8897	0.9945	0.9144

CONCLUSION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body and also to achieve promptly and then maintain the desired drug concentration over entire period of treatment. Microencapsulation is one of the widely used method for developing the new oral dosage form to control the drug release from the dosage form, improve the bioavailability, reduce adverse effects especially drug with small therapeutic range and prolong the action of drug. Ulcers are crater like sores which from in the lining of stomach, just below the stomach at the beginning of the small intestine in the duodenum. An ulcer is the result of imbalance between aggressive and defensive factors. Lansoprazole is a substituted benzimidazole derivative that targets gastric acid proton pumps, the final common pathway for gastric acid secretion. The drug covalently binding to the proton pumps causing prolonged inhibition of gastric acid secretion. Formulation of Lansoprazole microspheres by solvent evaporation method by using CAP, with a view to prevent the gastric side effects and to achieve oral sustained/ controlled release of the drug. It is concluded that the polymer of choice is suitable to prepare enteric microspheres of Lansoprazole as it doesn't interact with the drug to influence its pharmacological activity. The data suggest that a promising sustained release micro particulate drug delivery of lansoprazole can be developed. Our study has suggested that microencapsulation by solvent evaporation technique is inexpensive compared with other techniques and also advantages to prevent the drug related adverse effects of conventional dosage forms and maintain the sustained drug release over an extended period of time.

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