DEVELOPMENT AND EVALUATION OF FERROMAGNNETIC POLYMERIC PARTICLE SUSPENSION OF AMOXICILLIN AND CLARITHROMYCIN DUAL REGIMEN EFFECTIVE AGAINST *H. PYLORI* INFECTIONS.

THESIS SUBMITTED TO

ASSAM SCIENCE AND TECHNOLOGY UNIVERSITY, ASSAM

IN THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF

MASTER OF PHARMACY
IN
PHARMACEUTICS

Under the supervision of
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DECLARATION BY THE CANDIDATE

I hereby declare that the matter embodied in the dissertation entitled "**DEVELOPMENT AND EVALUATION OF FERROMAGNNETIC POLYMERIC PARTICLE SUSPENSION OF AMOXICILLIN AND CLARITHROMYCIN DUAL REGIMEN EFFECTIVE AGAINST *H. PYLORI* INFECTIONS"** is a bonafide and genuine research work carried out by me under the supervision of Dr. Bipul Nath, Assistant Professor, Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS), Hatkhowapara, Azara, Guwahati. The work embodied in this thesis is original and has not been submitted the basis for the award of any degree, diploma or fellowship in any other university or institution.

Signature of Student

Himangshu Deka

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Research is always a never ending process although for the successful completion of any research work it is a must needed work to acknowledge those members without whom the research work is incomplete.

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Place: Himangshu Deka

Date:

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1. Poster presentation

DEVELOPMENT AND EVALUATION OF FERROMAGNNETIC POLYMERIC PARTICLE SUSPENSION OF DRUG COMBINATION EFFECTIVE AGAINST *H. PYLORI* INFECTIONS.

Author's name: Himangshu Deka*, Dr. Bipul Nath, Jiban Debnath

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2. Review Article published

The article entitled **"MAGNETIC MICROSPHERES AS SITE SPECIFIC NOVEL DRUG DELIVERY SYSTEM: A REVIEW**" has been published in vol 7, issue 1, Jan 2018 in the journal **World journal of pharamaceutical research(WJPR).**

3. Research article

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DEVELOPMENT AND EVALUATION OF FERROMAGNETIC POLYMERIC PARTICLE SUSPENSION OF AMOXICILLIN AND CLARITHROMYCIN DUAL REGIMEN EFFECTIVE AGAINST *H. pylori* INFECTION

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ABSTRACT

The aim of the present study is to develop and evaluate ferromagnetic polymeric particle suspension of dual regimen Amoxicillin and Clarithromycin effective against Helicobacter pylori infection. Magnetic microsphere is a one of the newer approach of targeted drug delivery. It involves the use of particulate carriers to localize the drug at the diseased site. Site specific drug delivery enhances the efficiency of drug delivery and to reduce toxicity & side effects. The drug loaded chitosan microparticle was prepared by chemical cross linking using gluteraldehyde as cross linker. The crosslinked chitosan microparticles were coated with magnetite eudragit RL 100 polymeric dispersion. The drug polymer compatibility was studied by FTIR and DSC. The formulations were evaluated for % yield, particle size, % drug entrapment and drug release. The drug entrapment efficiency increased as the concentration of polymer increased. The optimum batch of the prepared microparticles is chosen based on good entrapment efficiency and in-vitro drug release characteristics and subjected for stability studies. Drug entrapment was found to be 91.8%. Drug release of the optimum batch was found to be 49.08% after 6 hours showing sustained release behavior at the stomach. In magnetic targeting, a drug or therapeutic radioisotope is bound to a magnetic compound, administered orally or injected into a patient's blood stream and can be localized with a powerful magnetic field in the target area. So, the present way of localized magnetic targeting of dual regimen would appear to be an effective method for the treatment of infection caused by *H. pylori*.

Keywords: Site specific delivery, microsphere, magnetic delivery

INTRODUCTION: A peptic ulcer is a mucosal lesion of the stomach or duodenum in which the acid and pepsin play major pathogenic roles. The major forms of peptic ulcer are gastric ulcer and duodenal ulcer, both of which are chronic diseases often caused by Helicobacter pylori . *H pylori*, a gram-negative, helical bacilli that live in the gastric epithelium It is the most common infectious human pathogen, infecting more than 50% of the populations worldwide (approximately 30% of children and 60% of adults), and is associated with 70% of benign gastric ulcers and 90% of duodenal ulcers

In magnetic targeting, a drug or therapeutic radioisotope is bound to a magnetic compound, administered orally or injected into a patient's blood stream and can be localized with a powerful magnetic field in the target area. Magnetic carriers receive their magnetic responsiveness to a magnetic field from incorporated materials such as magnetite, iron, nickel, cobalt, neodymium-iron-boron or samarium-cobalt. Magnetic microsphere were developed to minimize renal clearance and to increase target site specificity

The antiulcer potentials of most of these drugs in the novel delivery are still remain largely unexplored, despite their prospects evidenced by clinical research studies. No report was found in the literature that demonstrates the antiulcer properties of such combination in the form of magnetic microspheres. The focus of this work is to develop magnetic microspheres based on chitosan that shows a sustained release profile in a specific site in which the drug plays a role as a local therapeutic agent.

METHODOLOGY

1.Preformulation study

- **Orgenoleptic Properties**
- **Solubility study**
- **Melting point**
- **FTIR & DSC study**
- **Determination of absorption maxima & preparation of standard curve.**

2.Chitosan microparticle preparation

Chitosan microspheres were prepared by using emulsion method and glutaraldehyde was used as a cross-linker. A (2% w/v) chitosan solution was prepared with about 20 ml of 2% v/v aqueous acetic acid solution in which required amount of drugs was added gradually upon stirring. This drug polymer solution was stirred on a magnetic stirrer which was kept overnight. Aqueous phase (internal phase) was dispersed into 100 ml of liquid paraffin and glutaraldehyde, formed microspheres were dried at 50oC in hot air oven .

3. Magnetite preparation

To prepare magnetite, 500 mL round bottom flask fitted with condenser Charged the flask with 8.9 g (0.1 mol) of goethite, 9.94 g (0.05 mol) of $FeCl2.4H2O$ along with 250 mL deionized water. Added 50 mL of 2 M Sodium hydroxide. Reaction mixture was heated to reflux for 12 h. Its pH fell from 14 (orange) in to 8-9 (black precipitates). Particles washed and air dried.

4. Chitosan magnetic microparicle preparation

The crosslinked chitosan microparticles were coated with magnetite eudragit RL 100 :S100 polymeric dispersion .

5. Characterisation of chitosan microparicle

- **Determination of % yield**
- **Percentage entrapment efficiency**
- **Particle size analysis**
- **In vitro drug release**

6. Characterisation of chitosan magnetic microparticle

- **Determination of % yield**
- **Determination of flow properties**
- **Dissolution study**
- **Surface topography by SEM**

CONCLUSION: Chitosan microparticles were prepared by chemical cross linking method.The optimum batch of the prepared microparticles (F6) shown good yield, uniform size distribution and upto 91% entrapment of drug %, 49.08% drug release at 6hrs. This batch is subjected for coating with magnetic polymeric dispersion to develop chitosan magnetic microparticle. FM 3 batch has shown desired drug release characteristic and it is subjected for further studies. It can be concluded that the chitosan magnetic microspheres of amoxicillin & clarithromycin could deliver the drugs for a prolonged period of time at the target site and might be potentially used as carrier for targeted delivery.

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3.Nasra MK, Mohamed MM, Elblbesy MA, Hefney BA. Preparation of biocompatible magnetic microspheres with chitosan. J Biomater Nanobiotechnol ,2011; 2: 194-200.

RESULTS & DISCUSSION

1.Preformulation study

Organoleptic properties of drugs

Sample	Taste	Colour	Odour
Amoxicillin	Bitter	White	Penicillin type
Clarithromycin	Bitter	White	odorless

Solubility of drugs

Solvent	Amoxiciilin	Clarithromycin
Water	Freely soluble	Insoluble
Ethanol	Sparingly soluble	Slightly soluble
Acetone	Slightly soluble	Freely soluble
Acidic Buffer pH1.2	Freely soluble	Soluble

Drug excipient compatibility study by FT-IR & DSC

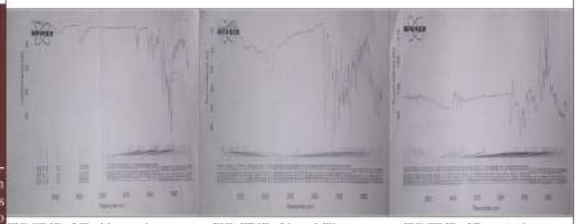

FIG:FT-IR of Clarithromycin FIG: FT-IR of Amoxicillin FIG:FT-IR of Drugs+polymer

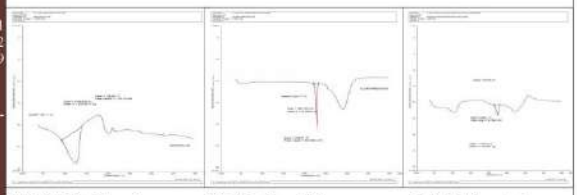

FIG:DSC of Clarithromycin FIG: DSC of Amoxicillin FIG:DSC of Drugs+polymer

2. Characterization of chitosaan microparicle

Formulation	Drug: polymer	Avg particle size(μm)	% yield	% Drug enreapment efficiency	% cumulative release at 6 hour
F1	1:1	112	71.3	43.07	-
F2	1:1	101	76.6	47.4	-
F3	1:2	141	83	74.9	64.34
F4	1:2	134	89.2	81	59.7
F5	1:3	188	91	84	56.11
F6	1:3	179	93.3	91.8	49.08

The optimum batch of the prepared microparticles (F6) is chosen for coating with magnetite

3. Chracterization of Chitosan magnetic microparticle

FORMULATION	COAT(RL100:S100)	CORE (Micro-sphere+magnetite)	% YIELD	% RELEASE IN 6 Hours
FM1	1:1	200+50 mg	73.33	20.06
FM2	2:1	200+50 mg	81.6	23.11
FM3	3:1	200+50 mg	77.11	47.33

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MAGNETIC MICROSPHERES AS SITE SPECIFIC NOVEL DRUG DELIVERY SYSTEM: A REVIEW

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ABSTRACT

The objective of this review on magnetic microspheres is to collect a new architecture that shows a great potential for future tests in the treatment of gastric ulcers. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres are chitosan, dextran etc. This review covers various reports on magnetic microspheres which can be prepared from a variety of carrier material. In the recent years, there has been keen interest in the development of magnetically target drug delivery system, because they aim to deliver the drug at a rate directed by the needs of the body during the period of treatment and target the activity entity to the site of action. Magnetic microsphere is a one of the newer approach of targeted drug delivery. It involves the use of particulate carriers to a localized diseased site. Site specific drug delivery enhances the efficiency of drug delivery and to reduce toxicity & side effects. The main objectives of this review are to highlight some important aspects of magnetic microspheres as a novel drug delivery system. The review shall cover definitions, concepts, types, mechanism of targeting, evaluation and characteristics of magnetic microspheres as well as various methods and techniques used in their preparations. The review also entails various applications and future prospects of magnetic microspheres.

KEYWORDS: Site specific delivery, magnetite, magnetic microsphere, approaches, magnetic delivery, anti-ulcer agents.

1. INTRODUCTION

Magnetite (Fe3O4) is a type of magnetic particle that has been the target of important studies on biomedicine because of its non-toxicity, high level of accumulation in tissues, interruption

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1. INTRODUCTION

1.1. PEPTIC ULCER

A peptic ulcer is a mucosal lesion of the stomach or duodenum in which the acid and pepsin play major pathogenic roles. The major forms of peptic ulcer are gastric ulcer and duodenal ulcer, both of which are chronic diseases often caused by Helicobacter pylori. The term peptic ulcer also encompasses gastric ulcers and duodenal ulcers associated with stress or the ingestion of drugs, most commonly aspirin and other non-steroidal antiinflammatory drugs (NSAIDs). Ulcer associated with Zollinger-Ellison Syndrome (ZES). caused by gastrin secreting islet cell tumors is also considered a form of peptic ulcer. Whether an ulcer develops, depends on the balance between aggressive factors principally gastric acid and pepsin) and factors that participate in mucosal defense or resistance to ulceration. Peptic ulcer develops when gastroduodenal mucosal defenses are unable to protect the epithelium from the corrosive effects of acid and pepsin. The proteolytic effects of pepsin in concert with the corrosive properties of secreted gastric acid contribute to the tissue injury that produces peptic ulcer. Gastric acid catalyses the cleavage of inactive pepsinogen molecules to proleolyticall) active pepsins and also provides the low pH for pepsin activity**.** Symptoms of peptic ulcer include abdominal discomfort and pain. Other symptoms include weight loss, nausea, vomiting, poor apettite, bolating. Some may also experience blood in stool and vomit and black stool that indicate gastrointestinal bleeding.

Peplic ulcers are remitting, relapsing lesions that are most ofien diagnosed in middleaged to older adults, but they may first become evident in young adult life. They may often appear without obvious precipitating influcnces and may then. after a period of weeks to months of active disease. heal with or without therapy. Even with healing, however, the propensity to develop peptic ulcers remains. Hence "once a peptic ulcer patient, always a peptic ulcer patient". Thus it is difficult to obtain accurate data on the prevalence of active disease. Rased on autopsy studies and population surveys, the best estimate indicates a prevalence of 6 to 14% for men and 2 to 6% for women. The male to female ratio for duodenal ulcers is about 3:l and for gastric ulcers about 1:5 to 1:2. Women are affected most often at or after menopause.[1,2,3]

1.1.1. COMMON CAUSES OF PEPTIC ULCER

- H pylori-The relationship between Helicobacter pylori infection and peptic ulcer disease has been studied exhaustively, and it is now accepted that the organism is the major cause, but not the only cause, of peptic ulcer disease worldwide. Eradicating the infection can alter the natural course of peptic ulcer disease by dramatically reducing its recurrence rate in treated patients, compared with untreated patients. It is the most common infectious human pathogen, infecting more than 50% of the populations worldwide (approximately 30% of children and 60% of adults), and is associated with 70% of benign gastric ulcers and 90% of duodenal ulcers.[2]
- Use of NSAIDs- NSAIDS inhibit certain prostaglandins that protect the stomach lining from the corrosive effect of gastric juice.[2]
- Alcohol consumption- Fermented and non distilled alcoholic bevarages increases gastric level and acid secretion.[2]
- Smoking and tobacco[3]
- Stress[3]
- Fasting condition[3]
- Radiation therapy[3]

1.1.2. TYPES OF PEPTIC ULCER [3]

The two most common types of peptic ulcer are called

- Gastric ulcers- Located in the stomach, characterized by pain in the upper abdomen and common in older aged group(especially in female).
- Doudenal ulcers- these are found at the beginning of small intestine and are characterized by severe pain in lower abdomen with burning sensation in the upper abdomen that awakens patients from sleep. Dounenal ulcer is more common in younger individuals and predominantly affects males.

1.1.3. DIAGNOSIS [3]

- X ray
- Endoscopy
- Test for the presence of *H pylori*- Serological test, breath analysis, stool analysis

1.2.1 *HELICOBACTER PYLORI*

Helicobacter pylori, a gram-negative, helical bacilli that live in the gastric epithelium was first isolated in 1983. It was discovered by Marshall and Warren who cultured Campylobacter pyloridis, which was later reclassified as Helicobacter pylori. It is transmitted via the fecal-oral, gastro-oral, or oral-oral routes. H. pylori is able to thrive in the gastric environment due to urease , motility , and adherence to gastric epithelium which allow it to neutralize gastric acid, penetrate through the mucus layer to the gastric epithelium, and colonize. It induces inflammation, leading to peptic ulcer disease (PUD) , gastric cancer, and gastric mucosa associated lymphoid-tissue (MALT) lymphoma . Although infection with H. pylori persists without treatment, the majority of infections do not lead to symptoms or gastrointestinal disease.[4,5]

1.2.2 Treatment

The first line, Food and Drug Administration (FDA) approved drug regimens for the treatment of H. pylori are listed in table below. These therapies include proton pump inhibitor (PPI) and two antibiotics or bismuth subsalicylate, acid suppressor, and two antibiotics. However, eradication rates using these regimens are a disappointing 75% in the United States due to increased H. pylori resistance to standard antibiotics . Clarithromycin and metronidazole show the highest rates of resistance and the factors associated with resistance include geographic region, sex, ethnicity, age, and active versus inactive ulcer disease . As a result of the declining eradication rates, other more promising therapies have been proposed. In a recent metaanalysis comprised mostly of trials from Italy, five days of a PPI with amoxicillin followed by five days of a PPI with clarithromycin and tinidazole had an eradication rate of 93.4% . This regimen has not yet been validated in the United States and it is not clear if it is superior to quadruple therapy. Sequential therapy (e.g. five days of PPI + amoxicillin followed by 5 days of PPI + clarithromycin and metronidazole) may increase medication adherence compared to quadruple therapy since it requires fewer medications. Another promising therapy that requires validation in the United States is PPI with levofloxacin and amoxicillin, with a reported 87% eradication rate in a recent meta-analysis.[6]

Table 1: FDA approved first line oral therapy for *H. pylori* treatment.

Therapy	Duration(Days)	Eradication rate
PPI twice a day, clarithromycin 500 mg twice a day, amoxicillin 1g twice a day	10-14	70-85%
PPI twice a day, clarithromycin 500 mg twice a day, metronidazole 500 mg twice a Day	10-14	70-85%
Bismuth subsalicylate 525 mg four times a day, metronidazole 250 mg four times aday, tetracycline 500 mg four times a day, PPI twice a day for 2 weeks, or H2RA for 4 weeks.	10-14	75-90%

PPI: Proton Pump Inhibitor, Lansoprazole 30 mg twice a day, Omeprazole 20 mg twice a day, H2RA: Histamine 2 receptor antagonist[3]

1.3.1. MAGNETIC MICROSPHERE

Magnetic microsphere is small spherical particle, with diameters in the micrometer range (1-1000 um in range). Magnetic microspheres are supramolecular particles that are small enough to circulate through capillaries without producing embolic occlusion but are sufficiently susceptible (ferromagnetic) to be captured in micro-vessels and dragged into the adjacent tissues by magnetic field of 0.5-0.8 tesla. Magnetic drug delivery by particulate carriers is a very efficient method of delivering a drug to a localized disease site. In magnetic targeting, a drug or therapeutic radioisotope is bound to a magnetic compound, injected into a patient's blood stream, and then stopped with a powerful magnetic field in the target area. Magnetic carriers receive their magnetic responsiveness to a magnetic field from incorporated materials such as magnetite, iron, nickel, cobalt, neodymium-iron-boron or samarium-cobalt. Magnetic microsphere were developed to minimize renal clearance and to increase target site specificity. Magnetic particles composed of magnetite which are well tolerated by the body, magnetic fields are believed to be harmless to biological systems and adaptable to any part of the body .[8,9,10]

The amount and rate of drug delivery via magnetic responsive microspheres can be regulated by varying (i) Size of microspheres; (ii) Drug content; (iii) Magnetite content; (iv) Hydration state; (v) Drug release characteristic of carrier.

The amount of drug and magnetite content of microspheres needs to be delicately balanced in order to design an efficient therapeutic system. Magnetic microspheres are characterized for different attributes such as (i) Particle size analysis including size distribution, surface topography, and texture etc. using scanning electron microscopy (SEM); (ii) Drug entrapment efficiency; (iii) % magnetite content; (iv) In vitro magnetic responsiveness; (v) Drug release.

1.3.2.ADVANTAGES OF MAGNETIC MICROSPHERE[9,11]

1) They facilitate accurate delivery of small quantities of potent drug and reduced concentration of drug at site other than the target organ or tissue.

2) They provide protection for unstable drug before and after administration, prior to their availability at the site of action.

3) They provide the ability to manipulate the in vivo action of the drug, pharmacokinetic profile, tissue distribution and cellular interaction of the drug. They enable controlled release of drug. Examples: Narcotic, Antagonist, Steroid hormones.

4) Difference occurs maximally in capillary network so efficient delivery of drug to diseased tissue is achieved.

5) Reduce the dosing frequency and thereby improve the patient compliance .

1.3.3. DISADVANTAGES OF MAGNETIC MICROSPHERES[9,11]

(1) One of the major limitations of this system is the drug cannot be targeted to deep-seated organism in the body. This approach is confined to the targeting of drugs to the superficial tissues like skin, superficial tumors or the joints.

(2) Thrombosis at the site of catheterization.

(3) The unknown toxicity of magnetic beads.

(4) The possible unwanted localization of the product in the liver and the regions of RES and the dangerous effect of self-flocculation of the magnetic particles causing vascular obstruction to vital organs in the body .

1.3.4. PRINCIPLES OF MAGNETIC TARGETING:-

Magnetic drug delivery by particulate carriers is a very efficient method of delivering a drug to a localized disease site. In magnetic targeting, a drug or therapeutic radioisotope is bound to a magnetic compound, injected into a patient's blood stream, and then localized with a powerful magnetic field in the target area. Depending on the type of drug, it is then slowly released from the magnetic carriers. It is thus possible to replace large amounts of freely circulating drug with much lower amounts of drug targeted magnetically to localized disease sites, reaching effective and up to several-fold increased localized drug levels. Magnetic carriers receive their magnetic responsiveness to a magnetic field from incorporated materials such as magnetite, iron, nickel, cobalt, neodymium– iron–boron or samarium–cobalt. Magnetic carriers are normally grouped according to size. At the lower end, we have the ferrofluids, which are colloidal iron oxide solutions.[9,11]

1.3.5. MAGNETITE

Magnetite is also called as ferric ferrous oxide, tri iron tetra oxide, and black iron oxide. Magnetic iron oxide chemical formula $FeOfe_20_3$ having a molecular weight of 231.55 with a chemical composition of Fe=72.36%, O=27.64%. The Ferro magnetic material when incorporated into microspheres makes them magnetically responsive, so that they can be concentrated to the desired site by applying some external magnetic field . Magnetite can be prepared by the following method[11,13]

Nitrogen gas flushed through 500 mL round bottom flask fitted with condenser.

↓

Charged the flask with 8.9 g (0.1 mol) of goethite, 9.94 g (0.05 mol) of $FeCl_2$.4H2O along with 250 mL deionized water.

↓

Added 50mL of 2 M Sodium hydroxide.

↓

Reaction mixture was heated to reflux for 12 h.

↓

Its pH fell from 14 (orange) in to 8-9 (black precipitates).

Particles washed and air dried

1.3.6. METHODS OF PREPARATION OF MAGNETIC MICROSPHERES

- **Continuous solvent evaporation**

In this method the drug and polymer are dissolved in appropriate volatile organic solvent and then magnetite (if magnetic microspheres) is added to this solution along with stirring in order to form a homogeneous suspension. This suspension is added to an immiscible auxiliary solution along with vigorous stirring. Now the volatile organic solvent is evaporated slowly at 22-30 °C to form microspheres. Microspheres are this centrifuged and freeze dried and stored at 4°C. [13]

- **Multiple emulsion method**

It involves the formation of the multiple emulsions of type w/o/w and is best suited to water soluble drugs, peptides, proteins and the vaccines. This method can be used with both natural as well as synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents. The continuous phase is generally consisted of the polymer solution that eventually encapsulates the protein contained in dispersed aqueous phase. The primary emulsion is the subjected to homogenization or sonication before addition to the aqueous solution of the poly vinyl alcohol. This results in the formation of a multiple emulsion. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction. A number of hydrophilic drugs like indomethacin, leutinizing hormone releasing hormone agonist, vaccines, proteins/peptides and conventional molecules are successfully incorporated into the microspheres using this method.[14,15]

- **Phase separation emulsion polymerization**

In this method, the aqueous solution of polymer, drug and magnetite are added to a vegetable oil which is then emulsified using a magnetic stirrer. The resultant emulsion is stabilized by heating at the temperature (100-150°C). The cross linking agent is then injected drop wise into the emulsion with continuous stirring. The formed magnetic microspheres are then separated from oil by washing procedures. The product is then freeze-dried & stord at 4°C. [14]

- **Emulsion solvent extraction method**

The preparation involved the dispersion of an aqueous phase, containing magnetite nanoparticles and a water-soluble homo-polymer, into droplets in an organic medium using an amphiphilic block copolymer as the dispersant. This was followed by water distillation at a raised temperature from the aqueous droplets to yield polymer magnetite particles. The structure of the particles was then locked in by a reagent being added to cross-link the water-soluble copolymer

block and homo-polymer. Since the hydrophobic block of the copolymer consisted of a protected polyester, the removal of the protective moieties from the coronal chains yielded poly (acrylic acid) or other functional polymers to render water dispensability to the spheres and to enable biomolecule immobilization.[15,18]

- **Cross linking method**

Nasra *et al*. prepared biocompatible magnetic microspheres with chitosan**.** Glutraldehyde-used as the cross-linker; sodium hydroxide solution-used as medium. They synthesised magnetic fluid by the following way - A 35% (w/v) ferrous sulfate solution, 54% (w/v) ferric chloride solution and 36% (w/v) sodium hydroxide solution were prepared using distilled water. Then the ferric salt and ferrous salt were mixed, stirred and heated. At 55 °C, the alkaline solution was added. The mixture was stirred for 30 min, and then 5 g of polyethylene glycol-10000 (PEG- 10000) was added. The temperature was raised to 80 °C and maintained for 30 min. The mixture was then neutralized while cooling, and the magnetic fluid was prepared. 1% (w/w) chitosan was dissolved in acetate buffer at pH 4.5. The dissolved chitosan was added drop wise on the magnetic fluid. Formed chitosan magnetic microspheres were washed with deionized water and soaked in 1, 3, and 5 mol % glutraldehyde solution for 2 h, and then washed with deionized water. [16,17]

- **Alkaline co precipitation method**

In this method, poly (acrylic acid-divinyl benzene) microspheres are treated with dilute aqueous NaOH solution (0.5 M) for hours at suitable temperature to transform the carboxylic acid groups to sodium carboxylates and then washed thoroughly with water to remove the excess NaOH till neutral pH. Purged the microsphere suspension with nitrogen for 30 min. To this suspension add an aqueous solution of FeCl3 and FeCl2 that had been purged with nitrogen. Stirred the mixture overnight under nitrogen atmosphere for ion exchange. The resulting microspheres were washed repeatedly with water under nitrogen atmosphere to remove excess iron salts. Added drop wise an aqueous NaOH solution (3 M) to a suspension of the microspheres taken up with iron ions under nitrogen atmosphere to adjust the pH value to be >12. The mixture was then heated to 60°C and kept for another 2h. The resulting magnetic microspheres were suspended in an aqueous HCl solution (0.1 M) to transform the –COONa to –COOH, and then washed thoroughly with water to neutral pH, dried under vacuum at 50°C overnight, giving magnetic microspheres.[19,20]

- **Sonochemical method**

The microspheres composed of iron oxide-filled and coated globular bovine serum albumin (BSA). The magnetic microspheres were prepared from BSA and iron penta carbonyl, or from BSA and iron acetate. Protein microspheres have a wide range of biomedical application, i.e. use as echo contrast agents for sonography. The microsphere were formed by either heat denaturation at various temperatures, or by cross linking with carbonyl compounds in the ether phase. Cross linking was done as the microspheres are formed by chemically cross-linking cysteine residues of the protein with HO2 radical formed around a non-aqueous droplet. The chemical cross-linking is responsible for the formation of the microspheres and is a result of the chemical ejects of the ultrasound radiation on an aqueous medium. Two sonochemical methods for the fabrication of iron oxide nanoparticles were (i) Water as the solvent and (ii) Decalin as solvent. Decane and iron pentacarbonyl Fe (CO)5 (7.43U1034 M) were layered over a 5% w/v protein solution. The bottom of the high-intensity ultrasonic horn was positioned at the aqueous organic interface. The mixture was irradiated for 3 min, employing a power of W150 W/ 32cm with the initial temperature of 23 °C in the reaction cell. The pH was adjusted to 7.0 by adding HCl. This procedure was performed again with an aqueous solution of iron acetate, Fe (CH3CO2)2 95% (Sigma) (7.66U1033 M). After the synthesis, the products were separated from the unreacted protein and from the residues of iron acetate or iron penta carbonyl by centrifugation (1000 r/min for 5 min). The magnetic microspheres were washed a few times with sufficient volumes of water to remove the residues of the precursors.[21,22]

- **Swelling and penetration method:-**

For swelling of polymer micro particles, 0.25 g of PS (Micron-size polystyrene) particles was mixed with 35 mL of a NMP/water solution in a specific v/v NMP (N-methyl2-pyrrolidone)-to-water ratio. In later preparations of magnetic microspheres, SDS (Sodium dodecyl sulfate) was added to the NMP/water solution. Whenever SDS was used, 0.025 g of SDS were added to each NMP/water solution. The NMP/water mixture with PS spheres was left soaking for 24 h at room temperature while stirring. 2.5 mL of the super paramagnetic nanoparticle dispersion (24 mg/mL or other specified concentration) was added to the mixture of PS sphere and NMP/water solution at 30°C while shaking (at 140 r/min) for 1-5 days to allow the magnetic nanoparticles to penetrate into the interior of the PS particles. Afterwards, the polymer particles were separated from the solution by centrifugation. Finally, particles were sequentially washed with methanol,

deionized water, and vacuum dried at room temperature for 1-2 days to yield the magnetic polymer microspheres.[26]

- **Hot melt microencapsulation**

The polymer is first melted and then mixed with solid particles of the drug that have been sieved to less than 50μm. The mixture is suspended in a non-miscible solvent (like silicone oil), continuously stirred, and heated to 5°C above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microspheres are washed by decantation with petroleum ether. The primary objective for developing this method is to develop a microencapsulation process suitable for the water labile polymers, e.g. poly anhydrides. Microspheres with diameter of 1-1000μm can be obtained and the size distribution can be easily controlled by altering the stirring rate. The only disadvantage of this method is moderate temperature to which the drug is exposed.[25]

1.3.7 APPLICATIONS OF MAGNETIC MICROSPHERES [28-42]

a. Magnetic microsphere carriers uses in the fields of biomedicine and bioengineering, biological and biomedical developments and trends such as enzyme immobilization, cell isolation, protein purification, and target drugs.

b. Magnetic vehicles are very attractive for delivery of therapeutic agents as they can be targeted to specific locations in the body through the application of a magnetic field gradient.

c. Magnetic microspheres are used in targeting drugs like mitoxantrone, paclitaxel and doxorubicin to tumor sites. Magnetic microsphere carriers labeled with radionuclide such as Rhenium-188 and Yttrium-90 have been also used in a preclinical study to treat liver and brain tumors .

d. Magnetic microspheres can be used for stem cell extraction and bone marrow purging .

e. Magnetic microspheres of cisplastin and paclitaxel were used in localized hyperthermia for treatment of cancer .

f. Magnetic polystyrene microspheres have been used as specific cell labeling .

g. Improvement in methods for isolating DNA, proteins, cells or cell organelles has been made and more recently, methods that rely on the use of solid phase have been proposed. Adsorbents such as silica that provide fast, efficient DNA purification are important for making this procedure amenable to automation. One of these kits involves isolation of DNA using silica coated magnetic particles.

h. Nowadays, several instruments are available from different companies that couples separation of biomolecules with its detection in terms of its quantification or its interactions with other biomolecules. These instruments either use directly ferromagnetic particle as label (magneto assay) or couples magnetic particles with other detection methods such as fluorescence or chemiluminescence .

i. Magnetic microspheres are now increasingly used as carriers for binding proteins, enzymes and drugs. Studies have shown that proteins and enzymes can be bound covalently to naked magnetic particles in the presence of carbodiimide. Such immobilization procedures for proteins, enzymes or drugs will have a major impact in various areas of medicine and biotechnology. The immobilized biomolecules can be used directly for a bioassay or as affinity ligands to capture or modify target molecules or cells.

j. Supra-magnetic iron oxide microspheres have been used for detection of metastases in non-enlarged lymph nodes .

k. Streptavidin coated magnetic beads were used for bacteria detection .

l. Magnetic Dynabeads have been used in immune-magnetic techniques for the enrichment and detection of isolated breast carcinoma cells in bone marrow and peripheral blood .

m. Magnetic microspheres carriers of contraceptive has been designed responsive to the changes in steroid secretions during menstrual cycle .

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1.4 DRUG PROFILE

1.4.1 Drug Profile: Amoxicillin

- **Description-**

Amoxicillin is an antibiotic useful for the treatment of a number -90%of bacterial infections.[1]It is the first line treatment for middle ear infections. It may also be used for strepthroat, pneumonia, skin infections, and urinary tract infections among others. It is taken

by mouth, or less commonly by injection. Common side effects include nausea and rash. It may also increase the risk of yeast infections and, when used in combination with clavulanic acid, diarrhea. It should not be used in those who are allergic to penicillin. While usable in those with kidney problems, the dose may need to be decreased. Its use in pregnancy and breastfeeding does not appear to be harmful. [43]

- **Chemical Structure:**

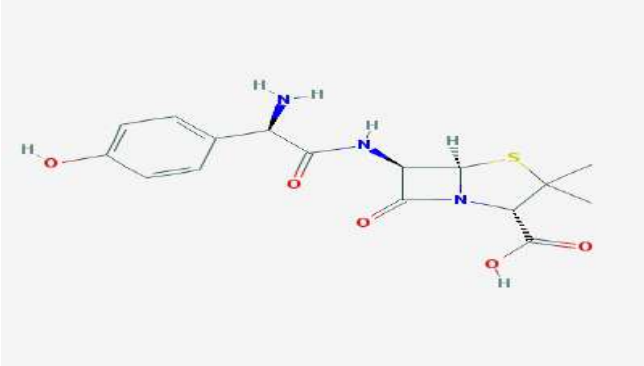

Fig 1: Chemical Structure of Amoxicillin

- **Molecular Formula-**

$C_{16}H_{19}N_3O_5S$

- **IUPAC Name-**

(2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

- **Molecular weight:** 365.404 g/mol
- **Melting Point- 194**
- **Color- white**
- **Odor- Penicillin-type odor**
- **Taste- Bitter tasting**
- **Drug Indication-**

For the treatment of infections of the ear, nose, and throat, the genitourinary tract, the skin and skin structure, and the lower respiratory tract due to susceptible (only b-lactamase-negative)

strains of *Streptococcus* spp. (a- and b-hemolytic strains only), *S pneumoniae*, *Staphylococcus* spp., *H. influenzae*, *E. coli*, *P. mirabilis*, or *E. faecalis*. Also for the treatment of acute, uncomplicated gonorrhea (ano-genital and urethral infections) due to *N. gonorrhoeae* (males and females).[43,44]

- **Mechanism of Action-**

Amoxicillin binds to penicillin-binding protein 1A (PBP-1A) located inside the bacterial cell well. Penicillins acylate the penicillin-sensitive transpeptidase C-terminal domain by opening the lactam ring. This inactivation of the enzyme prevents the formation of a cross-link of two linear peptidoglycan strands, inhibiting the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that amoxicllin interferes with an autolysin inhibitor.

- **Biological Half-Life:** 61.3 minutes

1.4.2 Drug Profile: Clarithrimycin

- **Description-**

Clarithromycin is a semisynthetic 14-membered ring macrolide antibiotic. Clarithromycin binds to the 50S ribosomal subunit and inhibits RNA-dependent protein synthesis in susceptible

organisms. Clarithromycin has been shown to eradicate gastric MALT (mucosa-associated lymphoid tissue) lymphomas, presumably due to the eradication of tumorigenic Helicobacter pylori infection. This agent also acts as a biological response modulator, possibly inhibiting angiogenesis and tumor growth through alterations in growth factor expression. [43]

- **Structure-**

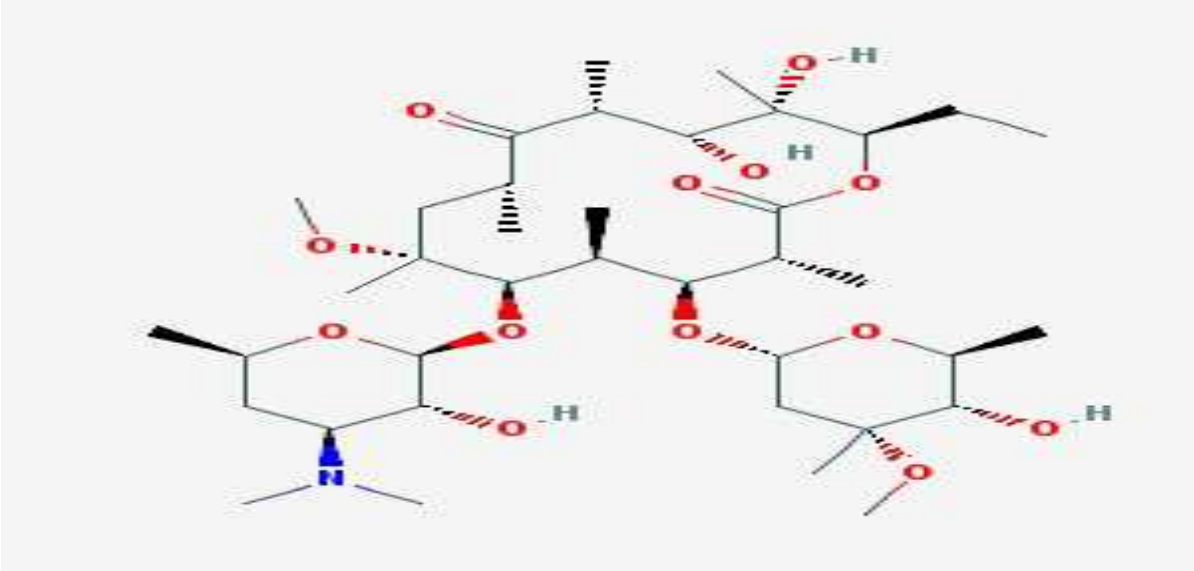

Fig 2: Chemical Structure of Clarithromycin

- **IUPAC Name**

(3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-6-[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-12,13-dihydroxy-4-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-7-methoxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione.[43]

- **Molecular Formula**

$C_{38}H_{69}NO_{13}$

- **Molecular Weight:**

1747.964 g/moL

- **Color: Colorless needles**
- **Melting Point**

220 dec °C

- **Drug Indication**

An alternative medication for the treatment of acute otitis media caused by *H. influenzae, M. catarrhalis, or S. pneumoniae* in patients with a history of type I penicillin hypersensitivity. Also for the treatment of pharyngitis and tonsillitis caused by susceptible *Streptococcus pyogenes*, as well as respiratory tract infections including acute maxillary sinusitis, acute bacterial exacerbations of chronic bronchitis, mild to moderate community-acquired pneuomia, Legionnaires' disease, and pertussis. Other indications include treatment of uncomplicated skin or skin structure infections, helicobacter pylori infection, duodenal ulcer disease, bartonella infections, early Lyme disease, and encephalitis caused by *Toxoplasma gondii* (in HIV infected patients in conjunction with pyrimethamine). Clarithromycin may also decrease the incidence of cryptosporidiosis, prevent the occurence of ?-hemolytic (viridans group) streptococcal endocarditis, as well as serve as a primary prevention for *Mycobacterium avium* complex (MAC) bacteremia or disseminated infections (in adults, adolescents, and children with advanced HIV infection).[43,44]

- **Mechanism of Action**

Clarithromycin is first metabolized to 14-OH clarithromycin, which is active and works synergistically with its parent compound. Like other macrolides, it then penetrates bacteria cell wall and reversibly binds to domain V of the 23S ribosomal RNA of the 50S subunit of the bacterial ribosome, blocking translocation of aminoacyl transfer-RNA and polypeptide synthesis. Clarithromycin also inhibits the hepatic microsomal CYP3A4 isoenzyme and P-glycoprotein, an energy-dependent drug efflux pump.

- **Biological Half-Life**

3-4 hours

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2. REVIEW OF LITERATURE

2.1 Magnetic microsphere

1. **Kakar et al. (J acute dis, 2009)** developed magnetic microspheres of mesalamine (5-aminosalicylic acid) for colon drug delivery. Microspheres were prepared by solvent evaporation technique. An attempt was made to target mesalamine to the colon. Eudrait s-100, ethyl cellulose and chitosan were used in different drug polymer ratio i.e 1:1, 1:2, 1:3. Microspheres were characterized in terms of particle size, percentage yield, drug content, encapsulation efficiency, in vitro release pattern and ex vivo study. Chitosan microspheres were found to be better retained at their target site. Flow characteristic are also better in case of chitosan magnetic microspheres.[1]

2. **Varma et al.(Int J Drug Delivery, 2011)** developed Magnetic nanoparticles encapsulated in Methyl methacrylate (Eudragit L-100) microspheres containing Indomethacin drug were prepared and their detailed structural and magnetic characteristics were studied. Iron oxide nanoparticles were obtained by chemical co-precipitation of Fe(II) and Fe(III) salts and stabilized with tetra-methyl ammonium hydroxide. Microspheres were prepared by solvent evaporation technique. They characterized the magnetic microspheres in terms of morphology, composite microstructure, size and size distribution, magnetic properties and *in-vitro* release patterns. The microspheres were uniform both in shape and usually also in size; their size distribution was narrow. All the magnetic parameters confirm superparamagnetic nature of the microspheres. Drug release in the first hour was found to increase and reached a maximum, releasing approximately 60-85% of the total drug content from the microspheres within 8 hours. From this study, it could be suggested that magnetic Methyl methacrylate microspheres could be retained at their target site in-vivo and such microspheres can be used in biomedical applications and research areas such as target drug delivery, selective blood detoxification, tissue engineering and replacement, and magnetic resonance imaging (MRI) contrast agents.[2]
3. **Sasidharan et al. (Int J Pharm Sci Rev Res, 2016)** developed sulfasalazine loaded magnetic microsphere coated with eudragit L-100 in the treatment of Inflammatory Bowel Disease and also to evaluate the total amount of magnetite present in microsphere.

Sulfasalazine loaded magnetic microsphere was prepared using simple cross linking method. The formulated magnetic microsphere was further evaluated for physiochemical property and was found to be within acceptable level. The magnetite solution was prepared using suitable method sulfasalazine microsphere was loaded with magnetite.. FTIR and DSC were evaluated for drugexcipient interaction, the morphological study was done with Scanning Electron Microscopy and the particles were found to be round, rough and discreet. Micrometric property revealed that all particles have better flow property. *In-vitro* study was carried out and it was found that the maximum drug release was found for formulation F4 which was 96.45±2.25% in 24hrs. Cell line study concluded that the optimized F4 formulation showed down regulation of COX -2as compared to control and marketed formulation. Stability study that F4 formulation was found to be stable for different temperature condition. They stated that in future, magnetic studies and *in-vivo* studies are essential to prove the site specific delivery and magnetic targeting effect of microsphere.[3]

4. **Nasra et al. (J Biomater Nanobiotechnol 2011**). In this study they prepared chitosan magnetic microspheres (CMMS) with simple crosslinking method. The obtained CMMS were in size range of 1000 - 2600 nm with average particle size of 1800 nm. All the essential characterizations of prepared CMMS were done and the results were in a good agreement with other magnetic microspheres prepared with different method. To test the biocompatibility of CMMS with blood, the effect of them on erythrocytes aggregation and blood hemolysis were studied. Their results showed that CMMS work as good compatible materials with blood.[4]
5. **Sundar et al. (Der Pharmacia Lettre ,2016)** formulate and evaluate magnetic microspheres of anastrozole using polycaprolactone as the encapsulating material to achieve targeted drug delivery. Anastrozole loaded magnetic PCL microspheres were prepared by O/W emulsion solvent evaporation technique and characterized in terms of morphology, particle size, entrapment efficiency, drug loading, FTIR, DSC studies, magnetite content, magnetic properties, in vitro drug release and in vitro release mechanism. Microspheres were smooth and spherical in shape with an average size of 10.2-11.24µm. Encapsulation efficiency and drug loading were found to be good and the formulations exhibited superparamagnetic behaviour with saturation magnetization of

7.66 emu/g. FTIR studies showed the absence of chemical interaction between polymer and drug. DSC studies revealed amorphous state of drug in the magnetic microspheres. The average magnetite content was 18.22%w/w which could be sufficient to direct the microspheres to their target site. The in vitro release studies showed initial burst effect followed by sustained effect over a period of 21 days. Among all the batches formulated, formulation F3 containing drug to magnetite ratio 1:3 shows more controlled release behaviour. Results suggest that the prepared magnetic microspheres might be potentially used as carrier for targeted delivery.[5]

6. **Varma et al. (Int J Pharm Anal ,2009)** prepared Diclofenac sodium-containing ethyl cellulose micro particles by the Emulsion-solvent evaporation method with a view for use in the application of magnetic carrier technology. The properties of these magnetic microspheres, such as morphological, magnetic susceptibility and polymer-drug interactions were characterized by different techniques (i.e. SEM, magnetometry and FT-IR). The loading efficiency and swelling kinetics magnetic microspheres were also studied. The formulated microspheres were below 5μm and spherical in nature as evidenced from SEM. FT-IR revealed that, there was no drug-polymer interaction. The in-vitro release profile was studied in normal saline medium up to 7 hours using USP XXII dissolution apparatus. Drug release in the first hour was found to increase and reached a maximum, releasing approximately 57.46% to 81.44% of the total drug content from the microspheres within 7 hours. A third order equation for the drug release was also calculated. Microspheres showed greater retention time under the influence of magnetic field created by an electromagnet with field strength 8000 G, when compared to the retention in the absence of magnetic field. From this study, it could be suggested that magnetic ethyl cellulose microspheres could be retained at their target site in-vivo, following the application of the magnetic field and are being capable of releasing the drug for an extended period of time, thus making them a suitable depot for delivering chemotherapeutic agent in-vivo.[6]
7. **Zhao et al. (J Magnetism Magnetic Materials ,2009)** developed Poly(lactic acid) (PLA)-coated magnetic nanoparticles using uncapped PLA with free carboxylate groups. The physical properties of these particles were compared to those of oleate-coated or oleate/sulphonate bilayer (W40) coated magnetic particles. Magnetic microspheres

(MMS) with the matrix material poly(lactide-co-glycolide) (PLGA) or PLA were then formed by the emulsion solvent extraction method with encapsulation efficiencies of 40%, 83% and 96% for oleate, PLA and oleate/sulfonate-coated magnetic particles, respectively. MMS made from PLA-coated magnetite were haemocompatible and produced no hemolysis, whereas the other MMS were hemolytic above 0.3mg/mL of blood.[7]

8. **Chung et al. (Reactive and Functional Polymers, 2008)** uses swelling and penetration process to prepare magnetic polymer microspheres. Micron-size polystyrene (PS) particles were swollen in an aqueous solution of *N*-methyl-2-pyrrolidone (NMP) and then mixed with superparamagnetic iron oxide nanoparticles. The magnetic nanoparticles were able to diffuse into polymer microspheres and were entrapped within the polymer microspheres. The saturation magnetization of resultant magnetic polymer microspheres increased as increasing magnetic nanoparticle concentrations were added to the swelling mixture. A higher ratio of NMP-to-water led to a greater swelling but a larger loss of polymer mass due to polymer chain dissolution in the NMP solution. Sodium dodecyl sulfate (SDS) in the NMP aqueous solution significantly enhanced the swelling and penetration process. The use of SDS could not only shorten the process time but also lower the required NMP concentration. The proposed method also worked well in preparing magnetic polymers microspheres with other styrene-based copolymer beads like poly(styrene–glycidyl methacrylate) (PS–GMA).[8]
9. **Wang et al.(Carbohydrate Polymers,2011)** prepared P(styrene-itaconic acid-divinylbenzene) microspheres (P(St-IA-DVB) microspheres) based on styrene (St), itaconic acid (IA) and divinylbenzene (DVB) via water-in-oil emulsions method (W/O) in the presence of emulsifiers with the size of 5–10 _m. The magnetic nanoparticles (i.e. Fe3O4) coated tightly on the surface of P(St-IA-DVB) microspheres were prepared in water with a continuous stirring. The morphology of blank microspheres and magnetic nanoparticles-coated microspheres was investigated in this work. In vitro drug release behavior was studied using doxorubicin as a model drug, and these magnetic nanoparticles-coated P(St-IA-DVB) (MNPSID) microspheres might have great potential application in magnetically targeted and thermal therapy.[9]

10. **Chandna et al.(J Acute Dis.2013)** studied magnetic microspheres as a newer approach in the pharmaceutical field. They discussed magnetic microsphere as an alternative to traditional radiation method**.** This type of delivery system is very much important for the localization of drugs to the disease site, by this system large amount of freely circulating drugs can be replaced by smaller amount of magnetically targeted drug. They concluded that this delivery system have several advantages such as reduction of dose and side effects. It is a challenging area for future research in drug targeting so more researches, long time toxicity study and characterization will ensure the improvement of magnetic drug delivery system.[10]
11. **Patil et al.(Indo American J Pharm Res,2016) studied** Magnetic microspheres as supramolecular particles that are small enough to circulate through capillaries without producing embolic occlusion (<4 μm) but are sufficiently susceptible (ferromagnetic) to be captured in micro vessels and dragged into the adjacent tissues by magnetic fields of 0.5-0.8 Tesla (T). Methods of preparation of magnetic microspheres are namely phase separation emulsion polymerization (PSEP) and continuous solvent evaporation (CSE). Ideally less than 125 p that can be suspended in aqueous vehicle and injected by an 18 or 16 number needle. The amount and rate of drug delivery via magnetic responsive microspheres can be regulated by varying (i) Size of microspheres; (ii) Drug content; (iii) Magnetite content; (iv) Hydration state; (v) Drug release characteristic of carrier. They concluded that it is the well developed new drug delivery system for targeting of drug in chronic diseases. It is safe and advantageous drug delivery system. The materials which are used in methods of preparation are economic and non toxic in nature so it is most convenient drug delivery system.[11]
12. **Vyas MB et al.(Int J Biopharmaceutics 2013)** developed magnetically responsive biodegradable microparticulate delivery system of cisplatin by phase separation emulsion polymerization technique by using bovine serum albumin. The formulations were evaluated with respect to particle size analysis, entrapment efficiency, magnetite content, *in vitro* magnetic responsiveness, *in vitro* drug release studies*, in vivo* drug targeting studies and stability studies. The formulated magnetic microspheres were found to be spherical with average particle size of 3-12 μm in diameter and incorporation efficiency up to 56.37%. Result of X-ray diffractrometry confirmed the presence of magnetite in

prepared cisplatin magnetic microspheres. The total percentage of Fe2O3 in the microspheres was found to be 42.53% to 55.48%. Highest *In vitro* drug release after 24 hours was 89.60%. Results of *in vitro* magnetic responsiveness and *in vivo* tissue targeting proved that the retention of microspheres in presence of magnetic field was significantly high than those in the absence of the magnetic field. Stability studies revealed that 4º is the most suitable temperature for storage of cisplatin loaded magnetic microspheres. Overall, this study shows that the magnetic albumin microspheres can be retained at their target site *in vivo*, following the application of magnetic field, and are capable of releasing their drug content for an extended period of time.[12]

13. **Farah (J Analytical & Pharm Res, 2016)** studied important aspects of magnetic microspheres as a novel drug delivery system. They cover definitions, concepts, types, mechanism of targeting, evaluation and characteristics of magnetic microspheres as well as various methods and techniques used in their preparations and also entails various applications and future prospects of magnetic microspheres. She concluded that it has the advantage of target specificity and better patient compliance. Its applications are enormous as they are not only used for delivering drugs but also for imaging tumors, detecting bio-molecular interaction etc. So in future by combining various other strategies, magnetic microspheres will find the central place in novel drug delivery, particularly in diseased cell sorting, diagnostics, gene & genetic.[13]

2.2 Anti H pylori Approaches

1. **Venkateswaramurthy et al.(Tropical J Pharml Res 2013)** formulated mucoadhesive microspheres of Amoxicillin trihydrate and clarithromycin by solvent evaporation method using Carbopol 974P, HPMC K4M and Eudragit RS 100.They discussed that a combination approach, i.e., mucoadhesive and extended/controlled drug delivery system, was explored for the effective and improved treatment of H. pylori infection in the study. Mucoadhesive drug delivery systems thus provide sustained drug release by localizing drug in the mucosal region where the bacteria reside . This would also increase the antibiotic concentration in the luminal region of the stomach by promoting the diffusion of the antibiotics in the epithelial cell layer and thus would be effective in inhibiting the growth of bacteria in that region.[14]

2. **Zawar et al.(Int J Pharma Bio Sci, 2010)** formulated and evaluated Floating–mucoadhesive tablets of clarithromycin for the treatment of Helicobacter pyloric (*H.pylori)* infection. Tablets were prepared by direct compression using directly compressible polymers such as HPMC K4M, HPMC K15M and carbopol 974P and they evaluated for drug - excipient compatibility, density, buoyancy test, mucoadhesion force, swelling study, drug content and *in-vitro* release profile. Sodium bicarbonate and citric acid were used for producing effervescent base for buoyancy of tablets. Analysis of drug release from tablet indicates drug release by zero order rate kinetics. No significant change was observed in physical appearance, drug content, floatability or *in vitro* dissolution pattern after storage at 45 °C / 75% RH for three months. They concluded that Stomach-specific antibiotic drug delivery would be highly beneficial in the treatment of *H. pylori* infection in peptic ulcer disease.[15]
3. **Arati et al**. (**Aaps Pharmscitech 2012**) A. vera gel powder is used for its cytoprotective action. Bilayer floating tablets were prepared by applying direct compression technique. The proportion of sodium bicarbonate and citric acid was adjusted to get the least possible lag time with good matrix integrity and total floating time. Polymer concentration was adjusted to get the maximum release in 8 h. The formulation was developed using hydroxypropyl methyl cellulose (HPMC) K4M and HPMC K100M in a ratio of 85:15 along with 1:4 ratio of effervescent agents was found to give floating lag time of less than 1 min with total floating time of more than 8 h and 97.0% drug release in 8 h. From the In vivo studies they found that A. vera and amoxicillin in combination have gastric acid inhibitory propertiesand suggests its usage in peptic ulcer treatment.[16]
4. **SambaT R et al. (Int J Pharm Pharm Sci, 2011)** prepared clarithromycin loaded microspheres for anti – *H.pylori* Therapy. The mucoadhesive microspheres prepared by using sodium alginate alone and in combination with HPMC K4M and carbopol 974 P. Clarithromycin loaded mucoadhesive microspheres were successfully prepared by emulsification-internal gelation technique with a maximum incorporation efficiency of 93 %. The scanning electron microscopic study indicated that the microspheres were spherical in shape. The in vitro wash-off test indicated that the microspheres had good mucoadhesive properties. *In vitro* were conducted in 0.1 N HCL. The preliminary results show great promise for this delivery strategy in the treatment of *H. Pylori* infection. [17]

5. **Malik et al. (The Pharma Innovation ,2012)** prepared clarithromycin loaded microspheres for anti – H.pylori Therapy. The microspheres were prepared by the w/o emulsification solvent evaporation method using mucoadhesive polymers sod. CMC and a release controlling polymer sod.alginate. The shape and surface morphology of prepared microspheres were characterized by optical microscopy . In vitro drug release studies were performed and drug release evaluated. They concluded that the prepared formulations possess good bioadhesive properties. These properties enable the microspheres to adhere to the gastric mucosal surface and stay in stomach for prolonged periods and could ensure the stability of drug in gastric environment, which eventually resulted in better eradication of *H. pylori* than the conventional dosage forms. Further studies are planned to examine the gastric residence time of the microsphere formulation and the efficacy in eradicating *H. pylori.*[18]
6. **Sanjay et al. (Acta Poloniae Pharmaceutica Ñ Drug Research**, **2006**) designed Floating matrix tablets to prolong the gastric residence time after oral administration, at a particular site and controlling the release of drug especially useful for achieving controlled plasma level as well as improving bioavailability. With this objective, floating dosage form containing clarithromycin as drug was designed for the treatment of *Helicobacter pylori*. Tablets containing hydroxypropylmethylcellulose (HPMC), drug and different additives were compressed using wet granulation and D-optimal design technique. The study shows that tablet composition and mechanical strength have great influence on the floating properties and drug release. Incorporation of gas-generating agent together with polymer improved drug release, besides optimal floating (floating lag time <30 s; total floating time >10 h). The drug release was sufficiently sustained (more than 8 h) and anomalous diffusion as well as zero-order was confirmed. Optimization of the evaluating parameters with ëdesign expertí software was employed to get final optimized formulation. The optimized formulation was obtained using 62.5% clarithromycin, 4.95% HPMC K15M, 18.09% HPMC K4M, 12.96% sodium bicarbonate which gave floating lag time < 30 s with a total floating time > 10 h.[19]
7. **Adeol et al. (Int J Pharmaceutics, 2014)** prepare and characterise ethylcellulose/chitosan microspheres containing clarithromycin with their surfaces functionalised with concanavalin A to produce a floating-mucoadhesive formulation. The

microspheres were prepared using an emulsification-solvent evaporation method. Particle size, surface morphology, *in vitro* buoyancy profile, zeta potential, drug entrapment efficiency, *in vitro* drug release and release kinetics of the particles were determined. Lectin was conjugated to the microsphere surface using two-stage carbodiimide activation and confirmed using FTIR, fluorescence studies and zeta potential measurements. Conjugation ranged from 11-15 μg Con A / mg microspheres which represents over 56 % efficiency although there was some drug loss during the conjugation process. Conjugation did not have a significant effect on the buoyancy and release of drug from the microspheres using a mucus diffusion model with 53 % and 40 % of drug released from unconjugated and conjugated microspheres within 12 h. Conjugation improved mucoadhesion and interaction with porcine gastric mucin compared to unconjugated microspheres. The buoyancy and improved mucoadhesion of the microspheres provides potential for delivery of CMN and other drugs to the stomach.[20]

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3. AIM & OBJECTIVE:

3.1 AIM:

Development and evaluation of ferromagnetic polymeric particle suspension of Amoxycillin and Clarithromycin dual regimen effective against *H. pylori* infection.

3.2 Objective of the study:

- Preformulation study of the drug and excipients.
- Formulation of cross linked chitosan microparticles by ionic gelation and cross linking method.
- Formulation optimization of the prepared cross linked chitosan microparticles.
- Preparation of magnetite particle by precipitation method.

- Microencapsulation of prepared cross-linked chitosan microparticles with magnetite and Eudragit RL100+S100 mixture by solvent evaporation.
- Evaluation of magnetic crosslinked chitosan microparticles.

3.3 Rational (Hypothesis) behind the study

Peptic ulcer disease (PUD) is a chronic, multifactorial disease characterized by benign lesions of gastric or duodenal mucosa. Estimates show that almost 20% of global population is affected by PUD during their lifetime (Levenstein et al, 2000). Thus, efforts are being re-directed towards finding effective alternatives that can be targeted towards ulcer healing, with minimal side effects. In this regard, effective novel drug delivery systems are promising alternatives for existing drug molecules; as a good number of anti-ulcer agents are available as medicine; but only few have shown or possess antiulcer properties with outcome over the last many years. The antiulcer potentials of most of these drugs in the novel delivery are still remain largely unexplored, despite their prospects evidenced by clinical research studies. No report was found in the literature that demonstrates the antiulcer properties of such combination in the form of magnetic microspheres. There is single report found on magnetic microparticles which uses covalent TiO2/pectin microspheres of Amoxycillin with Fe_3O_4 nanoparticles for magnetic field-modulated drug delivery against H.pylori infections.

The focus of this work is to develop magnetic microspheres based on chitosan that shows a sustained release profile in a specific site in which the drug plays a role as a local therapeutic agent. The concept of such a microsphere may be based on a tortuosity effect that sustains the release of the drug. This behavior is the result of the disposition of microparticles within the cross-linked polymer network. This is obtained by ionic cross-linking of chitosan microsphere approach using Fe_3O_4 as magnetic particle, and glutaraldehyde as crosslinker. Such architecture could show a versatile release profile. The release of the drug could be sustained for a longer time, and also controlled remotely. The proposed microsphere is addressed to the treatment of Helicobacter pylori (H. pylori)-associated ulcers that affect the mucosa of the stomach. Amoxicillin+Clarithromycin were used in the studies of release because it inhibits the growth of H. pylori. This antibiotic combination is effective against H. pylori in the in vitro therapy in which low doses of the drug are required over the oral administration. The magetic

chitosan microspheres are a new architecture that shows a great potential for future tests in the treatment of gastric ulcers.

4. MATERIALS AND METHODOLOGY

4.1 MATERIALS USED

All the drugs and chemicals used are of analytical grade and the manufacturers for respective chemical s are listed below

Table 2: List of chemicals and their manufacture

Chemicals	Manufacturer or supplier name
Acetone	Krishna enterprise, Guwahati

Amoxicillin	Yarrow chem. Product, Mumbai
Clarithromycin	Yarrow chem. Product, Mumbai
Chitosan	B S Trading, Kolkata
Eudragit S100	B S Trading, Kolkata
Liquid paraffin	Krishna enterprise, Guwahati
Glacial acetic acid	Infinity solution
Gluteraldehyde	Infinity solution
Hexane	Krishna enterprise, Guwahati
Ferric oxide	Yarrow chem. Product, Mumbai
Ferric chloride	Yarrow chem. Product, Mumbai
Conc. HCL	Research-lab fine chem., Mumbai
Potassium chloride	Yarrow chem. Product, Mumbai

4.2 INSTRUMENTS USED:

All the instruments used in the practical works conducted throughout the project and the respective manufacturers are enlisted below.

Table 3: list of instrument and their manufacturers

Instruments	**Company name**

Digital weighing balance	Citizen
UV spectrophotometer	Shimadju, model no-UV 1800240v
Magnetic stirrer	Rolex
FT-IR	Bruker, Alpha E
Melting point apparatus	Macro Scientific Works 10A/UA, Delhi 007
DSC apparatus	Perkin Elmer, USA
Hot air oven	Bruker AXS D8 Advanced
USP Dissolution apparatus	LAB INDIA

4.3 METHODOLOGY

4.3.1 Analytical method development[1-6]

4.3.1.1. Linearity and Range

Linearity, consisting of the basic elements input → converter → output, is the assumption that there is a straight line relationship between the input and output variables that can be written mathematically by the expression if the straight line crosses through the origin or by the expression if the straight line does not cross through the origin. The linear range corresponds to the valid interval of functional dependence of the signal on concentration or mass which assumes homoscedasticity of the measurements over the linear range. The linear response of rutin and resveratrol was determined by analyzing the calibration curve in the range of 2-40 μg/mL and 2-20μg/ml.

4.3.1.2 Precision:

The term precision is defined by the ISO International Vocabulary of Basic and General Terms in Metrology (ISO-VIM) and ICH as the closeness of agreement between quantity values obtained by replicate measurements of a quantity under specified conditions. Assessing the precision implies expressing numerically the random error or the degree of dispersion of a set of individual measurements by means of the standard deviation, the variance, or the coefficient of variation.

4.3.1.3 Repeatability (Within-Run Precision):

It is the concordance of a series of measurements of the same quantity when the experiments are conducted under same conditions (analyst, apparatus, instrument, and day) in a rapid succession. For this experiment, standard solution of Amoxicillin and CLarithromycin (5 + 5μg/mL) was prepared and analyzed six times as per the proposed method.

4.3.1.4Intermediate Precision (Between-Run Precision):

It is the concordance of a series of measurements of the same quantity when the experiments are conducted within the same laboratory under different conditions (analyst, apparatus, instrument, and day). Standard solution of Amoxicillin and Clarithromycin (5 + 5 μg/mL) was prepared and analyzed as per the proposed method.

4.3.1.5Accuracy (%recovery):

The accuracy was tested by recovery experiments. Recovery studies were carried out at 100 % level by adding a known quantity of pure drug to the preanalyzed formulation and the proposed method was followed. From the amount of drug found, percentage recovery was calculated.

Process: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The recovery experiments were carried out in triplicate by spiking previously analyzed samples with three different concentrations of standards.

4.3.1.6 Ruggedness:

Ruggedness was determined by carrying out analysis by two different analyst and the respective percentage recovery was noted and the results was indicated as % RSD.

- **Limit of Detection (LOD) and Limit of Quantification (LOQ):**

The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value. The quantification limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ of the proposed method were determined by using calibration curve:

$$\mathrm{LOD} = \frac{3.3\sigma}{S}, \qquad \mathrm{LOQ} = \frac{10\sigma}{S},$$

Where σ is the standard deviation of the response (Y intercept) and S is the slope of the calibration curve.[11,16]

4.3.1.7 Specificity:

Specificity of the methods was achieved by the analysis of different laboratory prepared mixtures of Amoxicillin and Clarithromycin within the linearity range.

4.3.2 <u>Preformulation study of the drugs:</u>

The pure drug was characterized for its identity, purity amd organoleptic properties

4.3.2.1 Orgenoleptic Properties

The organoleptic properties of drug for color, odour , taste were evaluated.

4.3.2.2.Solubility study

A minute quantity of the drug was taken on a test tube and the solubility of the drug was determined by dissolving the drug in 10 ml of various polar solvents like water, acetone, methanol, ethanol, acidic buffer.[7]

4.3.2.3.Melting point

A little amount of the drug sample in a dry capillary tube of 1 mm internal diameter forming a column about 3mm high. Heat the melting point apparatus to a temperature 5-10 ^{0}C below the expected temperature of melting and adjust the heating so that the temperature in the chamber rises about 1^{0}C per minute.

4.3.2.4.Fourier Transform Infrared Spectroscopy(FTIR)

The FT-IR analysis of the pure drug, excipients, drug-excipients mixture were carried out with FT-IR instrument (Bruker 10059736).

4.3.2.5.Differential Scanning Caloriemetry(DSC)

DSC can be used to determine the nature and specification of crystallinity of drug and exipients through measurement of glass transition temperature and melting point temperature and their associated enthalpies. This technique has been used to study the physical and chemical interaction between drug and excipients. Required amount of drug, drug polymer mixtures were taken, then the samples were sealed in aluminium pans analysed in an atmosphere of air flow rate 25ml/min. A temperature range of 30 ^{0}C to 4000 ^{0}C was used where the rate of heating is 10 ^{0}C/min.[7,8]

4.3.2.6 Determination of absorption maxima of amoxicillin and clarithromycin in acidic buffer 1.2

10 mg each drug was weight accurately and dissolved in 10 ml of acidic buffer solution in volumetric flask(stock solution 1 mg/ml). The stock solution was diluted to make concentration of 10mg/ml. The spectrum of this solution was run in 400-800 nm range in UV-visible spectrophotometer.[7]

4.3.2.7. Preparation of standard calibration curve of amoxicillin and clarithromycinn in acidic buffer pH 1.2

50 mg each drug was weight accurately and dissolved in 50 ml of acidic buffer solution in volumetric flask(stock solution 1 mg/ml). The stock solution was diluted to make concentration of 2 ug/ml , 4 ug/ml, 6ug/ml, 8ug/ml, 10ug/ml, 12ug/ml, 16ug/ml, 20ug/ml. The absorbance of each solution measured at their respective absorption maxima using acidic buffer pH 1.2 as blank. The standard curve was obtained by plotting the observed absorbances with the respective concentration.[7]

4.3.2.8. Determination of Iso-absorptive point

An iso-absorptive point (a wavelength of equal absorptivity of the two components) was determined by taking overlain spectrum of the solutions amoxicillin and clarithromycin (10 µg/ml each) in acidic buffer pH1.2 in UV range against the solvent blank.

4.3.3. Preparation of cross linked chitosan microsphere

Weigh amount chitosan and 750 mg of drug was dissolved in 40 ml 2% acetic acid. The drug-polymer dispersion was added in a 120 ml liquid paraffin containing 1.5 ml span 80 and it was stirred with the help of megnetic stirrer, then GA (25% aqueous solution) was added and stirred continuously till 2 h. Suspension of chitosan microspheres in paraffin oil, thus obtained was allowed to stand for 15 min to let the microspheres settle down under gravity. Supernatant was decanted and filtered. Microspheres obtained as residue were washed four times with solvent n-hexane to remove traces of the oil. They were finally washed with water to remove excess GA. The microspheres were dried at 40°C for 24 h.[11,12]

Table 4: Formulation table of chitosan microparticle

Formulation	Drug polymer ratio	Cross linking agent (ml)
F1	1:1	5
F2	1:1	10
F3	1:2	5
F4	1:2	10
F5	1:3	5
F6	1:3	10

4.3.4. Characterization of cross linked chitosan microspheres

4.3.4.1 Determination of percentage yield of microspheres

Thoroughly dried microsparticle were collected and weighed accurately. The percentage yield was calculated using formula.[13,14,15]

Percentage yield = (Practical yield/Theoretical yield) x 100.

4.3.4.2 In vitro drug release

A total of 100 mg equivalent chitosan microparticle were weighed and filled in the empty capsule shells. Dissolution tests were performed in a USP Dissolution Tester Apparatus II 4Paddle method) at 37 ± 0.5°C. The paddles were rotated at a speed of 50 rpm. The dissolution medium consisted of acidic buffer pH 1.2 (900 ml). Aliquots of 5 ml were withdrawn at different time intervals, filtered through Whatman filter paper and the content of drugs were determined spectrophotometricaly by simultaneous estimation method using ultraviolet (UV) spectrophotometer.[12,13,14]

4.3.5 Preparation of magnetite particles

A round bottom flask was charged with 8.9 g (0.1 mol) of FeO, 9.94 g (0.05 mol) of $FeCl_2 \cdot 4H_2O$ along with 250 mL deionized water and then 50 mL of 2 M NaOH was added while stirring vigorously. The reaction mixture was heated to reflux for 1-2 h. During the transformation of the pH, its pH fell from 14 to orange 8-9 and a black precipitate was formed. After precipitation was completed, the Fe_3O_4 particles were washed with distilled water, filtered and dried under vacuum at room.[17]

4.3.6. Formulation of chitosan magnetic microparticle

Drug loaded chitosan microparticles and magnetite particles were dispersed in 20 ml of 5% coating solution containing eudragit RL100 & S100 (1:2 ratio) in ethanol and the mixture was agitated for about 5 min at 500 rpm. 50 ml of n-hexane was added at a rate of 1 ml/min kept on stirring. The magnetitite coated chitosan microparticles were washed with n-hexane for 2-3 times and dried at room temperature.

Table 5

Batch specification for chitosan magnetic microparticles

Formulation code	% of Coating w/v	Coating mixture (RL100:S100+magnetite)	Core (Chitosan microparticle)
FM1	5%	1:1	4:1
FM2	5%	2:1	4:1
FM3	5%	3:1	4:1

4.3.7. Evaluation of chitosan magnetic microparticle

4.3.7.1 Particle size analysis

Particle size analysis of drug-loaded chitosan microsparticle was performed by optical microscopy using a compound microscope. A small amount of dry microspheres was suspended in purified water (10 ml). The suspension was ultrasonicated for 5 s. A small drop of suspension thus obtained was placed on a clean/ glass slide. The slide containing chitosan microspheres was mounted on the stage of the microscope and diameter of at least 300 particles was measured using a calibrated ocular micrometer

4.3.7.2 Percentage entrapment efficiency

A total of 25 mg microparticle were crushed and dispersed in 100 ml acidic buffer pH 1.2 and sonicated for 20 min. Dispersion was stirred on magnetic stirrer for 6 h. The dispersion was filtered and drug content was analyzed spectrophotometrically. The percentage drug entrapment efficiency was calculated using following equation:

$$\% \text{ Entrapment Efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

4.3.7.3. Flow properties

Where, ρt =Tapped density, ρb = bulk density

Angle of repose is determined by using funnel method. The accurately weighed microspheres are taken in a funnel and then height of funnel is adjusted in such as way that the tip of funnel just touches the apex of heap of blends. The blends are allowed to flow through funnel freely on to surface. The diameter of powder cone is measured and angle of repose is calculated by using following equation:

$\tan\theta = h/r$; Where θ - Angle of repose, h -height of pile, r - Radius of base.

4.3.7.4 Surface topography by scanning electron microscopy (SEM)

SEM of the microspheres shows the surface morphology of the microspheres like their shape and size.

4.3.7.5. **In vitro drug release study**

A total of 100 mg equivalent chitosan magnetic microparticle were weighed and filled in the empty capsule shells. Dissolution tests were performed in a USP Dissolution Tester Apparatus II (Paddle method) at 37 ± 0.5°C. The paddles were rotated at a speed of 50 rpm. The dissolution medium consisted of acidic buffer pH 1.2 (900 ml). Aliquots of 5 ml were withdrawn at different time intervals, filtered through Whatman filter paper and the content of drugs were determined spectrophotometricaly by simultaneous estimation method using ultraviolet (UV) spectrophotometer.

4.3.7.6 Stability studies

By placing the microspheres in screw capped glass container and stored them at following conditions: 1. Ambient humid condition; 2. Room temperature (27); 3. Oven temperature (40 ±2) ^{0}C; 4. Refrigerator (50-80 ^{0}C). It is carried out for 60 d and the drug content of the microsphere is analyzed.[17]

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5. RESULTS AND DISCUSSIONS

5.1 Analytical Method validation:

The solutions of 10μg/mL of amoxicillin and clarithromycin were analyzed and the λmax was found to be 227 nm and 202 nm, respectively. The isoabsorptive point 203 nm was found in overlaying spectra (Fig-). The calibration curve of amoxicillin and clarithromycin individually at 227 nm and 202 nm were plotted. The relationship between the absorbance and the concentration of amoxicillin and clarithromycin was found to be linear in the range of 2-40 µg/ml at wavelengths 227 nm and in the range of 2-20 µg/ml at wavelength 202 nm (table-2,fig-5)respectively. The representative linear equations and correlation coefficients have indicated very good linearity. Evaluation of repeatability, intermediate precision, accuracy, ruggedness, specificity done and percent relative standard deviation (%RSD) values were calculated. These values were found to be less than two (%RSD< 2), indicating \
good precision.

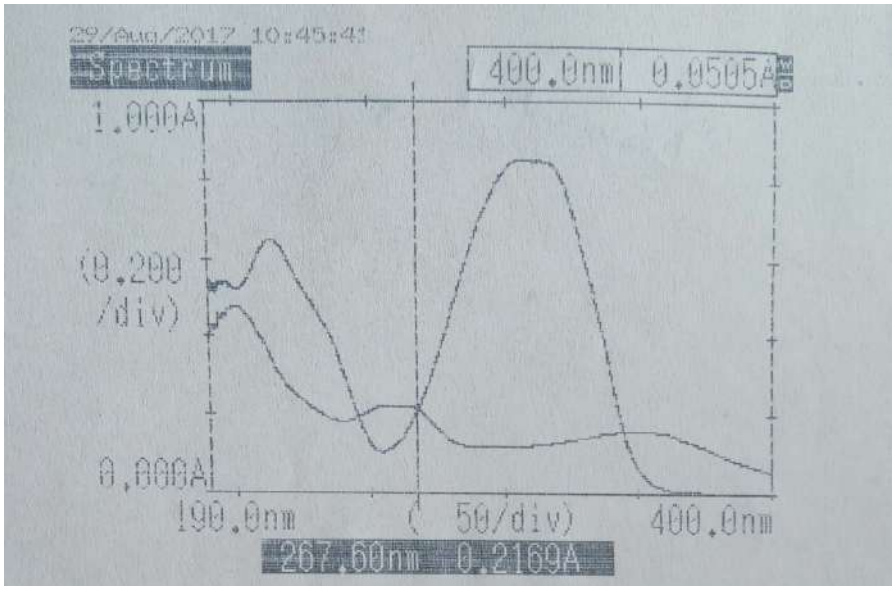

Fig 3: Determination of iso apsorptive point.

Table -6: Standard calibration curve of amoxicillin at 227nm

Serial no	Concentration(μg/ml)	Absorbance	%RSD
1	2	0.037	0.0112
2	4	0.0645	0.0131
3	6	0.0977	0.0146
4	8	0.1249	0.0211
5	10	0.1609	0.0213
6	12	0.1902	0.0134
7	16	0.2547	0.0764
8	20	0.3200	0.0478
9	40	0.6500	0.0853

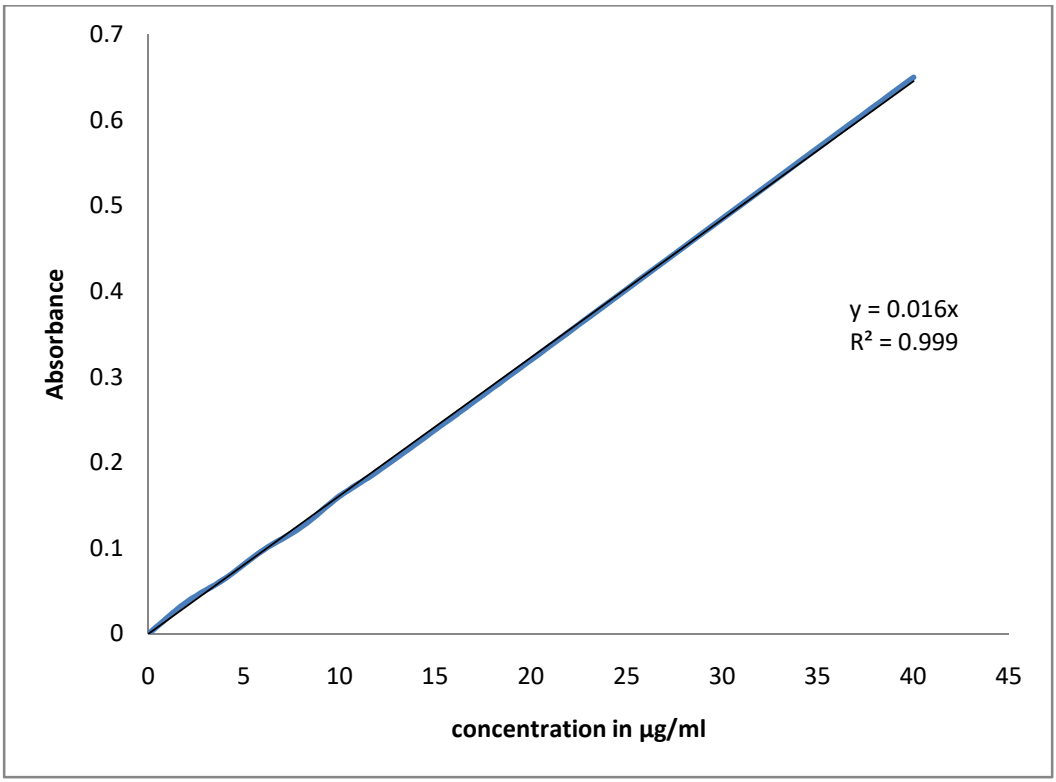

Fig 4: Calibration curve of amoxicillin at 227 nm

Table -7: Standard calibration curve of amoxicillin at 203nm.

Serial no	Concentration(µg/ml)	Absorbance	%RSD
1	2	0.0599	0.011
2	4	0.1323	0.0154
3	6	0.1463	0.0189
4	8	0.1873	0.0112
5	10	0.2502	0.0073
6	12	0.2937	0.0136
7	16	0.4017	0.0318
8	20	0.5106	0.0275

9	40	0.9782	0.0132

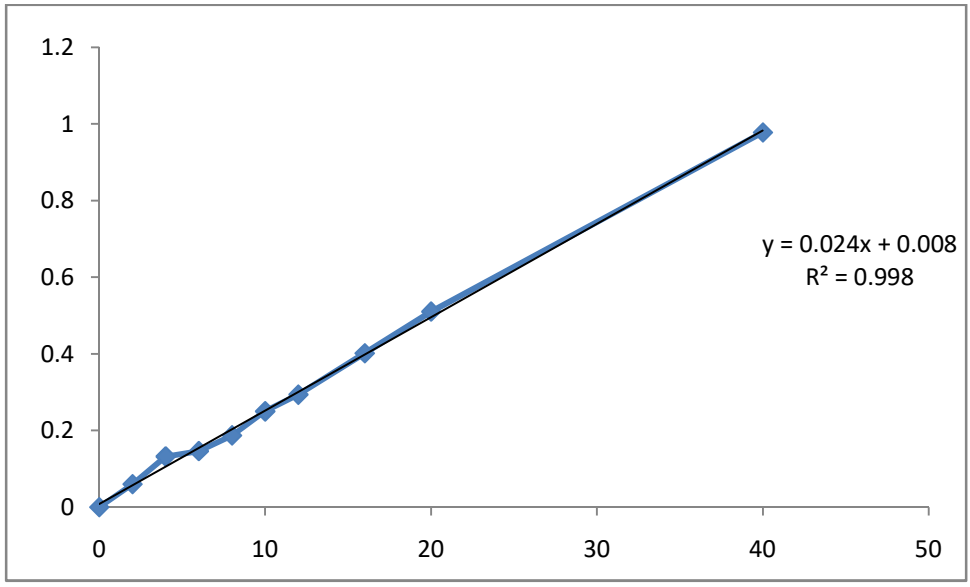

Fig 5: Calibration curve of amoxicillin at 203 nm

Table -8: Standard calibration curve of clarithromycin at 202 nm.

Serial no	Concentration(µg/ml)	Absorbance	%RSD
1	2	0.031	0.0149
2	4	0.0511	0.0231
3	6	0.0711	0.0064
4	8	0.1011	0.0370
5	10	0.122	0.0064

6	12	0.1434	0.0135
7	16	0.1811	0.0243
8	20	0.2298	0.0165

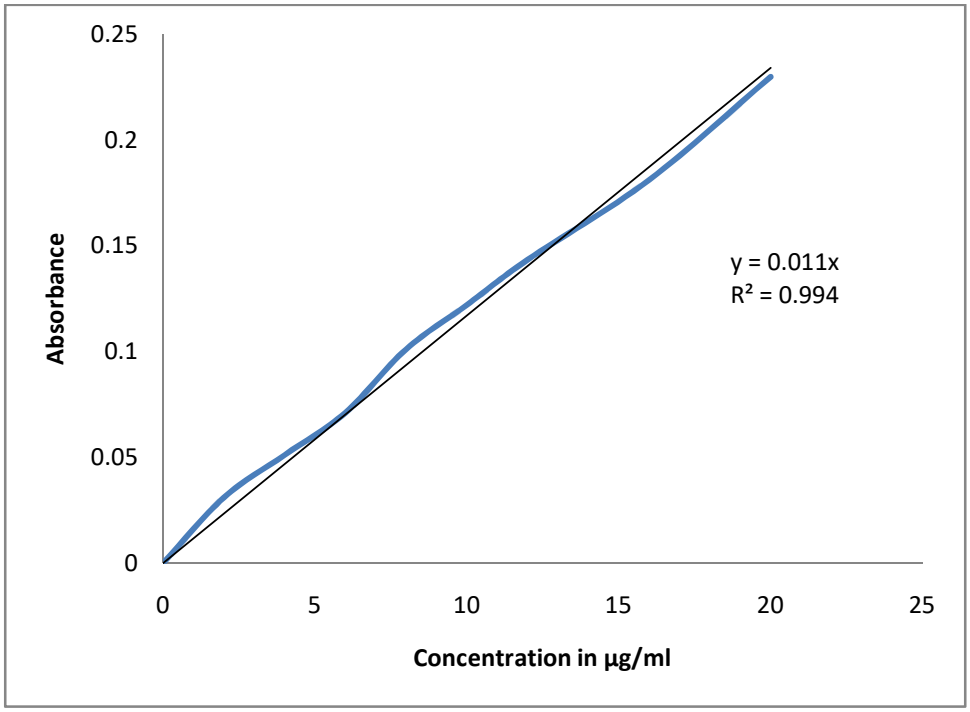

Fig 6: Calibration curve of clarithromycin at 202 nm

Table -9: Standard calibration curve of clarithromycin at 203 nm.

Serial no	Concentration(µg/ml)	Absorbance	%RSD
1	2	0.0317	0.0147
2	4	0.049	0.0263

3	6	0.068	0.0113
4	8	0.0928	0.0254
5	10	0.1244	0.0156
6	12	0.1514	0.0165
7	16	0.1734	0.0211
8	20	0.2029	0.0143

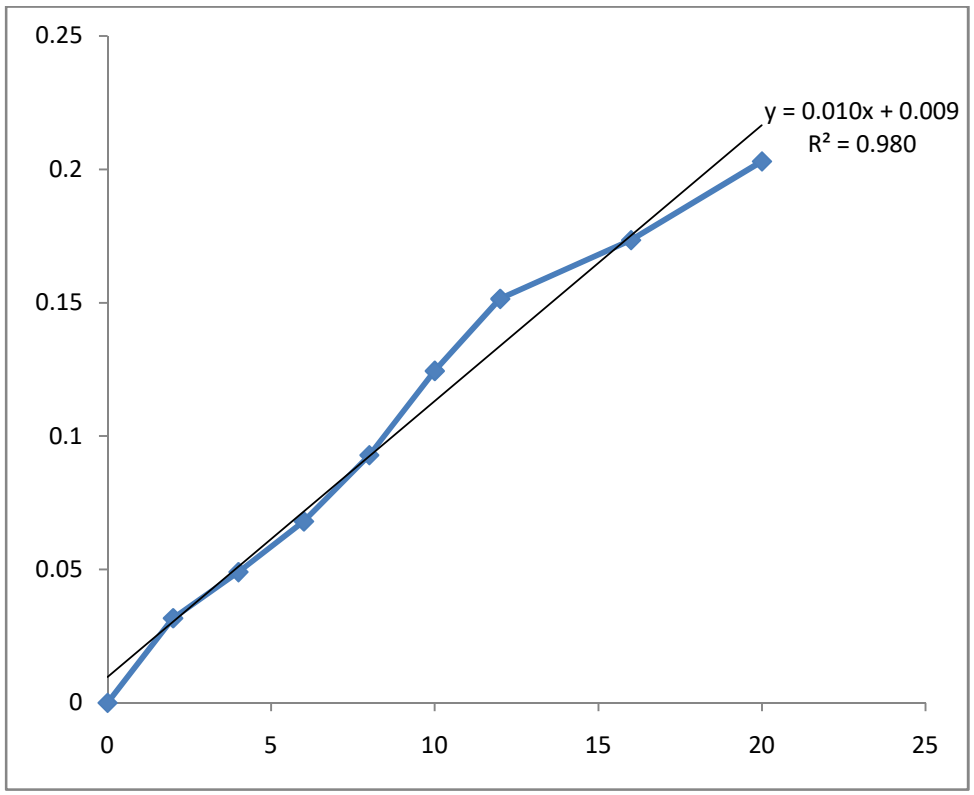

Fig 7: calibration curve of resveratrol at 203 nm

Precision

Table-10: Repeatability and Intermediate Precision study.

Serial no	Precision	% recovery of amoxicillin	% RSD	% recovery of clarithromycin	% RSD
1	Repetability	100.946	0.830	100.762	1.270
2	Intermediate precision (day1-day6)	104.289	0.535	101.979	0.617

Accuracy

Table -11: Results of recovery studies of amoxicillin and clarithromycin.

Serial no	Amount of mixture taken	Amount of amoxicillin added	% recovery	%RSD
1	5 µg/ml	1 µg/ml	103.166	0.686
2	5 µg/ml	2 µg/ml	103.745	0.262
3	5 µg/ml	3 µg/ml	104.493	0.592

Serial no	Amount of mixture taken	Amount of clarithromycin added	% recovery	%RSD
1	5 µg/ml	1 µg/ml	110.498	1.915
2	5 µg/ml	2 µg/ml	106.587	1.299
3	5 µg/ml	3 µg/ml	104.054	0.414

Ruggedness

Ruggedness was determined by carrying out analysis by two different analyst and the respective percentage recovery was noted and the results were indicated as % RSD .

Table-12: Results of Ruggedness of amoxicillin and clarithromycin.

Serial no		% recovery of amoxicillin	%RSD	% recovery of clarithromycin	%RSD
1	Analyst 1	105.027	0.712	100.75	1.270
2	Analyst 2	107.035	0.614	102.981	1.991

Table -13: Limit of Detection (LOD) and Limit of Quantification (LOQ).

Serial no	Name of the drug	227 nm	227 nm	202 nm	202nm	203 nm	203 nm
		LOD	LOQ	LOD	LOQ	LOD	LOQ
1	amoxicillin	0.331	0.109	-	-	0.080	0.243
2	clarithromycin	-	-	0.028	0.089	0.133	0.404

Specificity

Specificity of the methods was achieved by the analysis of different laboratory prepared mixtures of amoxicillin and clarithromycin within the linearity range.

Table-14 :Specificity of different laboratory prepared mixture.

Serial no	Ratio Rutin: Resveratrol	% recovery of rutin	%RSD	% recovery of resveratrol	%RSD
1	2:1	104.231	0.619	97.014	2.37
2	1:2	103.	0.291	110.846	0.454

5.2. Preformulation study

5.2.1 Organoleptic properties

The organoleptic properties like colour, odour, taste were observed and the results were compared with the official requirement and found to be acceptable. The results are shown in Table 15

5.2.2 Solubility study

The solubility was checked in common solvents depending on their polarity such as ethanol, water, methanol, acetone, acidic buffer pH 1.2. The results are shown in table 16

5.2.3 Melting point determination

The melting point was determined by using the melting point apparatus with the help of a capillary tube. The melting point of amoxicillin and clarithromycin were found to be 118 ^{0}C and 224^{0}C. The melting range was compared with the official requirement and is found to be acceptable.

5.2.4 Determination of absorption maxima for the drugs in acidic pH 1.2

The spectrum of the amoxicillin and clarithromycin solution was run in 400-800 nm range in UV-visible spectrometer. The maximum absorption maxima were found to be 227nm and 202 nm repectively.

5.2.5 Preparation of standard calibration curve of Amoxicillin and Clarithromycin in Acidic buffer pH 1.2

The standard curve was obtained by plotting the observed absorbance with the respective concentrations. The absorbance increases with the increase of concentrations and the curves obtained were linear with the equation y= 0.016x & y=0.011x and R^2 value were found to be 0.999 & 0.994 for Amoxicillin & Clarithromycin respectively.

TABLE 15: Organoleptic properties of drugs

Sample	Taste	Colour	Odour
Amoxicillin	Bitter	White	Penicillin type
Clarithromycin	Bitter	White	Odorless

TABLE 16: Solubility of drugs

Solvent	Amoxiciilin	Clarithromycin
Water	Freely soluble	Insoluble
Ethanol	Sparingly soluble	Slightly soluble
Acetone	Slightly soluble	Freely soluble
Acidic Buffer pH1.2	Freely soluble	Soluble

TABLE 17: Melting point of drugs

Drug	Melting point
Amoxicillin	188^0C
Clarithromycin	224^0C

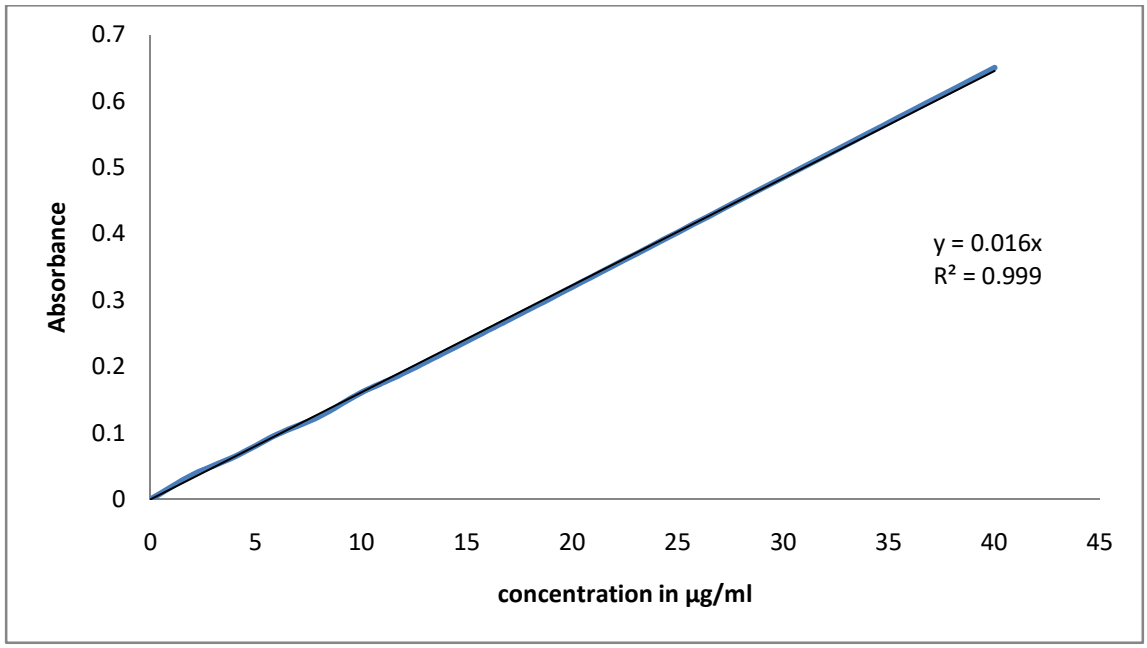

Fig 8: Standard curve of Amoxicillin in Acidic buffer pH 1.2

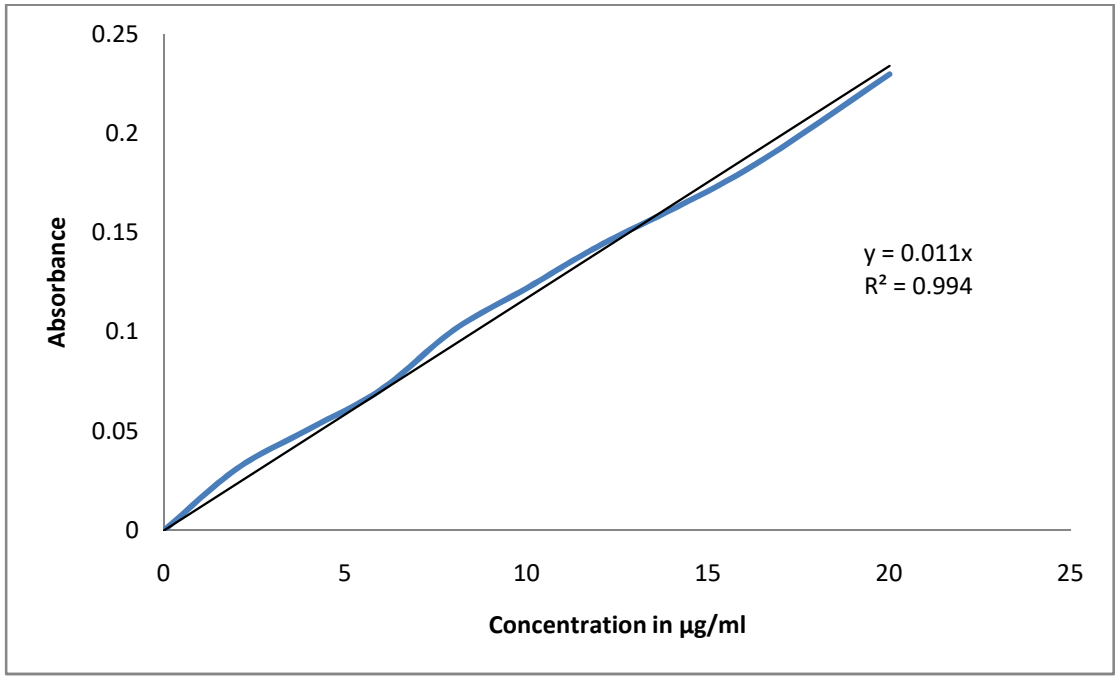

Fig 9: Standard curve of clarithromycin in acidic buffer pH 1.2

5.2.6 Drug –excipients compatibility study

The compatibility study was performed by FT-IR and DSC

FT-IR study

IR spectral analysis of amoxicillin shows peaks at 1770 cm^{-1} (c=o str), 3357 cm^{-1},3443 cm^{-1}(N-H str), 3564 cm^{-1}(O-H str), 3154 cm^{-1}(N-H str, secondary amide) confirming the purity of amoxicillin . IR spectrum of clarithromycin shows peak at 1068 cm^{-1},1106 cm^{-1}(C-O-C str), 1455 cm^{-1}(C-H_2 str),2875 cm^{-1}, 2937 cm^{-1}(C-H str),3465 cm^{-1}(O-H str) confirm the purity of the clarithromycin. In the IR spectrum of the drug and polymer physical mixture showed the peaks of pure drugs as well as the excipients with some variation in the same range indicating no interactuion.

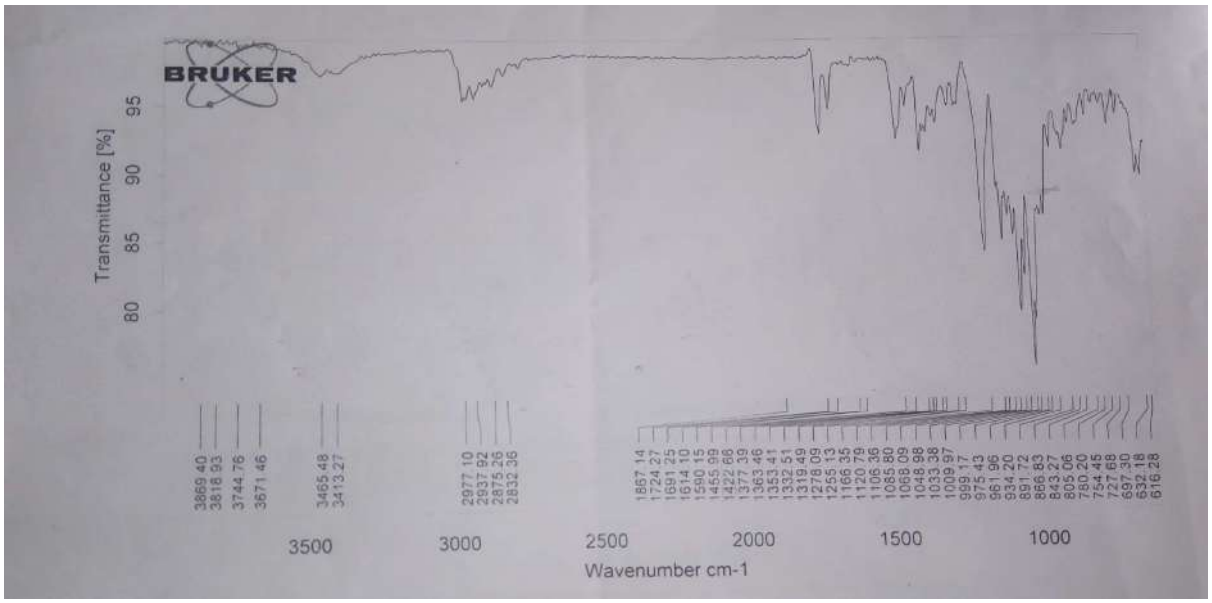

Fig 10: FT-IR of Clarithromycin

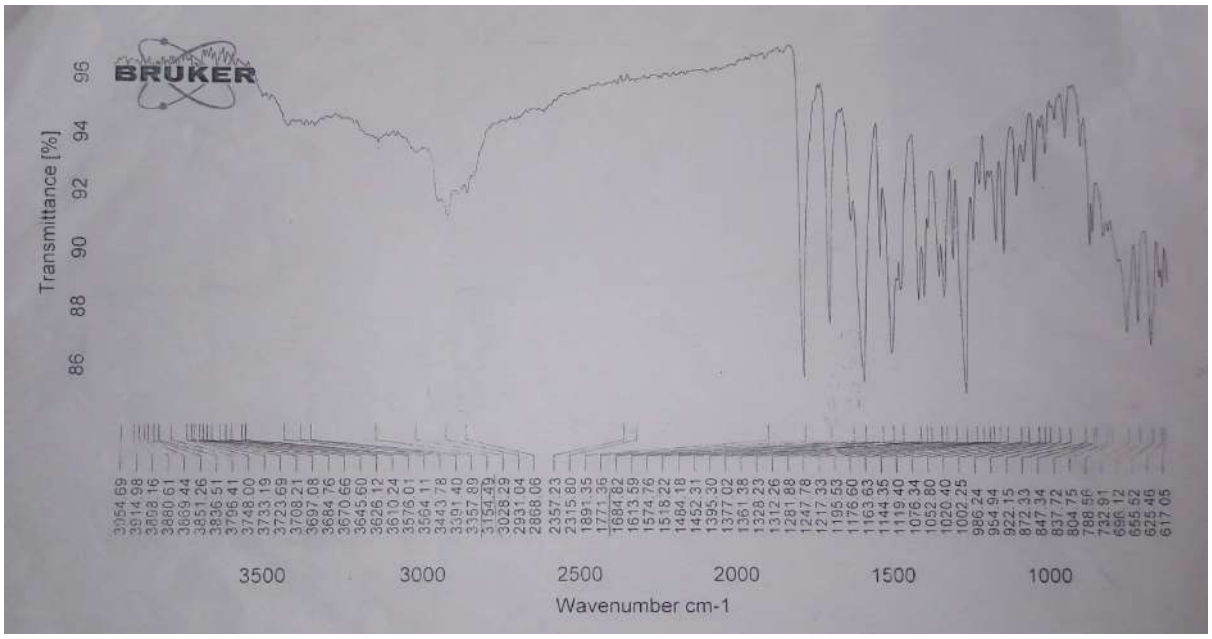

Fig 11: FT-IR of Amoxicillin

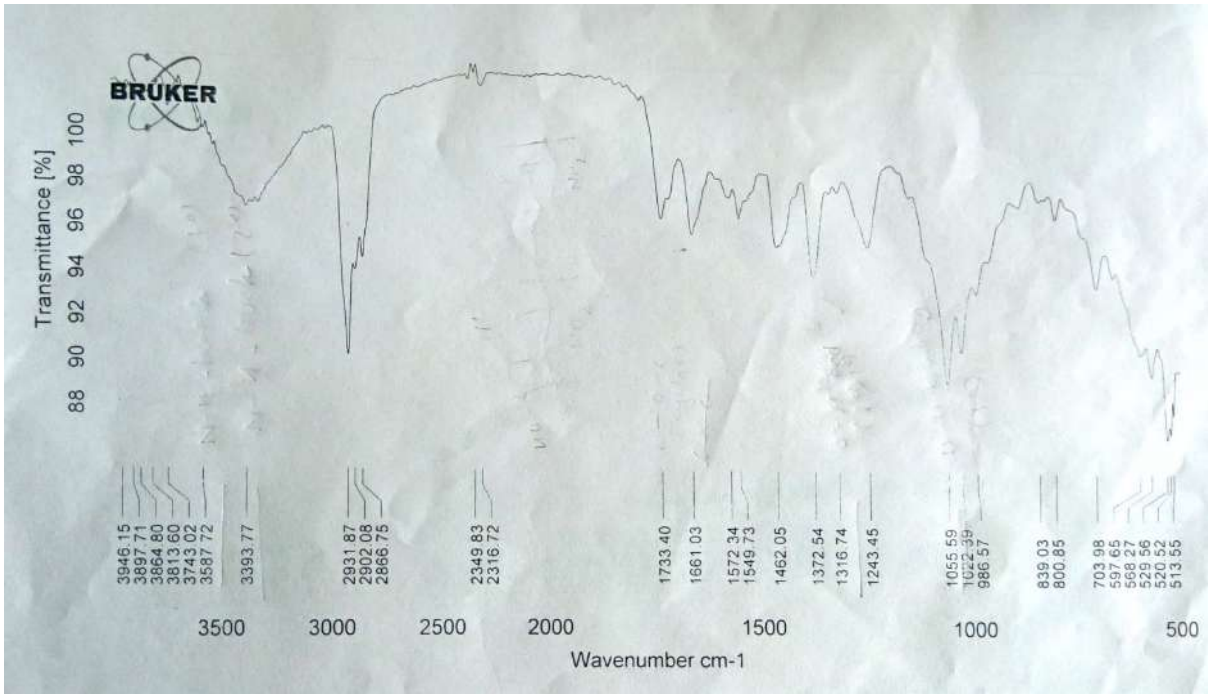

Fig 12: FT-IR of Chitosan

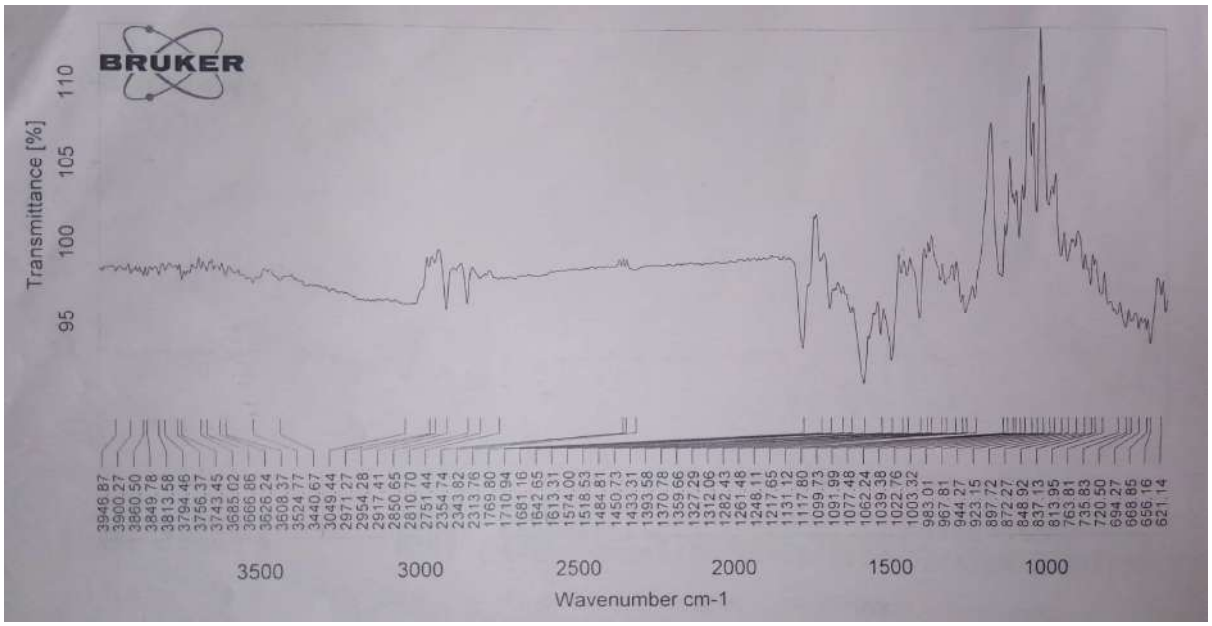

Fig 13: FT-IR of drugs and polymer mixture

DSC STUDY

The DSC thermogram of Amoxicillin and clarithromycin showed endothermic peak at 93.71 ^{0}C and 229.01^{0}C with onset of 188.38^{0}C and 224.17^{0}C which is attributed to its melting point. The physical mixture of drugs and chitosan showed endothermic peak at 224.21 ^{0}C and onset 216.94 ^{0}C. There is no significant deviation noticed indicating negative interaction between the drugs and polymer.

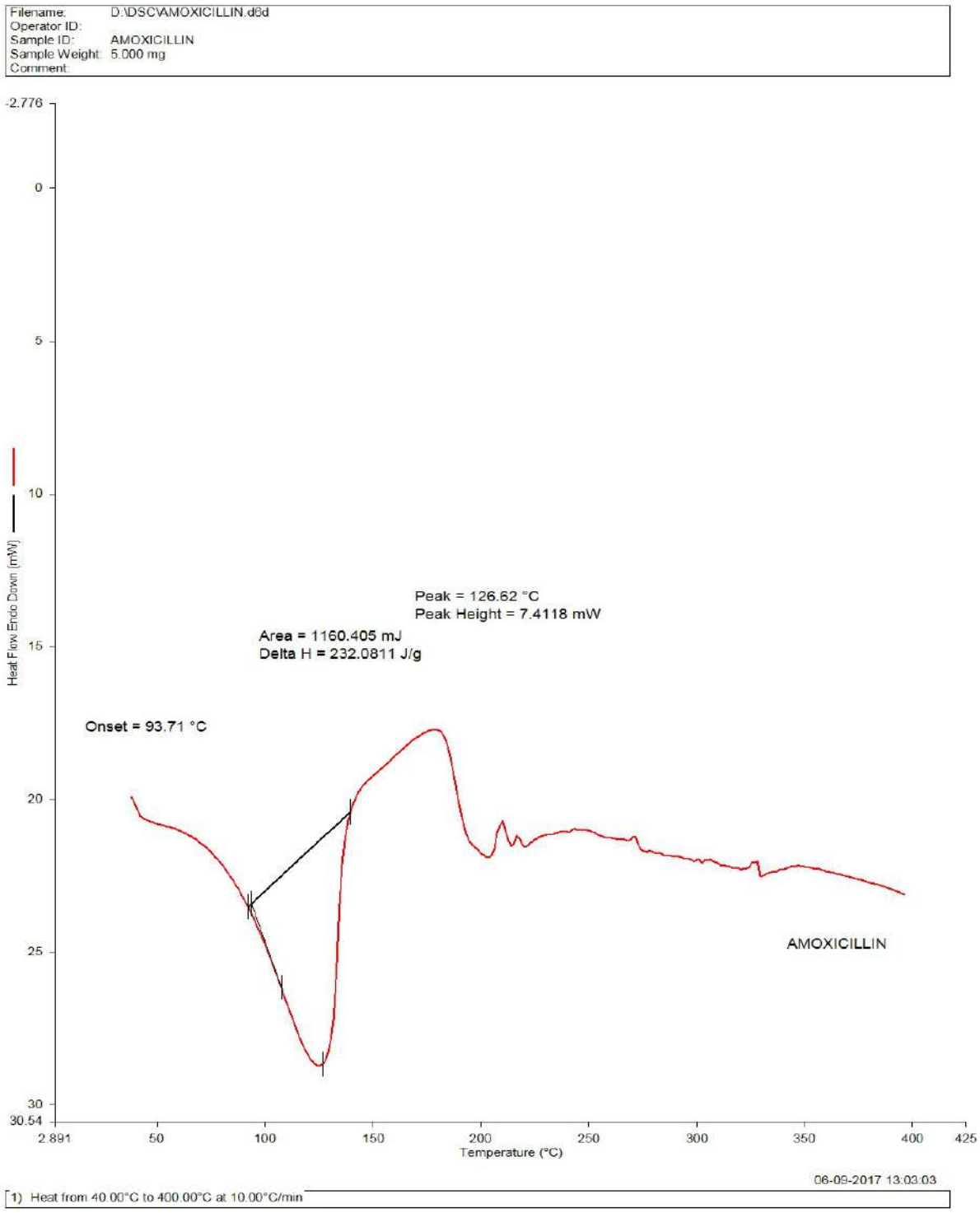

FIG 14: DSC THERMOGRAM OF AMOXICILLIN

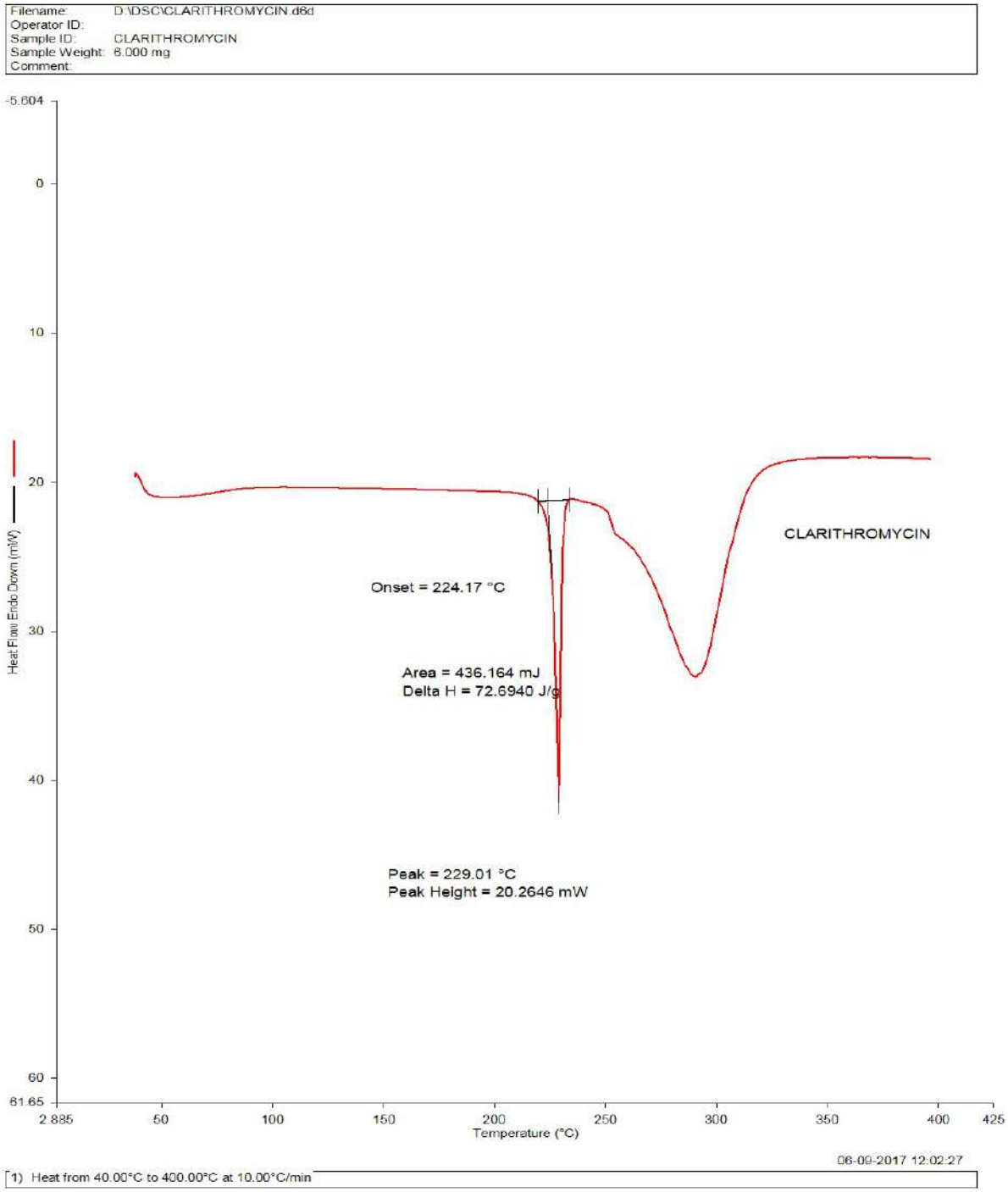

FIG 15: DSC THERMOGRAM OF CLARITHROMYCIN

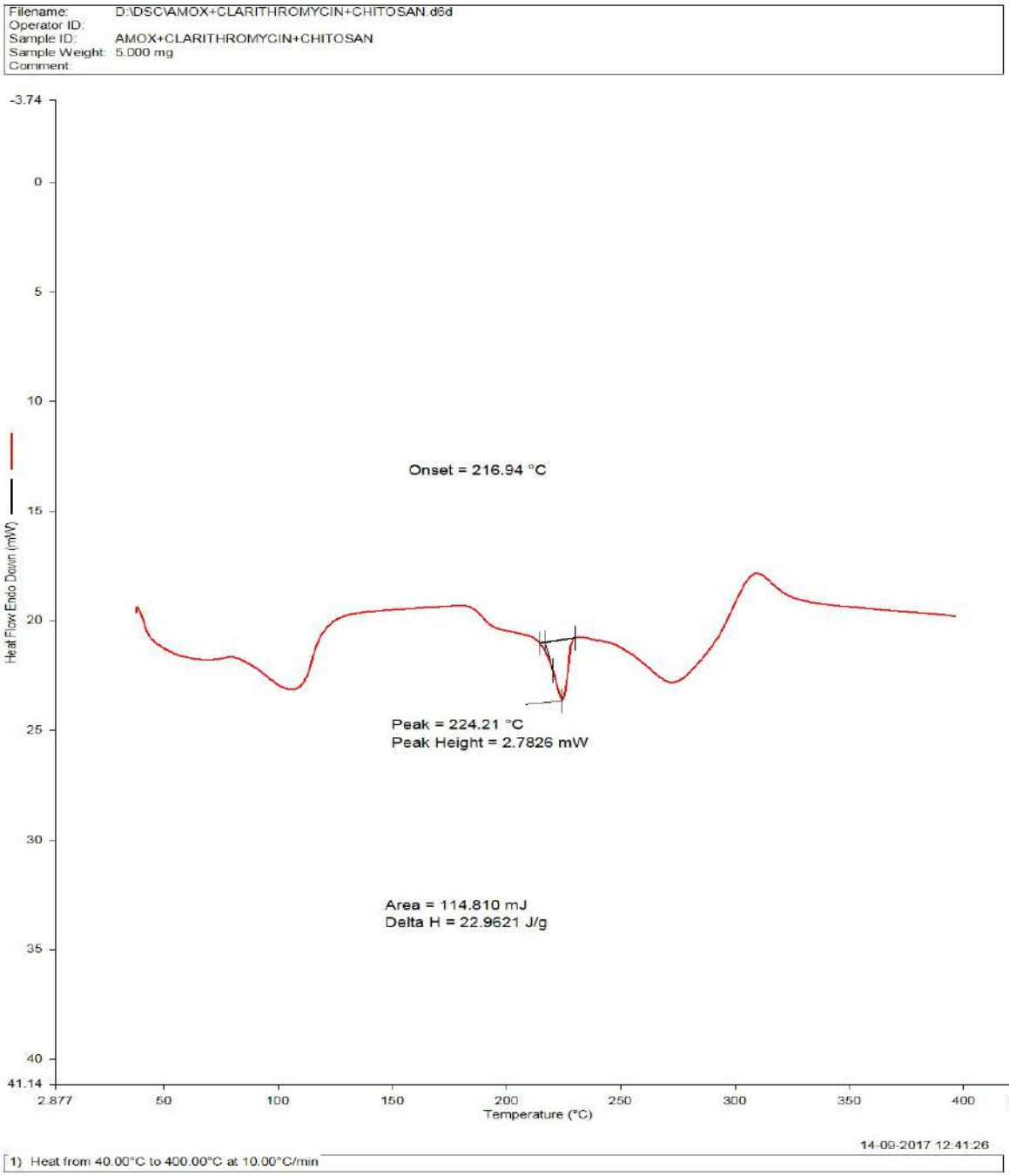

FIG 16: DSC THERMOGRAM OF AMOXICILLIN+ CLARITHROMYCIN+ CHITOSAN

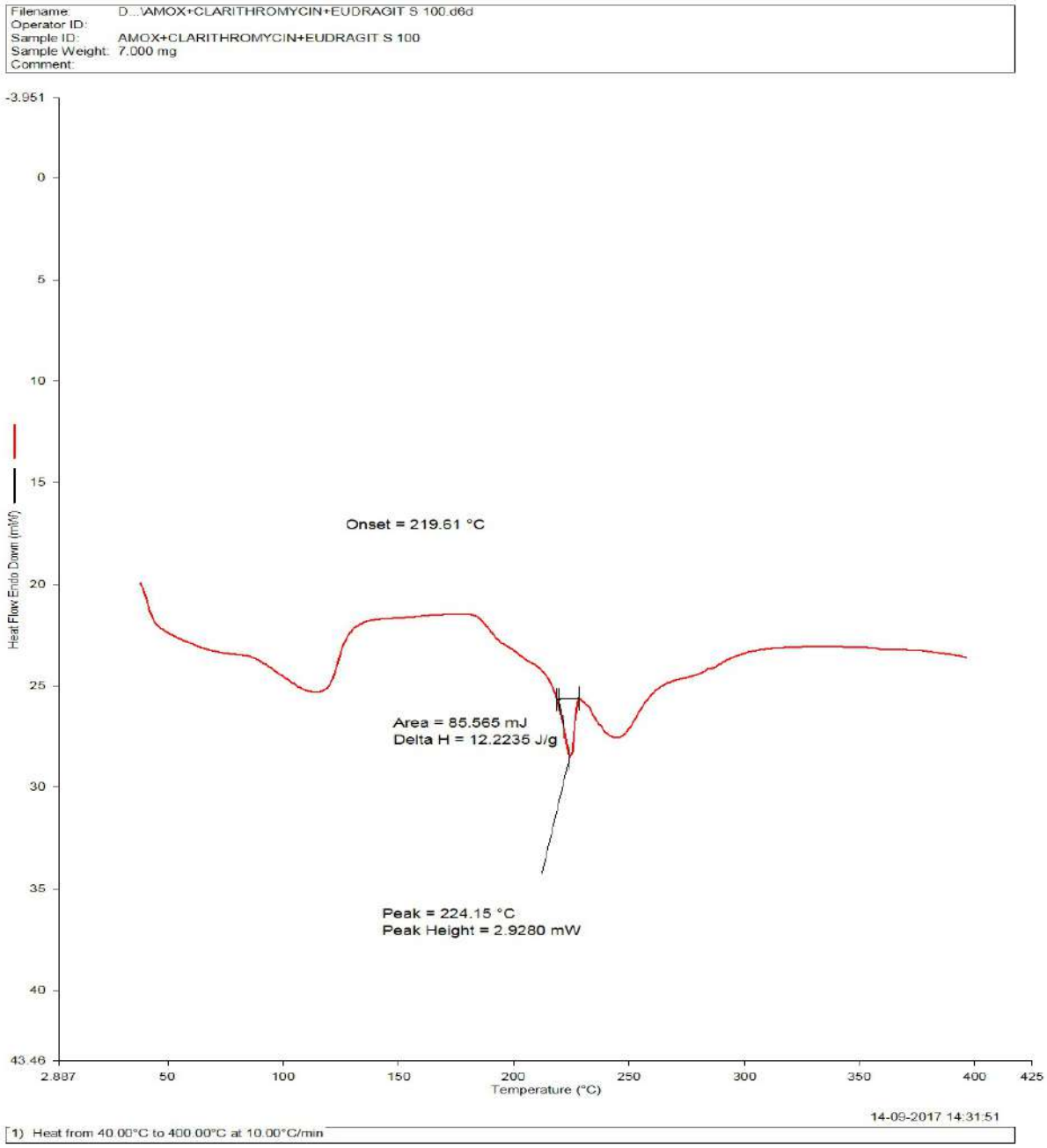

FIG 17: DSC THERMOGRAM OF AMOXICILLIN+ CLARITHROMYCIN+ EUDRAGIT S100+RL 100

5.3 EVALUATION OF CROSS LINKED CHITOSAN MICROPARTICLE

5.3.1Percent % yield:

The yield of the microparticle prepared by cross linking method was between 71-93.3%. The percentage yield increases with drug polymer ratio.

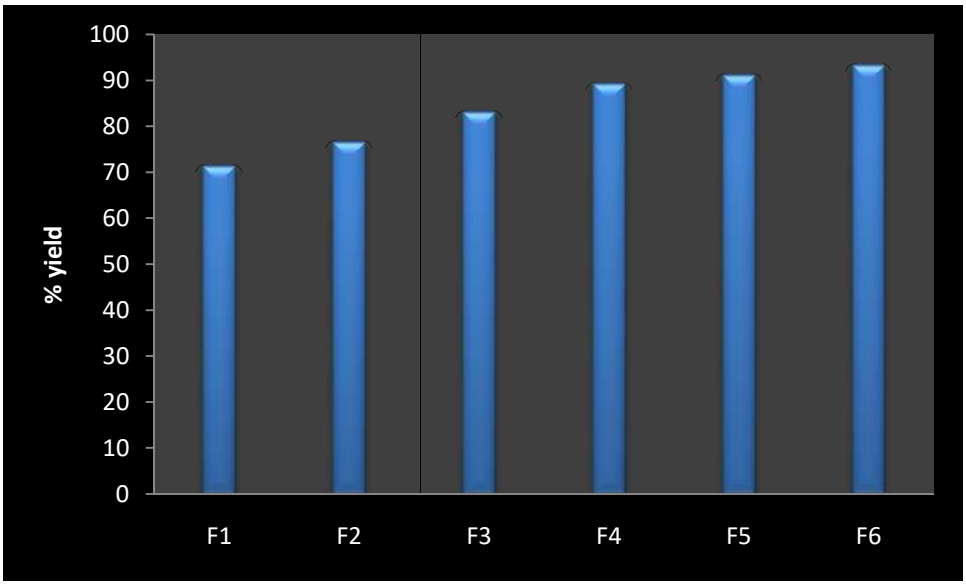

Fig 18 : Comparision of % yield of the formulations

5.3.2Drug entrapment efficiency

Drug:polymer ratio and volume of GA had positive effect on entrapment of drug.

Effect of drug:polymer ratio: Entrapment of drug was increased with increasing in drug:polymer ratio. It was occurred due to the increased in viscosity of aqueous phase with increasing the polymer concentration that stabilize droplets and which prevent out flow of drug during the hardening phase.

Effect of GA: Here % entrapment was increased with increasing the volume of GA. It can be explained by the higher degree of crosslinking occurred by higher concentration of GA. Increase

in amount of GA produces much denser matrix due to increased crosslinking with chitosan that reduces the outflow of drug during stirring and increases the encapsulation efficiency.

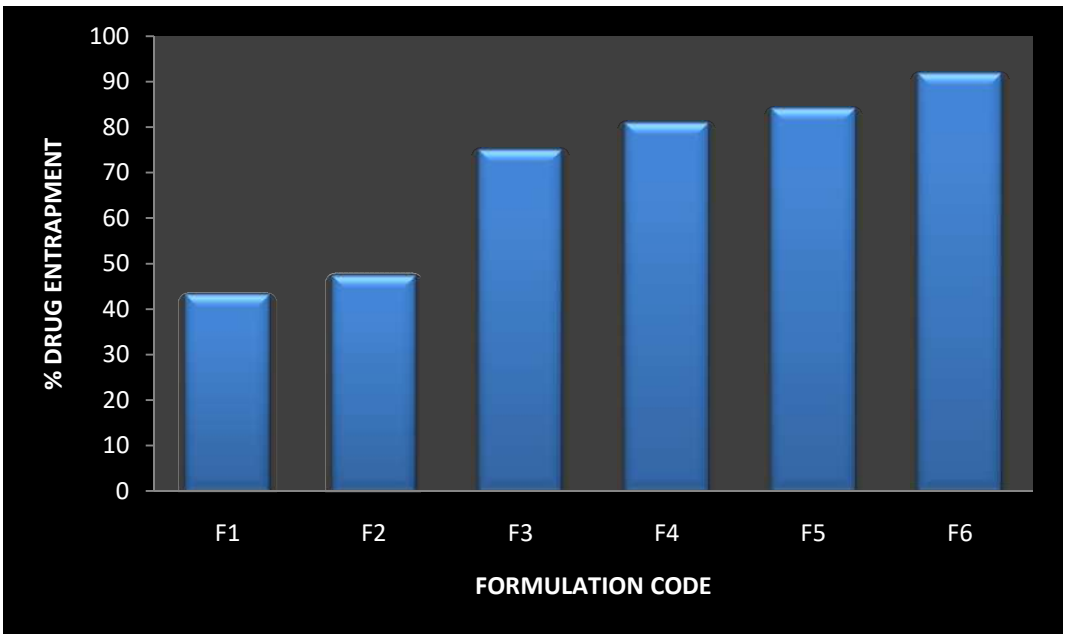

Fig 19:Comparision of % drug entrapment of formulations

TABLE 18 : % yield, % entrapment of the prepared microparticle

Formulation	% yield	%drug entrapment efficiency
F1	71.3	43.07
F2	76.6	47.4
F3	83	74.9
F4	89.2	81
F5	91	84
F6	93.3	91.8

5.3.3 In vitro Drug release Studies

The in vitro drug release study from the microspheres was studied for the 4 formulation F3, F4, F5 ,F6. The % cumulative release are shown in the table below. Here sustain release of drug was observed from the formulation in acidic buffer pH 1.2 for duration of 24 hours. Here increased in concentration of polymer in formulation drug release was sustained for longer period. Among

all thr formulations, F6 formulation showed better sustained release profile of the drugs for a period of 12 hours.

TABLE 19: In vitro drug release of chitosan microparticle

Time (hours)	% cumulative drug release			
	F3	F4	F5	F6
0.5	13.33	12.34	10.87	10.55
1	19.11	18.7	16.9	19.45
2	27.03	25	23.33	25.76
3	36.11	34.6	31.58	29.5
4	43	41.09	39.71	36.6
5	55.87	49.67	48.47	42.21
6	64.34	59.7	56.11	55.4
12	79.3	81.45	73.94	64.32

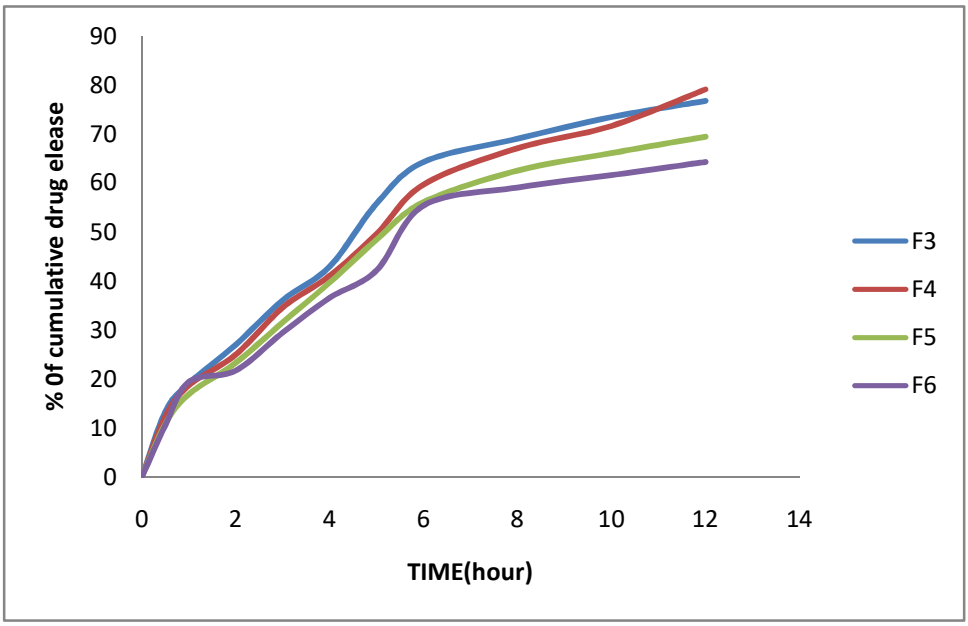

Fig 20: % cumulative release curve

5.3.4 Batch selection for formulation of magnetic microparticle

All the 6 batches were evaluated for % yield, % drug entrapment, % cumulative release. Among the batches F6 formulation shows better yield, entrapment efficiency and good sustained release behavior. This batch hence selected for coating along with magnetite to finaly formulate the chitosan magnetic microparticle.

5.4 Evaluation of chitosan magnetic microparticle

5.4.1 Particle size determination The prepared cross linked microparticle were predominantly round in shape with a mean

Table 20: Particle size of chitosan magnetic microparticle

Formulation	Eudragit RL100:S100	Avg. Particle size (µm)
FM1	1:1	134±1.6
FM2	2:1	188±.87
FM3	3:1	179±1.1

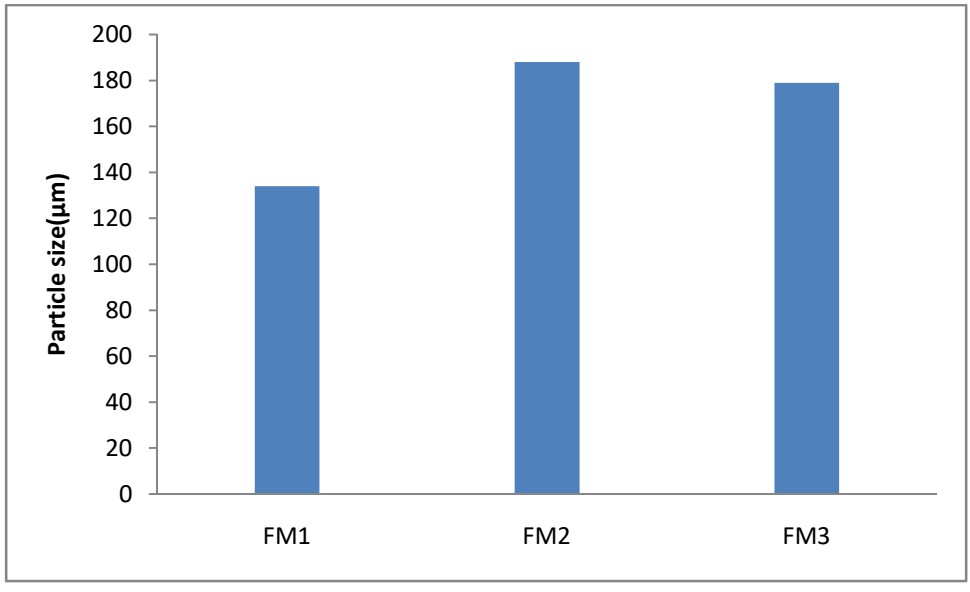

Fig 21: Particle size comparision

5.4.2 Flow properties of chitosan magnetic microparticle

Table 21: Flow properties of magnetic microspheres (as per USP30-NF25 specifications)

Formulation	FM1	FM2	FM3
Bulk density	o.61±0.22	0.70±0.64	0.68±0.3
Tapped density	0.70±.55	0.76±0.32	0.75±0.23
Hausner ratio	1.14	1.08	1.10
Carr'S Index(%)	12.23	11.34	9.33
Angle of repose	32.20	24.01	29.74

All the formulation shows good flow properties. Carr's index ranges from 9.33 to 12.23 %.

5.4.3 Surface characterization of chitosan magnetic microparticle

SEM images indicate that the microparticles are of spherical in shape with moderate rough surfaces,clumps were also present. SEM photographs revealed the absence of crystals of drug on the surface of the micrshperes, indicating uniform distribution of drugs on the surface of the microspheres.

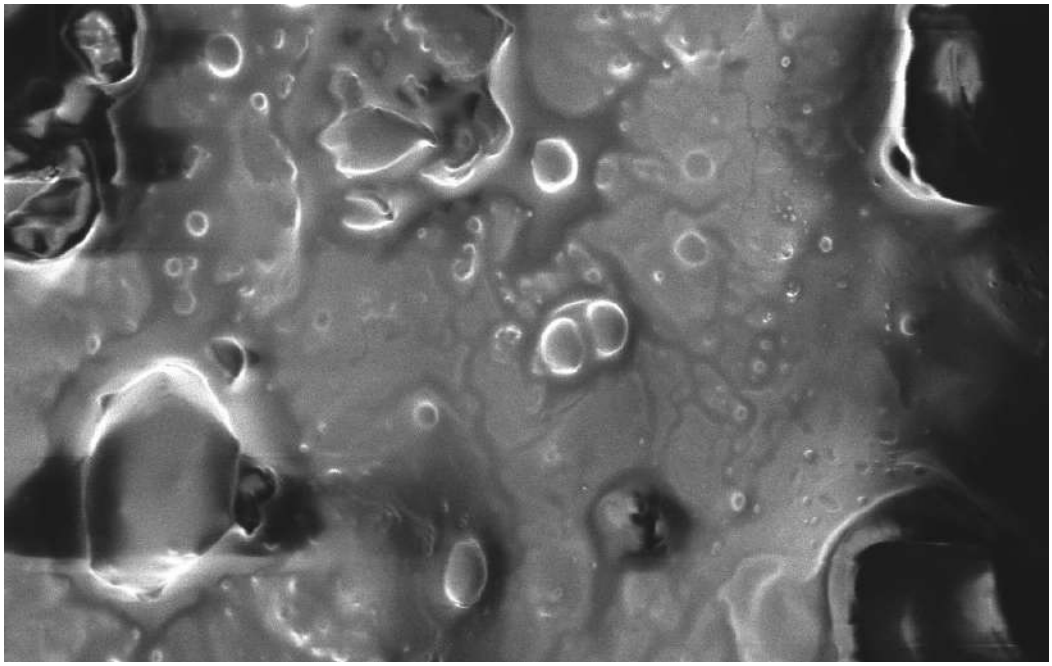

Fig 22: SEM image of FM1 formulation

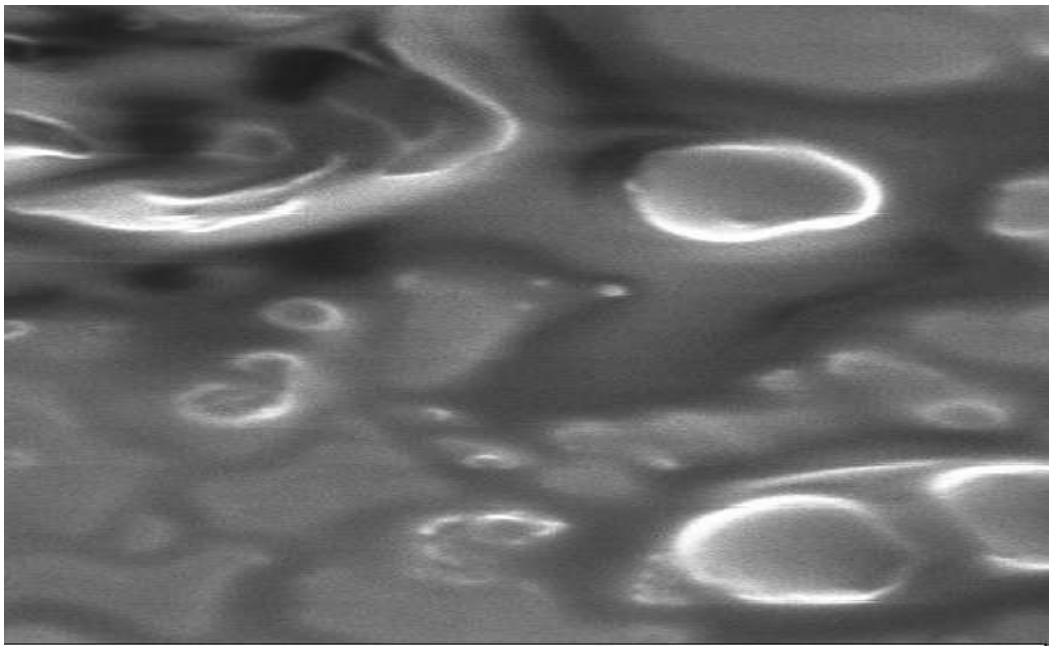

Fig23: SEM image of FM2 formulation

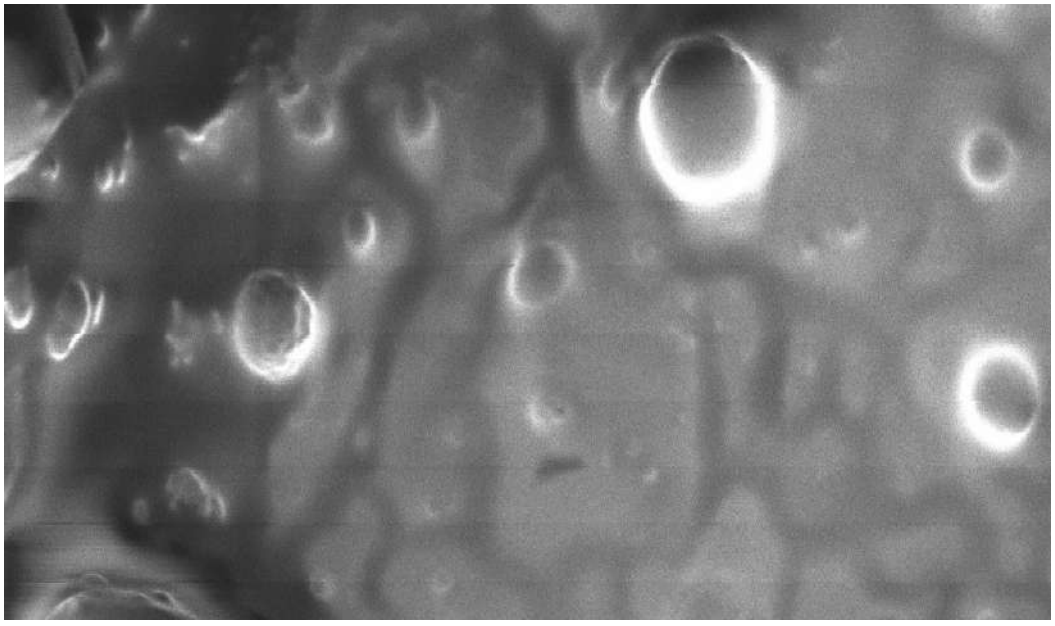

Fig 24: SEM image of FM3 formulation

5.4.4 In vitro drug release study of chitosan magnetic microparticle

Release studies reveals that the release behavior is governed by the ratio of eudragit RL 100 and S100, suggesting with higher amount of RL100 as coating polymers allows more drug diffusion from the microparticle. The FM11, FM2 formulation shows very minimal amount of drug release in 24 hours time period. The FM3 formulation shows a gradual increase in the drug release till 8

hours and after that the release was very slow. About 61% of the drugs was released in 12 hours. The % cumulative drug relase from the formulations is represented in Figure

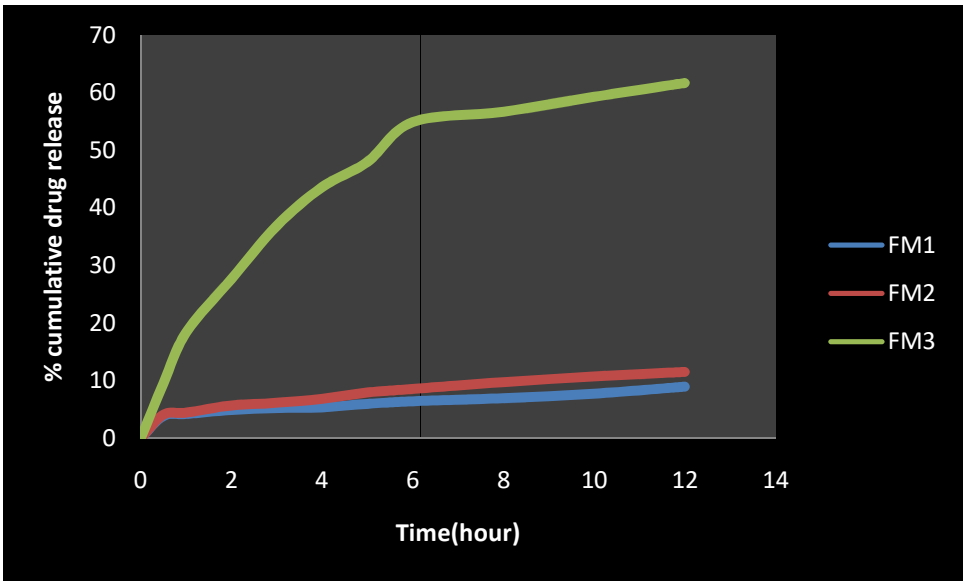

Fig 25: % cumulative release curve from magnetic microparticle

5.4.5 Kinetic evaluation of in vitro release data:

The in vitro release kinetics of the amoxicillin and clarithromycin loaded magnetic microparticles were plotted in various kinetic models and represented in table . The release constants for all the formulations were calculated from the slope of the appropriate plots and regression co-efficient (r^2) by linear regression analysis. All the formulations release regression values (r^2) were nest fitted with the krosmayer-peppas plot with a regression range of 0.9328-0.9771 and the release exponent (n) values were found in between 0.293-0.397(n<0.5) indicating a fickian tupe of release mechanism.

Table 22: Modeling and release kinetics of drugs from the magnetic microparticles

Model	Parameter	FM1	FM2	FM3
Zero order	R^2	-0.4078	-0.2180	0.1588
	K_0	0.620	0.796	3.988
First order	R^2	-0.3016	-0.0861	0.7844

	K_1	0.007	0.009	0.083
Higuchi	R^2	0.7990	0.8574	0.8991
	KH	2.485	3.190	15.854
Hixon-Crowell	R^2	-0.3365	-0.1292	0.6691
	KHC	0.002	0.003	0.024
Krosmayer-Peppas	R^2	0.9771	0.9761	0.9328
	K_{kp}	3.950	4.725	20.067
	n	0.293	0.326	0.397

5.4.6 Stability studies

Stability studies of the drug loaded microsphere were conducted over a period of 3 months. Drug stability in the microsphere formulation was assessed by calculating the % drug remaining of store formulation**.** There was no effective change in the drug content in the formulation stored at 40^0 C ±2 ^{0} C/70 % ± 5% RH.

Table 23: Stability study table

Drug Entrapment		Particle size		% Drug release at 12 hours	
At 0 time	After 3 months	At time 0	After 3 months	At time 0	After 3 months
91.6%	89.46	188 µm	185 µm	61.4%	59.84%

6. Conclusion

In the current research work, First chitosan microparticle and magnetite were prepared successfully. The FT-IR and DSC studies showed significant compatibility between the drug and the polymer. The chitosan microparticle were evaluated for % yield, % entrapment, particle size, % cumulative release study. The F6 formulation shows good entrapment efficiency and sustain release behavior. This batch is subjected for encapsulation by magnetie and Eudragit S100 & RL100 mixture provide site specific delivery of drug at the stomach region due to insolubility and intactness of eudragit RL100, which hold magnetite particles at the site and allow solubilization of eudragit S100 above pH 6. All the batches shows good flow properties, the particle size found in a range of 134 to 188 μm. SEM images confirmed that the prepared magnetic microparticle are spherical in shape. The FM3 formulation shows best release characteristic. The FM3 formulation shows drug release upto 61% after 12 hours. From the drug release kinetic s study it can be found that it follow fickian type of release mechanism. From the stability study data it can be concluded that there were no significant change in the formulation after storing at 40^{0} C $\pm 2^{0}$ c /75 $\pm$ 5% RH for 3 months period.
Hence it can be concluded that the magnetic microparticle of amoxicillin and clarithromycin could deliver the drug for a prolonged period of time at the target site specially in the lower pyloric region and duodenum region of alimentary tract where pH is 6.0-7.0 and would appear to be effective therapy in the form of polymeric magnetite particle suspension of the chosen dual regimen.