FORMULATION AND *IN-VITRO* EVALUATION OF METRONIDAZOLE MUCOADHESIVE MICROSPHERES

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MASTER OF PHARMACY

(Pharmaceutics)

Submitted by

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MELMARUVATHUR - 603 319

APRIL- 2013

CERTIFICATE

This is to certify that the research work entitled "**FORMULATION AND *IN-VITRO* EVALUATION OF METRONIDAZOLE MUCOADHESIVE MICROSPHERES''** submitted to The Tamil Nadu Dr.M.G.R. Medical University, Chennai in partial fulfillment for the award of the Degree of the Master of Pharmacy (Pharmaceutics) was carried out by **VIVEKANANDA RAO S R V (Register No. 26116014)** in the Department of Pharmaceutics under my direct guidance and supervision during the academic year 2012-2013.

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CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND *IN-VITRO* EVALUATION OF METRONIDAZOLE MUCOADHESIVE MICROSPHERES"** the confide research work carried out by **VIVEKANANDA RAO S R V (Register No. 26116014)** in the Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under the guidance of **Mr.K.SUNDARAMOORTHY,** B.Sc.,M.Pharm., professor, Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, during the academic year 2012-2013.

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Dedicated

To

My beloved parents...

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

%	----	Percentage
<	----	Less Than
>	----	More Than
°C	----	Degree Celsius
mcg	----	Microgram
cm	----	Centimeter
Avg	----	Average
DE	----	Dissolution Efficiency
DSC	----	Differential Scanning Calorimetry
F	----	Formulation
FTIR	----	Fourier Transform-Infra Red Spectroscopy
GIT	----	Gastrointestinal Tract
gm	----	Grams
HCl	----	Hydrochloric acid
HPMC	----	Hydroxy propyl methylcellulose
hrs	----	Hours
ICH	----	International Conference on Harmonization
IP	----	Indian Pharmacopoeia
LSC	----	Loose surface crystal
MDT	----	Mean Dissolution Time
mg	----	Milligram
ml	----	Milli liter

mm	----	Millimeter
N	----	Normality
Nm	----	Nanometer
NSAID	----	Non-Steroidal Anti-Inflammatory Drugs
PBS	----	Phosphate Buffer Solution
RH	----	Relative Humidity
rpm	----	Revolutions per Minute
S. No.	----	Serial Number
SEM	----	Scanning Electron Microscope
SSS	----	Standard Stock Solution
T	----	Time
USP	----	United State Pharmacopoeia
UV	----	Ultra Violet
W/v	----	weight/volume
λ max	----	Absorption Maximum

INTRODUCTION...

1. INTRODUCTION

1.1. Drug delivery systems *(Chien Y.W., 2009)*

The advancement of pharmacokinetics has established that the drug should be present above a certain minimum concentration in blood for as long a period as possible for optimum drug therapy.

Although, continuous infusion has been recognized as a superior mode of drug administration to maintain a constant and prolonged rug level in the body such mode of administration entails certain risk and hence requires hospitalization of the patient and close supervision.

As a result, solid oral dosage forms have become the most important and mostly used class of drug delivery system. Ordinary tablet and capsules known as conventional drug delivery system have to be administered several times a day depending on the biological half-life of the drug. Such multiple dosing may reduce invariably high plasma level of drug leading to waste of costly drugs and patient non-compliance.

Two important features as important while developing as drug delivery system. i.e., it should deliver the drug at a rate dictated by the needs of the body over the entire period of treatment and the drug should solely reach the site of action.

1.2. Sustained drug delivery systems *(Jain N.K.et al., 2004)*

The recognition of the fact that the absorption rate of the drugs into the body can be decreased by reduction of the rate of release of drug from the dosage forms. It

leads to develop some system to release their medications to the body slowly for prolonged drug release and sustained drug action.

Current efforts in the area of drug delivery include the development of targeted delivery in which the drug in only active in the body (for example, in cancerous tissues) and sustained release formulations which the drug is released over a period of time in a controlled manner form a formulation. These of sustained release formulations include liposomes, drug loaded biodegradable microspheres and drug polymer conjugates.

1. Improve patient's compliance and convenience due to less frequent dosing of drug.
2. Reduced 'See-saw' fluctuation and therefore helps in better control of disease condition.
3. Maximum utilization drug enabling reduction in total amount of dose administered.
4. Reduction in health care cost through improved therapy, shorter treatment period and less frequency of dosing.

The problem frequently encountered is the is the increase the residence time of the dosage form in the stomach and proximal portion of the small intestine, due to the rapid gastrointestinal transit phenomenon of the stomach which may consequently diminish the extent of absorption of many drugs since almost most of the drug entities areMostly absorbed from the upper part of the intestine, therefore it would be beneficial to develop a sustained release formulation which remains at the absorption site for an extended period of time.

Several approaches have been immersed to prolong the residence time of the dosage forms at the absorption site and one of these is the development of oral bioadhesive/mucoadhesive system. Various gastrointestinal mucoadhesive dosage forms, such as discs, microspheres, and bilayered tablets, have been thoroughly prepared and reported by several research groups.

1.3. Mucoadhesive drug delivery systems (*Shoba rani R et al., 2008)*

Mucoadhesive drug delivery system is a new system of drug delivery and has recently gained great concern in pharmaceutical sciences. The concept of mucoadhesives was introduced in the early 1980s. Mucoadhesion can be defined as the phenomenon of the attachment of natural or synthetic polymers to a mucosal surface. In general, the process involved in the mucoadhesion phenomenon can be described in three steps: first of all, the wetting and swelling of the polymer should allow an intimate contact with the tissue and secondly, interpenetration of the polymer chains and entanglement between the polymer and the mucin chains should be attained and finally, the formation of weak chemical bonds. Mucus is a viscous and heterogeneous biological product that coats many epithelial surfaces. Mucus-secreting cells are widely spread in different locations in the body, including the nasal, ocular, buccal area and the gastrointestinal, reproductive and respiratory tracts. Mainly, the mucus serves as a lubricant to minimize shear stresses and as a protection barrier against harmful substances. However, mucus can perform other important functions. Goblet cells located in the epithelium are unicellular mucus-Secreting glands. Mucus is stored in large granules in the goblet cell and can be released by exocytosis or exfoliation of the whole cell. Mucus granules are mainly stored in the apical side of the goblet cell, which results in the characteristic balloon shape of these cells. Although the secretion of mucus can vary depending on age, sex,

body location and health condition, the average mucus turnover is approximately 6 h. Mucus consists mainly of water (up to 95% weight), inorganic salts (about 1% weight), carbohydrates and lipids (less than 1%) and glycoproteins (no more than 5% weight). Mucus glycoproteins are also called mucins and consist of a protein core with branched oligosaccharide chains attached over 63% of its length. Approximately 80% by weight of the glycoprotein consists of oligosaccharides, which make the mucin more hydrosoluble and also protects the protein core from proteolytic degradation.

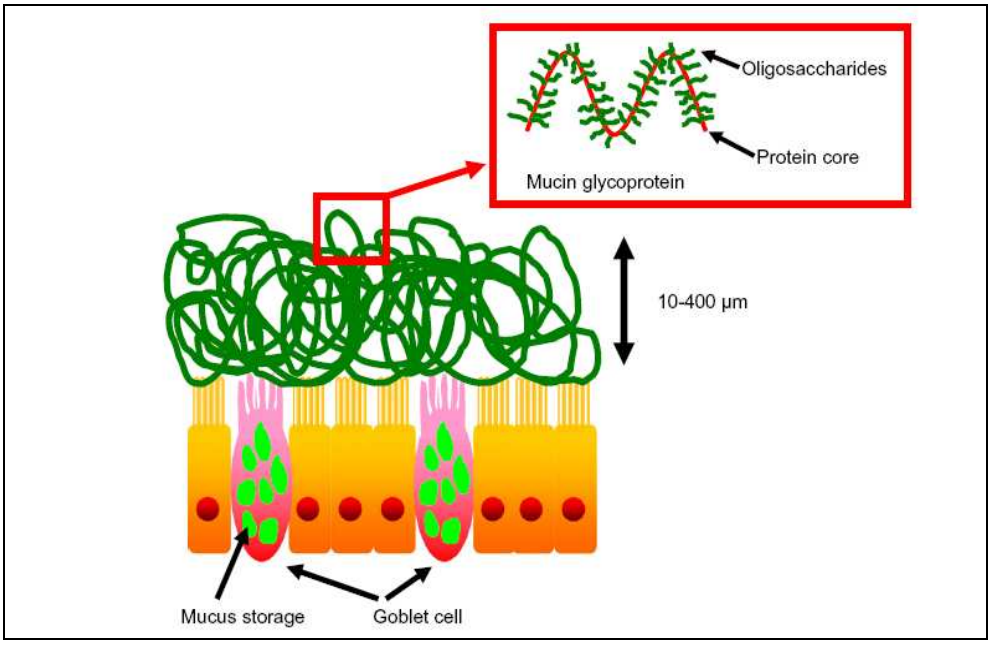


Figure 1.1: Mucus layer on epithelial surface

Bioadhesives are natural polymeric materials that act as adhesives. The term is sometimes used more loosely to describe glue formed synthetically from biological monomers such as sugars, or to mean a synthetic material designed to adhere to biological tissue. The term bioadhesion refers to any bond formed between two biological surfaces or a bond between a biological and a synthetic surface. It may be defined as attachment of synthetic biological macromolecules to a biological tissue. A more specific term than bioadhesion is mucoadhesion.

Mucoadhesion is the relatively new and emerging concept in drug delivery. Mucoadhesion is the special case of bioadhesion where the biological tissue is an epithelium covered by mucus. Most mucosal surfaces such as in the gut or nose are covered by a layer of mucus.

Adhesion of a matter to this layer is hence called mucoadhesion. Mucoadhesion keeps the delivery system adhering to the mucus membrane.

Mucoadhesion can be defined as the ability of synthetic or biological macromolecules to adhere to mucosal tissues. The concept of mucoadhesion is one that has the potential to improve the highly variable residence times experienced by drugs and dosage forms at various sites in the gastrointestinal tract, and consequently, to reduce variability and improve efficacy.

These systems remain in close contact with the absorption tissue, the mucous membrane, releasing the drug at the site of action leading to an increase in bioavailability.

Mucoadhesive drug delivery system prolong the residence time of the dosage form at the site of application or absorption and facilitate an intimate contact of the dosage form with the underline absorption surface and thus contribute to improved and / or better therapeutic performance of the drug.

The mucoadhesive drug delivery system may include the following

1. Buccal delivery system.
2. Sublingual Delivery system.
3. Vaginal delivery system.
4. Rectal delivery system.
5. Nasal delivery system.
6. Ocular delivery system.

7. Gastro Intestinal delivery system

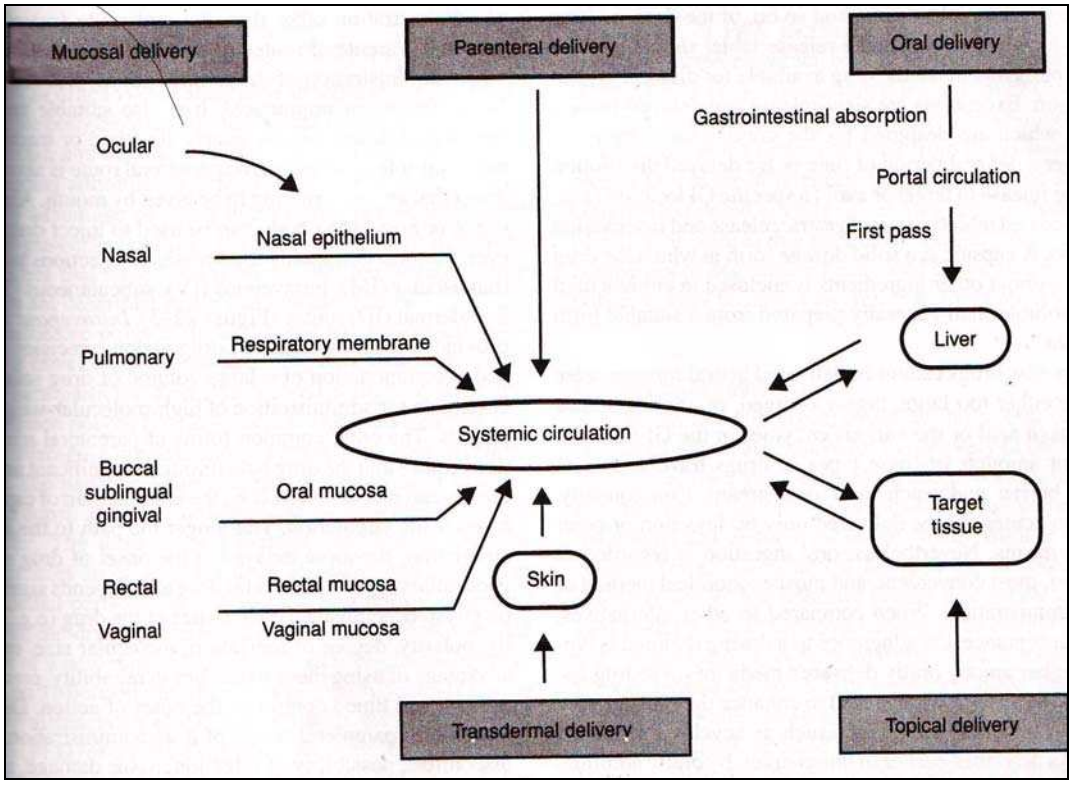


Figure 1.2. Potential sites for mucosal drug delivery

Their ability to stick to mucous membranes attracted attention as a pathway for resolving the problem of low bioavailability of traditional delivery systems used in the oral cavity and on the surface of the eye or other organs where movement of tissues or production of various secretions prevents prolonged retention of the medicinal agent. The reasons that the oral route achieved such popularity may be in part attributed to its ease of administration as well as the traditional belief that by oral administration the drug is well absorbed as the food stuffs that are ingested daily.

In the exploration of oral controlled release drug administration, one encounters three areas of potential challenge.

1. Development of a drug delivery system: To develop a viable oral controlled release drug delivery system capable of delivering a drug at a therapeutically effective rate to a desirable site for duration required for optimal treatment.

2. Modulation of gastro intestinal transit time: To modulate the GI transit time so that the drug delivery system developed can be transported to a target site or to the vicinity of an absorption site and reside there for prolonged period of time to maximize the delivery of a drug dose.

3. Minimization of hepatic first pass elimination: If the drug to be delivered is subjected to extensive hepatic first pass elimination, preventive measures should be devised to either bypass or minimize the extent of hepatic metabolic effect.

Definition of mucoadhesion *(Amit Alexander.et al., 2011)*

Adhesion can be defined as the bond produced by contact between a pressure - sensitive adhesive and a surface The American Society of testing and materials has defined it as the state in which two surfaces are held together by interfacial forces, which may consist of valence forces, interlocking action or both. When the adhesion involves Mucus or mucus membrane it is termed as mucoadhesion.

Concepts of mucoadhesion

In biological systems, four types of bioadhesion can be distinguished as follows:-

1. Adhesion of a normal cell on another normal cell.
2. Adhesion of a cell with a foreign substance.
3. Adhesion of a normal cell to a pathological cell.
4. .Adhesion of an adhesive to a biological substance.

Mucous membrane

Mucous membranes are the moist linings of the orifices and internal parts of the body that are in continuity with the external surface. They cover, protect, and provide secretory and absorptive functions in the channels and extended pockets of the outside world that are incorporated in the body. Mucus is a translucent and viscid secretion, which forms a thin, continuous gel blanket adherent to mucosal

epithelial surface. The mean thickness of this layer varies from about 50-450 μm in humans. It is secreted by the goblet cells lining the epithelia or by special exocrine glands with mucus cells acini. The exact composition of the mucus layer varies substantially, depending on the species, the anatomical location and pathological states. They secrete a viscous fluid known as mucus, which acts as a protective barrier and also lubricates the mucosal membrane. Mucosal membranes of human organism are relatively permeable and allow fast drug absorption they are characterized by an epithelial layer whose surface is covered by mucus. The primary constituent of mucus is a glycoprotein known as mucin as well as water and inorganic salts. However, it has general composition.

Table 1.1: Composition of Mucous Membrane

S.NO.	COMPOSITION	% AMOUNT
1	Water	95
2	Glycoproteins and Lipids	0.5-5.0
3	Mineral Salts	1
4	Free Proteins	0.5-1.0

Table 1.2: Comparative properties of Gastrointestinal, Dermal andTransmucosal drug administration (*Khar R K. et al., 2002*)

	Gastrointestinal	Dermal	Nasal	Oral mucosal	Vaginal
Accessibility	+	+++	++	++	+
Surface area	+++	+++	+	++	+++
Surface Environment	+	++	++	+++	+
Permeability	+++	+	+++	++	+++
Reactivity	++	++	+	+++	++
Vascular Drainage	+++	+	+++	++	+++
First pass clearance	+	+++	+++	+++	+
Patient acceptability	++	+++	++	+++	+++

Examples of mucosa

- Buccal mucosa.
- Oesophageal mucosa.
- Gastric mucosa.
- Intestinal mucosa.
- Nasal mucosa.
- Olfactory mucosa.
- Oral mucosa.
- Bronchial mucosa.
- Uterine mucosa.
- Endometrium (mucosa of the uterus).
- Penile mucosa.

1.4 Mucoadhesive polymers

Mucoadhesive polymers are water-soluble and water-insoluble polymers, which are swellable networks, jointed by cross-linking agents. These polymers possess optimal polarity to make sure that they permit sufficient wetting by the mucus and optimal fluidity that permits the mutual adsorption and interpenetration of polymer and mucus to take place.

Mucoadhesive polymers that adhere to the musin-epithelial surface can be conveniently divided into three broad classes,

1) Polymers that become sticky when placed in water and owe their muco-adhesion to stickiness.

2) Polymers that adhere through nonspecific, noncovalent interactions are primarily electrostatic in nature (although hydrogen and hydrophobic bonding may be significant).

3) Polymers that bind to specific receptor site on tile self surface.

Examples of some Mucoadhesive polymer

Natural /Semi-synthetic	Na alginate,	Agarose,	Chitosan,
	Pectin,	Tragacanth,	Gelatin,
	Xanthan gum,	Carragenan,	Starch
Synthetic	Poly vinyl alcohol,	Polyamides,	Polycarbonates,
	Poly alkylene glycols,	Poly vinyl ethers,	Esters and halides
	Poly methacrylic acid,	PMMA,	Methyl cellulose,
	Ethyl cellulose,	HPC,	HPMC
	Methyl cellulose,	Sod. CMC	
Bicompatible	Esters of haluronic acid,		
	polyvinyl acetate,		
	Ethylene glycol.		
Biodegradable	Poly (lactides),	Poly (lactide-coglycolides),	
	Poly caprolactones,	Poly alkyl cyanoacrylates.	
	Poly orthoesters,	Poly (glycolides),	
	Poly phosphoesters,	Poly anhydrides,	
	Poly phosphazenes,	Chitosan,	

Ideal characteristics of a mucoadhesive polymer

1. The polymer and its degradation products should be nontoxic and nonabsorable from the GIT.
2. It should be nonirritant to the mucous membrane.
3. It should preferably form a strong noncovalent bond with the mucin-epithelial cell surfaces.
4. It should adhere quickly to most tissue and should possess some site-specificity.
5. It should allow daily incorporation to the drug and offer no hindrance to its release.
6. The polymer must not decompose on storage or during the shelf life of the dosage form.
7. The cost of polymer should not be high so that the prepared dosage form remains competitive.

1.5. Factors affecting mucoadhesion

1) Polymer Related Factors

a) Molecular weight: The interpenetration of polymer molecules into the mucus layer is variable, for low molecular weight polymers penetration is more than high molecular weight polymers because entanglements are favored in high molecular weight polymers.

b) Concentration of active polymer: For solid dosage forms such as tablets, the higher the concentration of polymer, the stronger the bioadhesion force.

c) Spatial Conformation: Bioadhesive force is also dependent on the conformation of polymers, i.e., helical or linear. The helical conformation of polymers may shield many active groups, primarily responsible for adhesion, thus reducing themucoadhesive strength of the polymer.

d) Chain flexibility of polymer: Chain flexibility is important for interpenetration and enlargement. As water-soluble polymers become more and more cross linked, the mobility of the individual polymer chain decreases, also as the cross linking density increases, the effective length of the chain which can penetrate into mucus decrease even further and mucoadhesive strength is reduced.

e) Degree of Hydration: Another important factor affecting the mucoadhesive strength of polymeric components is the degree of hydration. In this respect many polymers will exhibit adhesive properties under conditions where the amount of water is limited. However in such a situation, adhesion is thought to be a result of a combination of capillary attraction and osmotic forces between the dry polymer and the wet mucosal surface which act to dehydrate and strengthen the mucus layer. Although this kind of "sticking" has been referred to as mucoadhesion it is important to clearly distinguish such processes from "wet-on-wet" adhesion in which swollen mucoadhesive polymers attach to mucosal surfaces. Hydration is essential for the relaxation and interpenetration of polymer chains, excess hydration could lead to decreased mucoadhesion and/orientation due to the formation of slippery mucilage. In this situation cross linked polymers that only permit a certain degree of hydration may be advantageous for providing a prolonged mucoadhesive effect. The attachment and bonding of bioadhesive polymers to biological substrates occurs mainly through interpenetration followed by secondary non-covalent bonding between substrates. Given that secondary bonding mainly arises due to hydrogen bond formation, it is well accepted that mucoadhesive polymers possessing hydrophilic functional such as, carboxyl (COOH), hydroxyl (OH), amide (NH_2) and sulphate groups (SO_4H) may be more favorable in formulating targeted drug delivery platforms. Typically, physical entanglements and secondary interactions (hydrogen bonds)

contribute to the formation of a strengthened network; therefore polymers that exhibit a high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins.

2)Environmental–Related Factors

a) pH: pH influences the charge on the surface of both mucus and polymers. Mucus will have a different charge density depending on pH, because of difference in dissociation of functional groups on carbohydrate moiety and amino acids of the polypeptide backbone, which may affect adhesion.

b) Applied strength: To place a solid bioadhesive system, it is necessary to apply a defined strength. Whichever the polymer may be the adhesion strength of those polymers increases with the increase in the applied strength.

c) Initial contact time: The initial contact time between mucoadhesive and the mucus layer determines the extent of swelling and the interpenetration of polymer chains. The mucoadhesive strength increases as the initial contact time increases.

d) Selection of the model substrate surface: The handling and treatment of biological substrates during the testing of mucoadhesive is an important factor, since physical and biological changes may occurs in the mucus gels or tissues under the experimental conditions.

3) Swelling: The swelling characteristic is related to the polymer itself, and also to its environment. Interpenetration of chains is easier as polymer chains are disentangled and free of interactions. More the swelling of polymeric matrix higher the adhesion time of polymers.

4) Physiological variables: Mucin turnover and disease state of mucus layer are physiological variables, which may affect bioadhesion.

Functions of mucous layer *(Jain N.K.et al., 2004)*

The mucous layer, which covers the epithelial surface, has various roles.

1. Protective Role.
2. Barrier Role.
3. Adhesion Role.
4. Lubrication Role.
5. Mucoadhesion Role.

1. **Protective Role:** The Protective role results particularly from its hydrophobicity and protecting the mucosa from the lumen diffusion of hydrochloric acid from the lumen to the epithelial surface.

2**. Barrier Role:** The role of mucus layer as barrier in tissue absorption of drugs and other substances is well known as it influence the bioavailibity of the drugs.The mucus constitutes diffusion barrier for molecules, and especially against drug absorption diffusion through mucus layer depends on molecule charge, hydration radius, ability to form hydrogen bonds and molecular weight

3. **Adhesion Role:** Mucus has strong cohesive properties and firmly binds the epithelial cells surface as a continuous gel layer.

4. **Lubrication Role:** An important role of the mucus layer is to keep the membrane moist. Continuous secretion of mucus from the goblet cells is necessary to compensate for the removal of the mucus layer due to digestion, bacterial degradation and solubilisation of mucin molecules

5. **Mucoadhesion Role:** One of the most important factors for bioadhesion is tissue surface roughness. Adhesive joints may fail at relatively low applied stresses if cracks, air bubbles, voids, inclusions or other surface defects are present. Viscosity and wetting power are the most important factors for satisfactory bioadhesion.

At physiological pH, the mucus network may carry a significant negative charge because of the presence of sialic acid and sulphate residues and this high charge density due to negative charge contributes significantly to the bioadhesion.

Need of mucoadhesive

- Controlled release.
- Target & localised drug delivery.
- By pass first pass metabolism.
- Avoidance of drug degradation.
- Prolonged effect.
- High drug flux through the absorbing tissue.
- Reduction in fluctuation of steady state plasma level.
    - An ideal dosage form is one, which attains the desired therapeutic concentration of drug in plasma and maintains constant for entire duration of treatment. This is possible through administration of a conventional dosage form in a particular dose and at particular frequency. In most cases, the dosing intervals much shorter than the half life of the drug resulting in a number of limitations associated with such a conventional dosage form are as follows:
- Poor patient compliance; increased chances of missing the dose of a drug with short half-life for which frequent administration is necessary.
- A typical peak plasma concentration time profile is obtained which makes attainment of steady state condition difficult.
- The unavoidable fluctuation in the drug concentration may lead to under medication or over medication as the steady state concentration values fall or rise beyond in the therapeutic range.

- The fluctuating drug levels may lead to precipitation of adverse effects especially of a drug with small therapeutic index whenever overmedication occurs

Advantages of mucoadhesives (*Punitha S and Ganga S., 2010*)

- A prolonged residence time at the site of drug action or absorption.
- A localization of drug action of the delivery system at a given target site.
- An increase in the drug concentration gradient due to the intense contact of particles with the mucosal.
- A direct contact with intestinal cells that is the first step before particle absorption.
- Ease of administration.
- Termination of therapy is easy.{except gastrointestinal}
- Permits localization of drug to the oral cavity for a prolonged period of time.
- Can be administered to unconscious patients. Except gastrointestinal}
- Offers an excellent route, for the systemic delivery of drugs with high first pass metabolism, there by offering a greater bioavailability
- A significant reduction in dose can be achieved there by reducing dose related side effects.
- Drugs which are unstable in the acidic environment are destroyed by enzymatic or alkaline environment of intestine can be administered by this route. Eg. Buccal sublingual, vaginal.
- Drugs which show poor bioavailability via the oral route can be administered conveniently. It offers a passive system of drug absorption and does not require any activation.
- The presence of saliva ensures relatively large amount of water for

drug dissolution unlike in case of rectal and transdermal routes.

- Systemic absorption is rapid.
- This route provides an alternative for the administration of various hormones, narcotic analgesic, steroids, enzymes, cardiovascular agents etc.
- The buccal mucosa is highly perfused with blood vessels and offers a greater permeability than the skin.
- Less dosing frequency.
- Shorter treatment period.
- Increased safety margin of high potency drugs due to better control of plasma levels.
- Maximum utilization of drug enabling reduction in total amount of drug administered.
- Improved patient convenience and compliance due to less frequent drug administration.
- Reduction in fluctuation in steady state levels and therefore better control of disease condition and reduced intensity of local or systemic side effects.
- Despite the several advantages associated with oral controlled drug delivery systems, there are so many **disadvantages,** which are as follows:
- Basic assumption is drug should absorbed throughout GI tract
- Limited gastric residence time which ranges from few minutes to 12 hours which lead to unpredictable bioavailability and time to achieve maximum plasma level.

1.6. Limitations of mucoadhesion

1. Drug administration via the buccal mucosa has certain limitations
2. Drugs, which irritate the oral mucosa, have a bitter or unpleasant taste, odour, cannot be administered by this route.
3. Drugs, which are unstable at buccal pH cannot be administered by this route.
4. Only drugs with small dose requirements can be administered.
5. Drugs may swallow with saliva and loses the advantages of buccal route.
6. Only those drugs, which are absorbed by passive diffusion, can be administered by this rout.
7. Eating and drinking may become restricted.
8. Swallowing of the formulation by the patient may be possible.
9. Over hydration may lead to the formation of slippery surface and structural integrity of theformulation may get disrupted by the swelling and hydration of the bioadhesive polymers.

1.7. Stages of mucoadhesion *(Vyas .S. P.,2002)*

1. Contact Stage
2. Consolidation Stage.

Contact Stage: The first stage is characterized by the contact between the mucoadhesive and the mucous membrane, with spreading and swelling of the formulation, initiating its deep contact with the mucus layer.

Consolidation Stage: In the consolidation step (Figure 1), the mucoadhesive materials are activated by the presence of moisture. Moisture plasticizes the system, allowing the mucoadhesive molecules to break free and to link up by weak Vander Waals and hydrogen bonds.

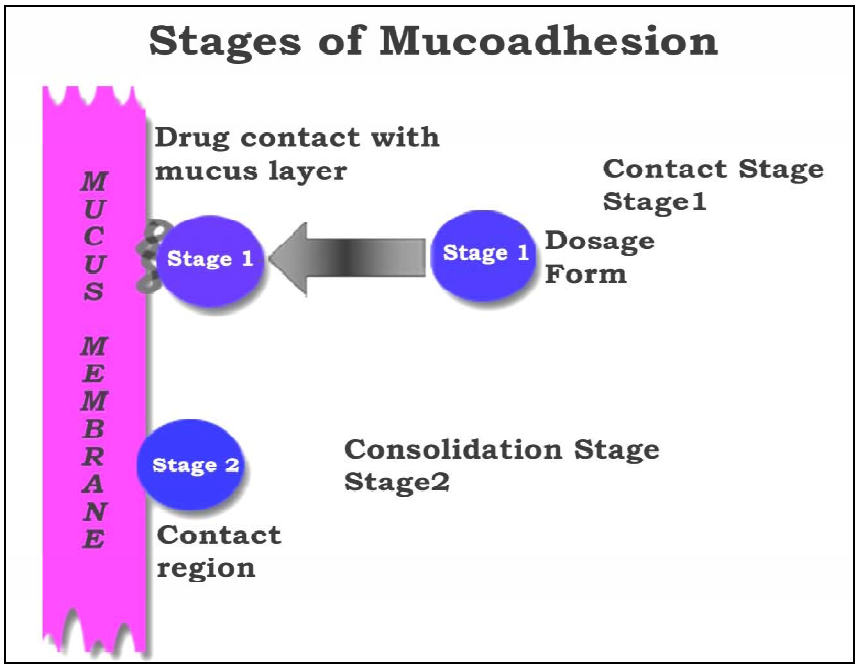


Figure 1.3: The two steps of the mucoadhesion process

1.8. Theories of mucoadhesion *(Jain N.K. et al., 2004)*

1. Electronic Theory

The adhesive polymer and mucus membrane strategically have different electronic characteristics. When the two surfaces contact each other, a double layer of electrical charge is formed at the interface, and then adhesion developed between the double layers due to electrical charge.

2. Adsorption Theory

The adsorption theory of bioadhesion proposes two bond theories:

(i) Primary chemical bonds permanent and therefore undesirable in bioadhesion

(ii) Secondary chemical bonds are found to be van-der Waals, hydrogen, hydrophobic and electrostatic forces.

3. Wetting Theory

The wetting theory emphasizes mainly on the intimate contact between the adhesive and mucus. Thus, a wetting surface will be controlled by structural similarity, degree of cross linking of the adhesive polymer, or use of a surfactant.

4. Diffusion Theory

A semi permanent adhesive bond is formed because of the chains of adhesive and the substrate interpenetrates one another to a sufficient depth and it is considered as the essence of this theory. The diffusion coefficient of both interacting polymers and the diffusion co-efficient are the factors responsible for the penetration rate. In addition mobility, flexibility of the bioadhesive polymer, mucus glycoprotein, and the expanded nature of both network are other important parameters considered.

1.9. Mechanism of mucoadhesion *(Bhatt j.h..et al.,2009)*

The concept of mucoadhesion is one that has the potential to improve the highly variable residence times experienced by drugs and dosage forms at various sites in the gastrointestinal tract, and consequently, to reduce variability and improve efficacy.Intimate contact with the mucosa should enhance absorption. The mechanisms responsible in the formation of bioadhesive bonds are not fully known, however most research has described bioadhesive bond formation as a three step process.

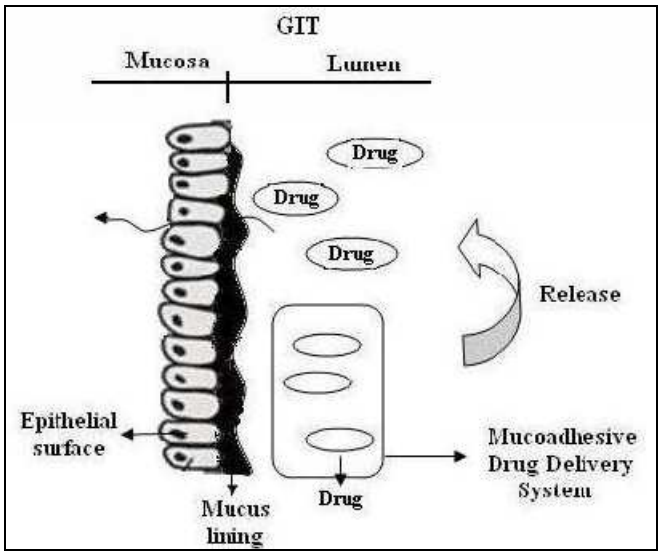


Figure 1.4: Interaction of mucoadhesive drug delivery system with mucous layer

STEP 1: Wetting and swelling of polymer

STEP 2: Interpenetration between the polymer chains and the mucosal membrane.

STEP 3: Formation of Chemical bonds between the entangled chains**.**

Step 1

The wetting and swelling step occurs when the polymer spreads over the surface of the biological substrate or mucosal membrane in order to develop an intimate contact with the substrate.

This can be readily achieved for example by placing a bioadhesive formulation such as a tablet or paste within the oral cavity or vagina. Bioadhesives are able to adhere to or bond with biological tissues by the help of the surface tension and forces that exist at the site of adsorption or contact. Swelling of polymers occurs because the components within the polymers have an affinity for water.

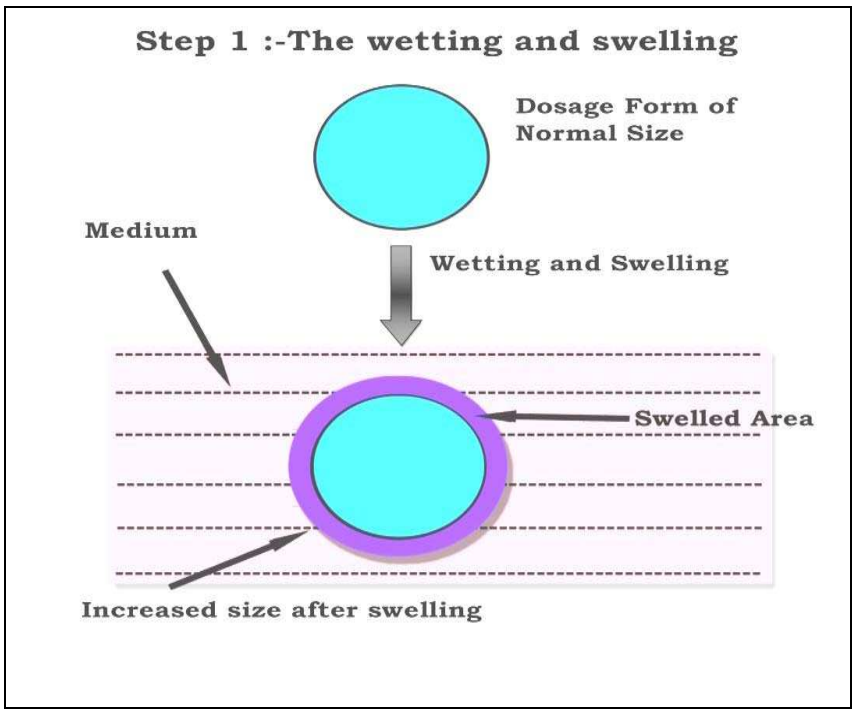


Figure 1.5: Wetting and Swelling of Polymer

Step 2

The surface of mucosal membranes is composed of high molecular weight polymers known as glycoproteins. In this step interdiffusion and interpenetration take place between the chains of mucoadhesive polymers and the mucous gel network creating a great area of contact. The strength of these bonds depends on the degree of penetration between the two polymer groups. In order to form strong adhesive bonds,onepolymer

Group must be soluble in the other and both polymer types must be of similar chemical structure.

Interdiffusion and interpenetration

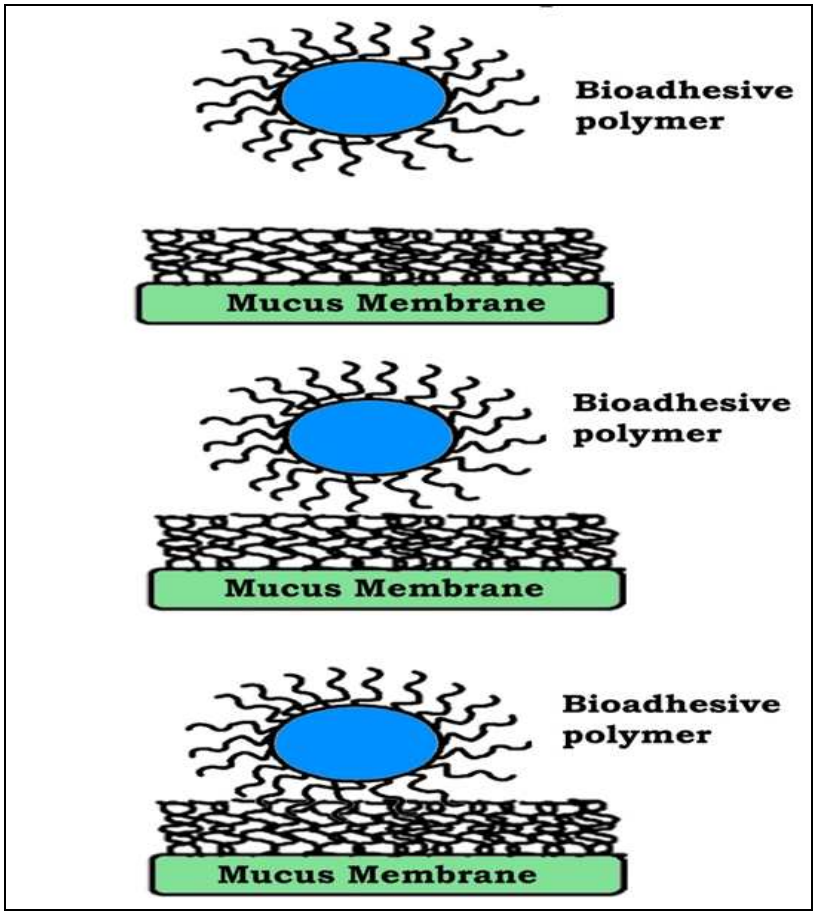


Figure 1.6: Interdiffusion and Interpenetration of Polymer and Mucus

Step 3

In this step entanglement and formation of weak chemical bonds as well as secondary bonds between the polymer chains mucin molecule The types of bonding formed between the chains include primary bonds such as covalent bonds and weaker secondary interactions such as van-der Waals Interactions and hydrogen bonds. Both

primary and secondary bonds are exploited in the manufacture of bioadhesive formulations in which strong adhesions between polymers are formed.

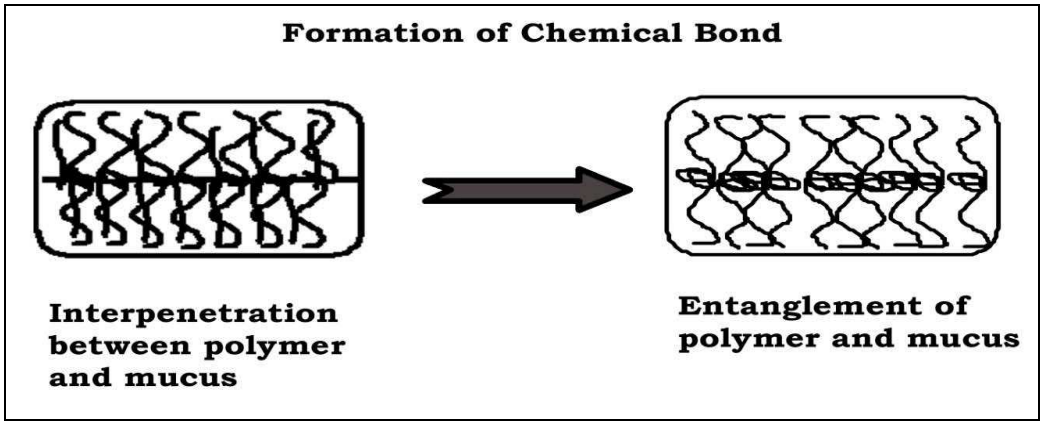


Figure.1.7: Entanglement of Polymer and Mucus by Chemical bonds

1) **Ionic bonds**—where two oppositely charged ions attract each other via electrostatic interactions to form a strong bond (e.g. in a salt crystal).

2) **Covalent bonds**—where electrons are shared, in pairs, between the bonded atoms in order to fill the orbital in both. These are also strong bonds.

3) **Hydrogen bonds**—here a hydrogen atom, when covalently bonded to electronegative atoms such as oxygen, fluorine or nitrogen, carries a slight positively charge and is therefore is attracted to other electronegative atoms. The hydrogen can therefore be thought of as being shared, and the bond formed is generally weaker than ionic or covalent bonds.

4) Van-der-Waals bonds—these are some of the weakest forms of interaction that arise from dipole– dipole and dipole-induced dipole attractions in polar molecules, and dispersion forces with non-polar substances.

5) Hydrophobic bonds—more accurately described as the hydrophobic effect, these are indirect bonds (such groups only appear to be attracted to each other) that occur when

non-polar groups are present in an aqueous solution. Water molecules adjacent to non-polar groups form hydrogen bonded structures, which lowers the system entropy.

Micro particles are of two types *(Khar R K. et al., 2002)*

1. *Microspheres*: The adsorbed substance is dispersed through out the microsphere matrix
2. *Micrcapsules:* The entrapped substance is completely surrounded by a distinct capsule wall

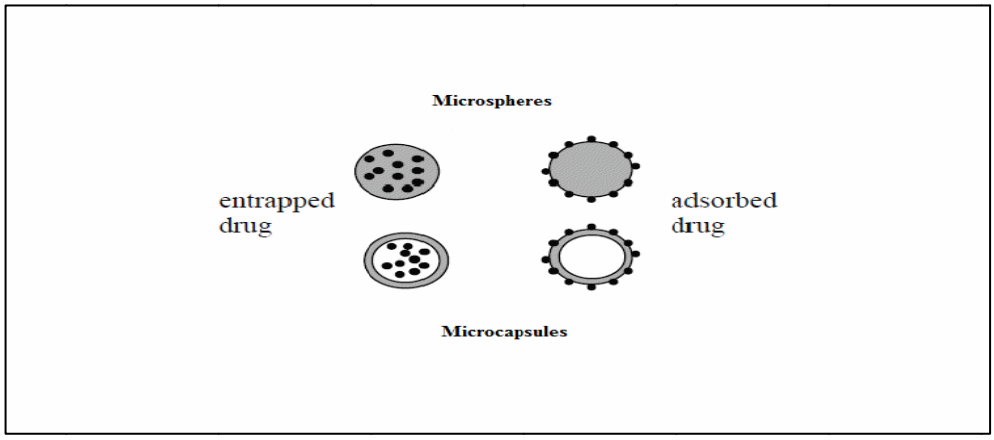


Figure 1.8: Differentiation between microspheres and microcapsules

1.10. Loading of drug

The active components are loaded on to the microspheres principally using two methods either during the preparation or after the formation of microsphere by incubating them with drug.

The active components can be loaded by means of physical entrapment, chemical Linkage or surface absorption. Entrapment largely depends on the method of preparation And the nature of drug and polymer.

Maximum loading can be achieved by incorporating the drug at the time of preparation but it may get affected by many other process variables such as method of preparation, presence of additives heat of polymerization, agitation intensity etc. drug in loading in pre-formed microspheres is relatively less but the major advantage of the loading method is that there is no effect of process variables, loading is carried out in preformed microsphere by incubating them with high concentration of drug in a suitable solvent. The drug in these microspheres is loaded by penetration or diffusion through the pores.

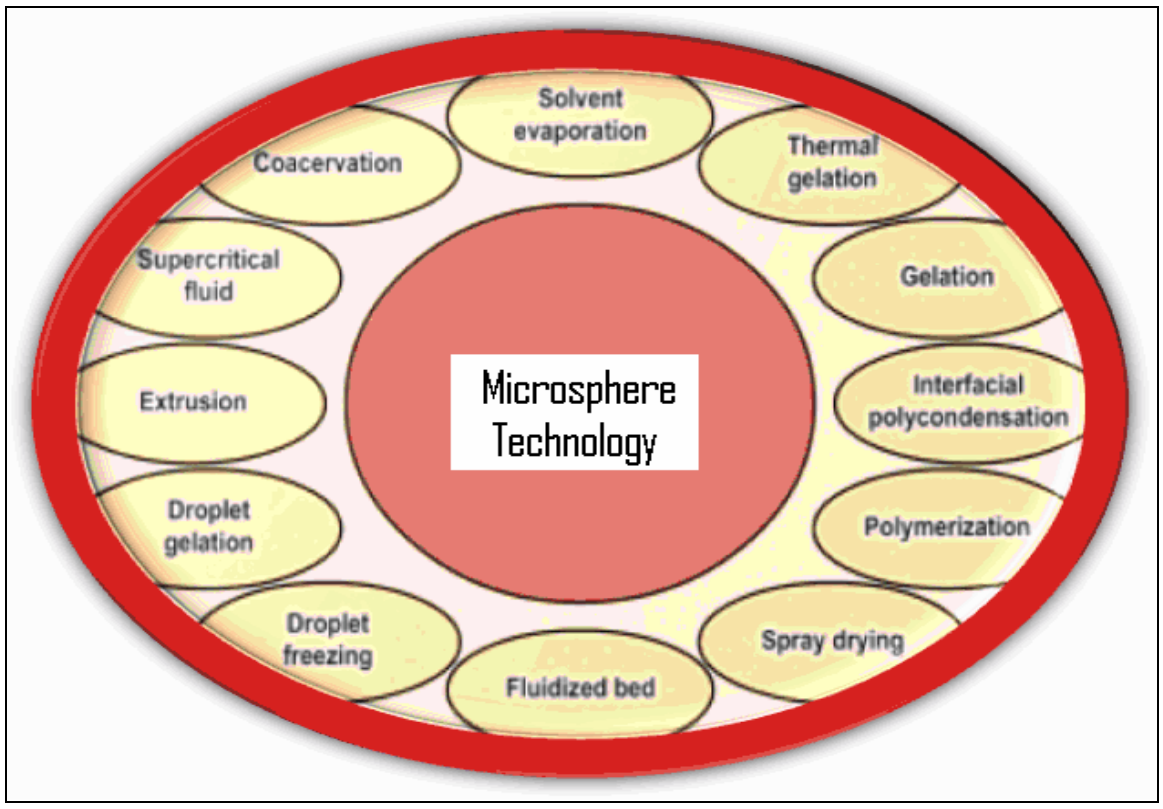


Figure 1.9: Different methods employed for microspheres

1.11. Methodology *(Shoba rani R. et al.,2008)*

The selected methods of Mucoadhesive microspheres was prepared by,

Ionic Orifice Gelation Technique

In this technique cross linking was done with calcium chloride solution to release the drug in a controlled manner. Microspheres were prepared by using Orifice ionic gelation technique in which sodium alginate, HPMC and M etylcellulose in different ratios as mentioned above was added to 32 ml of water the above solution with continuous stirring to form homogenous solution.

After the solution by sonicating the mixture for 20 minutes the Metronidazole was then added to the above solution to form a clear solution. The drug polymer mixture is poured in 1.5% calcium chloride solution by using 22# needle by stirring at 50rpm the microspheres thus formed are allowed 30 min for curing in calcium chloride solution then were decanted and washed with distilled water and air dried over night at room temperature.

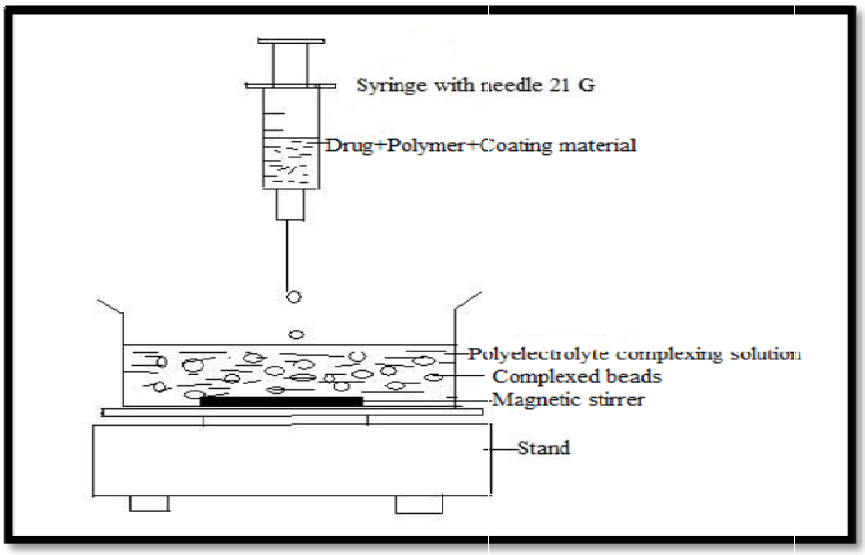


Figure 1.10: Beads prepared by the ionic orifice gelation method

1.12.Techniques to manufacture microspheres

A.physical methods

Air-suspension coating

The particles are coated while suspended in an upward-moving air flow stream.just sufficient air is only permitted to rise through the outer annular space to fluidie the particles settling most of the rising air (usually heated) flows inside around the cylinder causing the particles rising rapidly.

At the top surface, as the air stream diverges and slows,they settle back on to the outer bed and move down ward to repeat the cycle.in this process,as ability of applying coatings in the form of solvent solutions,aqueous solution,emulsions,and dispersions.core materials comprised of micron particles can be effectively encaps lated by air suspension techiniques,but agglomeration of the particles to some larger size is achieved.

Coacervation-Phase Separation

The general outline of the processes consists of three steps carried out under continuous agitation.

1**. Formation of three immiscible chemical phases**

A liquid manufacturing phase, a core material and a coating material. To form the three phases, the core material dispersed in a solution of the coating polymer, the solvent for the polymer being the liquid manufacturing vehicle phase.

2. **Deposition of the coating**

It is mainly of depositing the liquid polymer coating upon the core material. This is obtained by controlled, physical mixing of the material in the manufacturing vehicle. Deposition of the liquid polymer coating around the core material occurs only if the polymer is adsorbed at the interface between the core material and the liquid vehicle phase. The continued deposition of the coating material is improved by a reduction in the total free interfacial energy of the system.

3**. Rigidization of the coating**

It involves mainly in rigidizing the coating, usually by thermal, cross-linking, or desolvation techniques, to form a self-sustaining microspheres.

Pan coating

The pan coating process, mainly used in the pharmaceutical industry, is among the oldest industrial procedures for forming small, coated particles or tablets. The particles are mainly tumbled in a pan or other device while the coating material is applied steadily and slowly. The particles has been tumbled in pan, while the coating material is applied slowly with respect to microspheres, solid particles are greater.

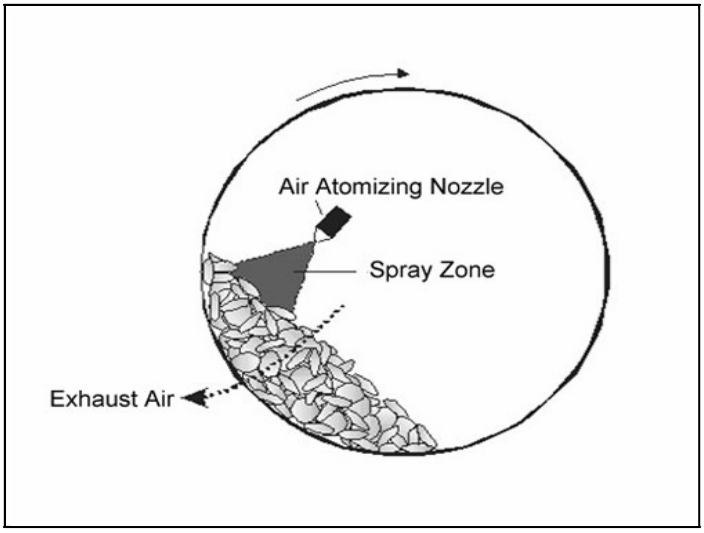


Figure 1.11: Pan coating and its process

Spray–drying

Spray drying is an important microspheres technique in this an active material is dissolved or suspended in a melt or polymer solution and becomes trapped in the dried particle. The main advantages of this ability to handle labile materials because of the short contact time in the dryer, in addition, the operation is economical. In modern spray dryers, the solutions are to be sprayed can be as high as 300mPa.s. Spray drying and spray congealing processes are similar in that both involve dispersing the core material in a liquid coating substance and spraying the core - coating mixture into some environmental condition, whereby relatively rapid solidification (and formation) of the coating is affected. The principal difference between the two methods is the coating solidification is obtained.

Coating solidification in the case of spray drying is effected by rapid evaporation of a solvent, by thermally congealing a molten coating material or by solidifying a dissolved coating by introducing the coating - core material mixture into a non-solvent.

Removal of the non-solvent or solvent from the coated product is then accomplished by sorption, extraction, or evaporation techniques.

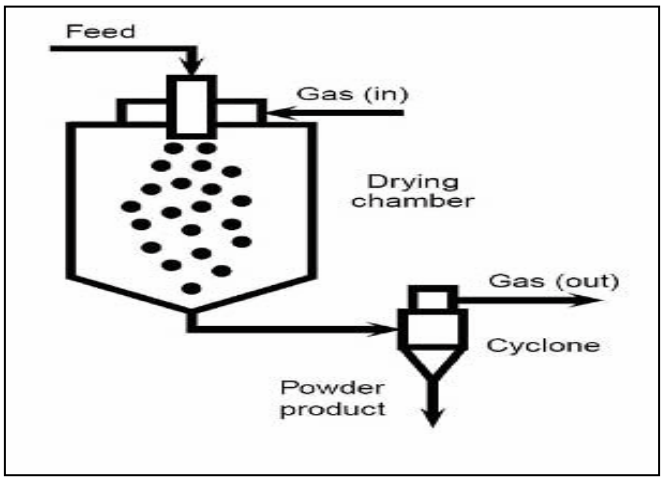


Figure 1.12: Spray drying technique and its process

Chemical process

Solvent Evaporation

The liquid manufacturing vehicle is the mainly used for the formulation. The coating for the microspheres will be dissolved in a volatile solvent, which has to be immiscible with the liquid manufacturing vehicle phase. A core material will be either dissolved or dispersed in the coating polymer solution. On agitation, the core coating material mixture will be dispersed in the liquid manufacturing vehicle phase to obtain the microspheres of appropriate size. The mixture will be then heated (if necessary) to evaporate the solvent in the polymer. In the case in which the core material is dispersed in the polymer solution, polymer shrinks around the core.

In the case in which core material is dissolved in the coating polymer solution, a matrix - type microsphere is formed. Once all the solvent evaporated, the liquid vehicle temperature is reduced to ambient temperature (if required) with continued agitation. At this stage, the microspheres can also be used in suspension form, coated on to substrates or isolated as powders.

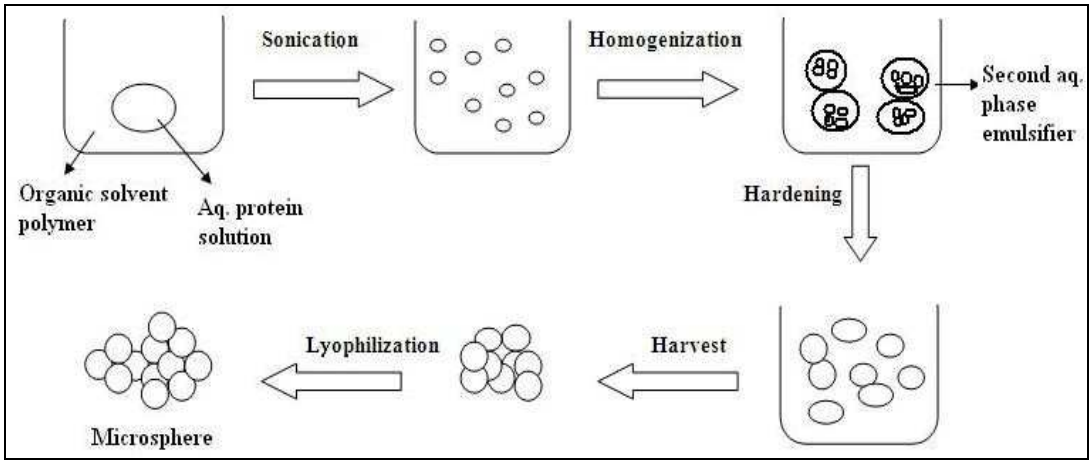


Figure 1.13: Solvent evaporation process

The solvent evaporation technique is used to produce microspheres which are applicable to a wide variety of liquid and solid core materials. The core materials may be either water - soluble or water - insoluble materials. e.g. “Evaluation of Sucrose Esters as Alternative Surfactants in Microspheres of Proteins by the Solvent Evaporation Method”.

Centrifugal extrusion

Liquids are been encapsulated using a rotating extrusion head containing concentric nozzles. In this particular process, a core liquid is surrounded by a sheath of wall solution or melt. As the jet moves through the air it will break, owing to Rayleigh instability, core droplets are formed, each coated with the wall solution. From the droplets formed, a molten wall will be hardened or a solvent may be evaporated

from the wall solution. This process is excellent for forming making particles 400–2,000 μm (16–79 mils) in diameter.

Vibrational Nozzle

Micro granulation (matrix-encapsulation) can be done by using a laminar flow through a nozzle and an additional vibration of the nozzle. The vibration has to be done in resonance and Rayleigh instability leads to form uniform droplets. The liquid should be with limited viscosities (0-10,000 mPa·s have been shown to work), e.g. solutions, emulsions, suspensions, melts etc. The solidification can be done with an internal gelation (e.g. sol-gel processing, melt) or an external (additional binder system, e.g. in a slurry). The process works for generating droplets between 100–5,000 μm (3.9–200 mils), for preparing smaller and larger droplets are known.

Inter facial polymerization

In Interfacial polymerization, polycondensation of the two reactants occur in between. The basis of this method termed as the classical Schotten-Baumann reaction between compound an acid chloride containing an active hydrogen atom, such as an amine or alcohol, polyesters, polyurea, polyurethane.

Matrix polymerization

In this method, the particle is formed by evaporation of the solvent from the matrix material. However, the solidification of the matrix also can be caused by a chemical change in a number of processes; a core material is dissolved in a polymeric matrix during formation of the particles. A simple example of this kind is actually Spray Drying method, in which the particle is formed by evaporation of the solvent from theMatrix material. However the chemical change can also cause solidification of materials.

1.13. Release kinetics patterns of microspheres

Although, the aim of the microsphere is to protect the core by surrounding wall. The wall may get ruptured at the time of usage. microsphere contents may get ruptured by melting the wall, dissolving it under particular conditions , as in the case of an enteric coating , in other system it get ruptured by solvent action , enzyme action.

Micro sphere can be used to slow the release of a drug into the body. This may help for the controlled release dose to substitute for several doses of non-encapsulated drug and also may decrease toxic side effects for some drugs by preventing high initial concentrations in the blood.

1.14. Applications of microspheres *(Jain N. K.et al., 2004)*

1. Microspheres in vaccine delivery.
2. Targeting using micro particulate carriers
    a. Targeting may be provided by:
    b. By controlling the size of the microspheres
    c. By conjugation with antibodies
    d. By incorporation of magnet particle
3. Monoclonal antibodies mediated microspheres targeting
4. Chemo embolisation
5. Imaging
6. Topical porous microspheres
7. Surface modified microspheres

LITERATURE SURVEY…

2. LITERATURE SURVEY

Literature review indicating advancement in Microsphere drug delivery system is given by:

Akanksha Garud., et al.(2010) Metronidazole microcapsules with a coat consisting of alginate and the natural cationic polymer, chitosan were formulated by using tripolyphosphate cross-linking method and were investigated with a view to develop mucoadhesive microcapsules. The microcapsules were evaluated for their surface morphology, microencapsulation efficiency, in-vitro wash-off test, swelling behavior and in-vitro drug release. The microcapsules formed had rough surface morphology in scanning electron microscopy. The drug entrapment efficiency was found to be in the range 75.2±1.31% and 82.1±0.75%. Chitosan microcapsules displayed a limited amount of swelling which is supposed to be related to the degree of cross-linking with tripolyphosphate. The microcapsules showed better mucoadhesive property at intestinal pH 7.4 than at gastric pH 1.2 in the in-vitro wash-off test. The drug release was found to be slow and extended over long duration of time.

Bhatt jh., et al. (2009) were prepared Metronidazole Microsphere employing sodium alginate in combination with four mucoadhesive polymers – sodium CMC, Methylcellulose, Carbopol and HPMC-K4M as coat materials with different polymers ratios. The microspheres were found to discrete, spherical, free flowing, and of the monolithic matrix type. The mucoadhesive microspheres were evaluated by in vitro and in vivo methods using Gamma Scintigraphy for controlled release.

Devraj., et al. (2010) present article deals with an oral dosage form proposed for the attainment of timed release drug delivery of metronidazole. Metronidazole containing matrix tablets coated with 3,4,5 & 6% w/v cellulose acetate phthalate in acetone were examined for applicability as timed

release tablets with a predetermined lag time of 4-5 hrs. Different types of enteric coated tablets were prepared and there drug dissolution profile was studied in 0.1 N HCl (0 to 2hr) and PBS 6.8 (2-24 hr) as dissolution media at 37±0.5 ºC,100 rpm by USP Apparatus-1(Basket assembly). The result indicated that the tablets with timed release functions could be prepared and,that the lag time were increased as the coat concentrations increased (3% to 6%w/v).The different kinds of timed release enteric coated tablets that showed lag time of 2 to 5.4 hrs in in- vitro dissolution in 2% w/v rat caecal content in 6.8 PBS(Phosphate buffer saline). The lag time showed a good agreement between the in- vitro test in PBS 6.8 and in -vitro test in 2% w/v rat caecal content. However the lag times were 4.5 hrs in in-vitro test in 2%w/v rat caecal content medium.

Gupta AK ., et al. (2011) The aim of current work to develop and evaluate sustained release mucoadhesive (SRM) microspheres of Repaglinide using emulsification solvent evaporation technique. Effects of formulation variables i.e. polymer concentration and phase volume ratio on particle size, % mucoadhesion and drug release were investigated in this study. Scanning electron microscopy of microspheres with maximum drug content (FormulationCH1:8) demonstrated smooth surface spherical particles with mean diameter of 64.78 ± 3.26 μm. The mean Particle size, % drug loading and mucoadhesion were found to vary by changing the formulation variables. Microspheres size was significantly increased as increasing the polymer concentration in the aqueous phase while size of microspheres decrease as increase in volume of continuous phase. Decrease in size of microspheres leads to decrease in mucoadhesion time, % drug loading and faster the drug release. It can be concluded that the present mucoadhesive microspheres can be an ideal system to deliver the Repaglinide in sustained release manner for management of Type II Diabetes Mellitus.

Madhavi Boddupalli B., et al. (2010) *was* to formulated and evaluated mucoadhesive microsphers of Venlafaxine Hydrochloride by using carbopol and HPMC K4M as mucoadhesive polymers. There was sustained release up to 12 hours and almost 70% of mucoadhesion was observed after 12 hours. The results were encouraging and further studies are required for in-vivo efficiency.

Malay Das K., et al. (2008) were developed of diltiazem-loaded mucoadhesive microspheres successfully prepared by emulsification-internal gelation technique using different polymers. 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. The wash-off was faster at simulated intestinal fluid (phosphate buffer, pH 7.4) than that at simulated gastric fluid (0.1 M HCl, pH 1.2). The *in vitro* drug release mechanism was non-fickian type controlled by swelling and relaxation of polymer.

Mahendra Singh., et al. (2011) had prepared by emulsion cross linking method using Glutaradehyde as a cross linking agent. Gelatin A and Chitosan were used as polymer and co polymer respectively. All the prepared microspheres were evaluated for physical characteristics, such as particle size, incorporation efficiency, swelling index, *in vitro* bioadhesion using rat jejunum and *in vitro* drug release in pH 6.6 phosphate buffer. The data indicates the verapamil hydrochloride release followed Higuchi's matrix and Peppas model. Stability studies showed stability of formulation at all the conditions to which the formulations were subjected.

Nagda Chirag., et al. (2009) were designed, characterized and evaluated bioadhesive microspheres of ACE employing polycarbophil as bioadhesive polymer. Bioadhesive microspheres of ACE were prepared by double emulsion solvent evaporation method. The *in-vitro* release studies were performed using pH 6.8 phosphate buffer. The drug loaded

microspheres in a ratio of 1:5 showed 38 % of drug entrapment, percentage mucoadhesion was 79 % and 89 % release in 10 h. The in vitro release profiles from microspheres of different polymer-drug ratios followed Higuchi model.

Nishanth Kumar N., et al. (2011) was formulated and evaluated gliclazide mucoadhesive microsphere using hydroxypropylmethylcellulose K4M and carboxymethylcellulose as polymers were prepared by simple emulsification phase separation technique using glutaraldehyde as across-linking agent. Twenty preliminary trial batches, F1 to F20 batches of microspheres were prepared by using different volume of cross-linking agent, cross-linking time and 3:1 polymer-to-drug ratio. Among the two polymers, the best batch was hydroxypropylmethylcellulose K4M exhibited a high drug entrapment efficiency of 69% and a swelling index1.16 % mucoadhesive after 1hour was 70% and the drug release was also sustained for more than 10 h.

Ofokansi KC., et al.(2007) had formulated ceftriaxone sodium-loaded mucoadhesive microspheres by the emulsification cross-linking method using arachis oil as the continuous phase. The release profile of ceftriaxone sodium from the microspheres was evaluated in both simulated gastric fluid (SGF) without pepsin (pH 1.2) and simulated intestinal fluid (SIF) without pancreatin (pH 7.4). Release of microspheres by diffusion following non-Fickian transport mechanism and was higher and more rapid in SIF than in SGF. The results obtained from this study may indicate that ceftriaxone sodium could be successfully delivered rectally when embedded in microspheres formulated with either type a gelatin alone or its admixtures with porcine mucin.

Patil P.B., et al. (2009) Mucoadhesive microspheres were prepared by an inter polymer complexation poly(acrylic acid) (PAA) with poly(vinyl pyrrolidone) (PVP) to increase gastric residence time and a solvent diffusion method. The complexation between poly(acrylic acid) and

poly(vinyl pyrrolidone) as a result of hydrogen bonding wasconfirmed by the shift in the carbonyl absorption bands of poly(acrylic acid) using FT-IR. A mixture of ethanol/water was used as the internal phase, corn oil was used as the external phase of emulsion, and span 80 was used as the surfactant. Spherical microspheres were prepared. Theparticle size increased as the content of water was increased. The mean particle size increased with the increase in polymer concentration. The adhesive force ofmicrospheres was equivalent to that of Carbopol. The release rate of atenolol from the complex microspheres was slower than the PVP microspheres at pH 2.0 and 6.8.

Rajeshwar kamal kant ., et al. (2010) had characterized of mucoadhesive microspheres with Famotidine as model drug for prolongation of gastric residence time Using mucoadhesive polymers sodium CMC and sodium alginate. *In vitro* drug release studies were performed and drug release evaluated. The prepared microspheres exhibited prolonged drug release (8h). The *In vitro* studies demonstrated diffusion-controlled drug release from the microspheres.

Ram Chand Dhakar., et al. (2010) were prepared and evaluated by emulsification solvent evaporation method using Sodium carboxy methyl cellulose (SCMC), Carbopol 934P (CP), and Hydroxyl propyl methyl cellulose K4M (HPMC) as a mucoadhesive polymers. Microspheres prepared were found discrete, spherical and free flowing. Among all the formulation, formulation F1 containing SCMC and F2 containing CP showed the best reproducible results and mucoadhesive profile with good surface morphology.

Sambathkumar R., et al. (2011) had formulated and systematically evaluated *in vitro* performances of Furazolidone mucoadhesive microspheres were prepared by simple emulsification phase separation technique using Eudragit RS100 as matrix and Carbopol 974P and Hydroxy propyl methyl cellulose K4M as mucoadhesive polymer. The prepared

microspheres were evaluated with respect to the particle size, encapsulation efficiency, shape and surface properties, mucoadhesive property, *in vitro* drug release and suitability for anti *Helicobacter pylori* effect. The best batch exhibited a high drug entrapment efficiency of 82.12 % and percentage mucoadhesion after 1 h was 93.35 %. The drug release was also sustained up to 12 h.

Senthil A., et al. (2011) were prepared by glipizide microspheres containing chitosan simple emulsification phase separation technique using glutaraldehyde as a cross-linking agent. Microspheres were discrete, spherical, and free flowing. The microspheres exhibited good mucoadhesive property in the in vitro wash-off test and also showed high percentage drug entrapment efficiency. A 3^2 full factorial design was employed to study the effect of independent variables, polymer-to-drug ratio (X1), and stirring speed (X2) on dependent variables percentage mucoadhesion, t80, drug entrapment efficiency, and swelling index. Percentage mucoadhesion after 1 hour was 78%. The drug release was also sustained for more than 12 hours.

Saravanakumar K., et al. (2011) were formulated and developed of naproxen sodium microsphere two investigated factors (independent variables) were the stabilizer agent concentration in the aqueous phase (%w/v PVA) and the polymer concentration in the organic phase (%w/v HPMC K15M).The results showed that encapsulation efficiency was significantly affected by the two investigated factors, with PVA concentration having a highly negative effect, probably due to naproxen sodium's solubility enhancement in the aqueous phase in the presence of higher amounts of stabilizer. These results demonstrate that it is possible to control the quantity of drug loaded in the microspheres.

Shiv Shankar Hardenia., et al. (2011) were prepared and evaluated ethylcellulose microspheres containing ciprofloxacin for in-vitro performance of ciprofloxacin. Ciprofloxacin microspheres containing ethylcellulose were prepared by emulsion solvent diffusion evaporation method. The best cumulative release was achieved after 24 hrs i.e. 91.6%. The Mucoadhesive property of the ethylcellulose microspheres was evaluated by in-vitro wash off test. The microspheres exhibited 75% mucoadhesion and showed good drug entrapment efficiency. By, above results it was concluded that ethylcellulose microspheres showed reproducible results, with good Mucoadhesive properties and good surface morphology.

Venkateswaramurthy N., et al. (2010) was to designed and characterized mucoadhesive microspheres containing Clarithromycin as an anti-*H. pylori* agent to deliver the drug specifically to mucus layer where *H.pylori* resides and evaluate the effectiveness of the mucoadhesive microspheres for *H. pylori* eradication therapy. Microspheres were prepared by using Eudragit RL100 as matrix and Carbopol 974P as a mucoadhesive polymer. The microspheres were prepared by emulsion solvent evaporation technique. The prepared microspheres were evaluated with respect to the particle size, production yield, encapsulation efficiency, shape and surface properties, mucoadhesive property, *in vitro* drug release and suitability for anti *Helicobactorpylori* effect.

Veena Belgamwar., et al. (2009) was to prepared and evaluated mucoadhesive multiparticulate system for oral drug delivery using ionic gelation technique. Microspheres composed of various mucoadhesive polymers including HPMC of various grades like K4M, K15M, K100M, E50LV, Carbopol of grades 971P, 974P and polycarbophil were prepared. In this technique cross linking of sodium alginate with calcium chloride was done which retarded the release of drug from the mucoadhesive polymer. Metoprolol release from the multiparticulate

system was regulated and extended until 12 hours and exhibited a non fickian drug release kinetics approaching to zero order, as evident from the release rate exponent values which varied between 0.57 to 0.73. The stability studies performed on the optimized batches at 40°C / 75% RH for 90 days.

Yadav N., et al. (2011) had to formulate and evaluated sustained release mucoadhesive microspheres of Acyclovir loader Sodiumcarboxymethylcellulose and hydrxypropylmethylcellulose were used as mucoadhesive polymers. The microspheres were prepared using solvent evaporation technique. The results of mucoadhesion study showed better retention of Sodium CMC microspheres (8.0±0.8 h) in duodenal and jejunum regions of intestine. Overall, the result indicated prolonged delivery with significant improvement in oral bioavailability of acyclovir from mucoadhesive microspheres due to enhanced retention in the upper GI tract.

DRUG AND POLYMER PROFILE…

3. DRUG PROFILE

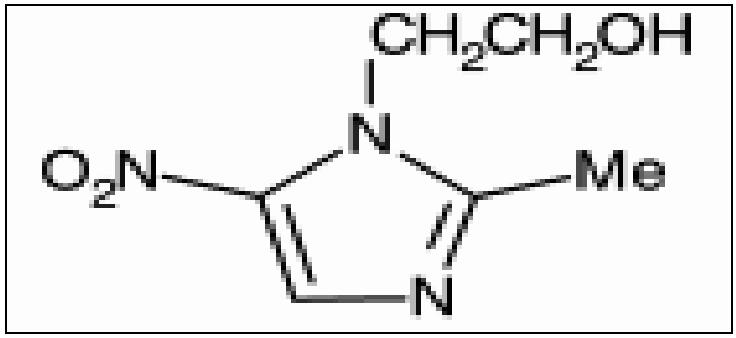
3.1METRONIDAZOLE *(IP,2007)*

Metronidazole is a 5-nitroimidazole derivative with activity against protozoa like *Balantidium coli, Blastocystis hominis, Entamoeba histolytica, Giardia intestinalis and Trichomonas vaginalis.*

The drug also has well-established bactericidal activity against obligate anaerobic bacteria *invitro*, including Gram-negative organisms.

Chemistry

Metronidazole belongs to the class of nitroimidazoles. It's full chemical name is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol or 1(2-hydroxyethyl) 2 methyl-5- nitroimidazole



Pharmacology

The mode of action involves reduction of Metronidazole by bacterial nitroreductases to an unstable intermediate, which interacts with DNA, effectively preventing further replication.

Pharmacokinetics

Absorption

After oral administration, Metronidazole is well absorbed from the gastrointestinal tract, to the extent of about 100%. Peak plasma concentrations of approximately 5 and 10mcg/ml

are achieved usually within 1 to 2 hours, after single doses of 250 and 500mg respectively.

Distribution

Metronidazole is widely distributed. It appears in most body tissues and fluids including saliva, bile, seminal fluid, breast milk, CSF bone, liver and liver abscesses, lungs and vaginal secretions, crosses the placenta and blood brain barrier. The volume of distribution is 0.55 L/kg in adults and 0.54- 0.81 L/kg in neonates. Protein binding of metronidazole is less than 20%.

Metabolism

Metronidazole is metabolized in the liver primarily by side chain oxidation and glucuronide conjugation. The principal oxidative metabolites are 1-(2-hydroxyethyl)-2- hydroxymethyl-5-nitroimidazole, which has antibacterial activity and is detected in the plasma and urine, and 2-methyl-5-nitroimidazole -1- acetic acid, which has no antibacterial activity and is often not detected in plasma, but is excreted in urine.

Elimination

The mean elimination half – life of Metronidazole is about 6- 8 hours (range 6.0 to 12.0h). the majority of a dose of Metronidazole is excreted in the urine, mainly as metabolites, a small amount appears in the faeces.

Dosing Information

Metronidazole is prescribed in:

1. **Amoebiasis**: Metronidazole acts as an amoebicide at all sites of infection with *Entamaoeba histolytica*. Because of its rapid absorption it is probably less effective against parasites in the bowel lumen and is therefore used in conjunction with a luminal amoebicide such as diloxanide furoate.

Metronidazole is given in doses of 400 to 800mg three times daily by mouth for 5 to 10 days. Children aged 1 to 3 years may be given one quarter, those aged 3 to 7 years one third and those aged 7 to 10 years one half the adult dose. An alternative adult dose is 1.5to 2.5 g as a single dose daily for 2 to 3 days.

2. Giardiasis: The usual dose is 2g once daily by mouth for 3 successive days, or 400mg three times daily for 5 day, or 500mg twice daily for 7 to 10 days.

3. Trichomoniasis: Metronidazole is given orally either as a single 2 g dose, as a 2-day course of 800mg in the morning and 1.2g in the evening, or as a 7-day course of 600mg to 1g daily in two or three divided doses.

4. Bacterial vaginosis: the usual dose is a single 2g dose or a 500mg twice daily for 5 to 7 days.

5. Anaerobic bacterial infection: Metronidazole is given by mouth in an initial dose of 800mg followed by 400mg every 8 hours, usually for about 7 days. A regimen of 500mg every 8 hours is alternatively used.

6. Peptic Ulcer disease: Metronidazole is used in combination therapy to eradicate Helicobacter pylori. The usual dose is 400mg twice daily. When given along with omeprazole and amoxycillin, the dose is 400mg thrice daily. The treatment is continued for 1 week.

Contraindications

- Hypersensitivity to Metronidazole
- Pregnancy
- Breast-feeding Concominant anti-coagulant therapy
- Blood dyscrasia, prior or current history
- Severe hepatic function impairment

Adverse Effects

The adverse effects of Metronidazole are generally dose related. The most common are gastrointestinal disturbances, especially nausea and an unpleasant metallic taste. Vomiting and diarrhoea or constipation may also occur. Furred tongue, glossitis and stomatatis may be associated with an overgrowth of *Candida*. Weakness, dizziness, ataxia, headache, drowsiness, insomnia and changes in mood or mental state such as depression or confusion have also been reported. Peripheral neuropathy, usually presenting as numbness or tingling in the extremities and epileptiform seizures are serious adverse effects on the nervous system that have been Associated with high doses of metronidazole on prolonged treatment.

3.2. POLYMERS PROFILE:

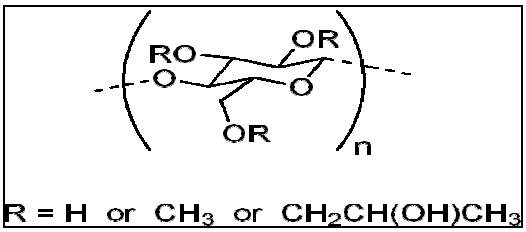
(Raymond C,Rowe.,et al., 2003)

3.2.1. HYDROXY PROPYL METHYL CELLULOSE

Synonyms: Benecel, HPMC, Methocel, Hydroxy propyl methyl cellulose

Molecular weight: 10,000-15,000

Structure:



Description : slightly off-white to beige powder in appearance and may be formed into granules.

Colour : white to yellowish white

Odour : odorless or nearly odorless

Taste : bland taste

Texture : powder

Acidity / Alkalinity : pH 5.5-8.0 for a 1%w/w aqueous solution.

V**iscosity for 2 %(w/v) aqueous solution:** 4000mpas (Viscosity measured at 200C)

Solubility:

Soluble in cold water, forming a viscous colloidal solution, practically insoluble in mixtures of ethanol and dichloromethane, mixtures of alcohol and water

Functional category:

Coating agent, film former, and rate controlling polymer for sustained release, stabilizing agent, suspending agent and viscosity builder.

Applications in pharmaceutical technology:

High viscosity grades may be used to retard the release of drugs from a matrix at levels of 10-80%w/w in tablets and capsules.

Stability and Storage:

Stable between pH 3-11, should be stored in a well-closed container in a cool and dry place.

Incompatibilities:

Incompatible with some oxidizing agents such as hydrogen peroxide, potassium permanganate.

3.2.2SODIUM ALGINATE

(Raymond C,Rowe.,et al., 2003)

Nonproprietary Names:

BP: Sodium Alginate

PhEur: Sodium Alginate

USP-NF: Sodium Alginate

Synonyms:

Alginato sodico; algin; alginic acid, sodium salt; E401; Kelcosol; Keltone; natrii alginas; Protanal; sodium polymannuronate.

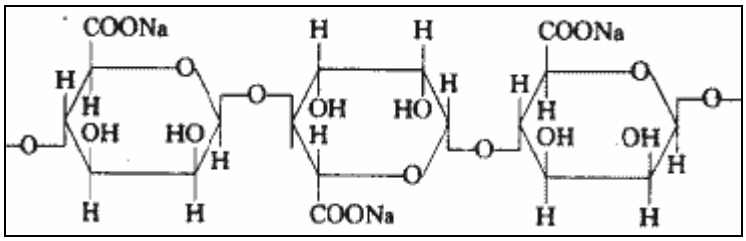
Chemical Name: Sodium alginate

CAS Registry Number: [9005-38-3]

Empirical Formula and Molecular Weight:

Sodium alginate consists chiefly of the sodium salt of alginicacid, which is a mixture of polyuronic acids composed of residues of Dmannuronicacid and L-guluronic acid.

Structural Formula:



Functional Category

Stabilizing agent; suspending agent; tablet and capsule disintegrant;

Description

Sodium alginate occurs as an odorless and tasteless, white to pale yellowish-brown colored powder

Colour **:** pale yellowish-brown

Odour : odorless.

Taste : tasteless

Texture **:** powder

Acidity/alkalinity: pH _ 7.2 (1% w/v aqueous solution)

Solubility:

Practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water mixtures in which the ethanol content is greater than 30%. Also, practically insoluble in other organic solvents and aqueous acidic solutions in which the pH is less than 3. Slowly soluble in water, forming a viscous colloidal solution.

Stability and Storage Conditions:

Sodium alginate is a hygroscopic material, although it is stable if stored at low relative humidities and a cool temperature. Aqueous solutions of sodium alginate are most stable at pH4–10. Below pH 3, alginic acid is precipitated. A 1% w/v aqueous solution of sodium alginate exposed to differing temperatures had a viscosity 60–80% of its original value after storage for 2 years. Solutions should not be stored in metal containers. Sodium alginate solutions are susceptible on storage to microbial spoilage, which may affect solution viscosity. Solutions are ideally sterilized using ethylene oxide, although filtration using a 0.45 mm filter also has only a slight adverse effect on solution viscosity. Heating sodium alginate solutions to temperatures above 70°C causes depolymerization with a subsequent loss of viscosity. Autoclaving of solutions can cause a decrease in viscosity, which may vary depending upon the nature of any other substances present. Gamma irradiation should not be used to sterilize sodium alginate solutions since this process severely reduces solution viscosity. Preparations for external use may be preserved by the addition of 0.1% chlorocresol, 0.1%

chloroxylenol, or parabens. If the medium is acidic, benzoic acid may also be used. The bulk material should be stored in an airtight container in a cool, dry place.

Incompatibilities:

Sodium alginate is incompatible with acridine derivatives, crystal violet, phenyl mercuric acetate and nitrate, calcium salts, heavy metals, and ethanol in concentrations greater than 5%. Low concentrations of electrolytes cause an increase in viscosity but high electrolyte concentrations cause salting-out of sodium alginate; salting-out occurs if more than 4% of sodium chloride is present.

Applications in Pharmaceutical Formulation or Technology:

1. Sodium alginate is used in a variety of oral and topical pharmaceutical formulations.
2. In tablet formulations, sodium alginate may be used as both a binder and disintegrant ; it has been used as a diluent in capsule formulations.
3. Sodium alginate has also been used in the preparation of sustained-release oral formulations since it can delay the dissolution of a drug from tablets, capsules and aqueous suspensions.
4. The effects of particle size, viscosity and chemical composition of sodium alginate on drug release from matrix tablets have been described.
5. In topical formulations, sodium alginate is widely used as a thickening and suspending agent in a variety of pastes, creams, and gels, and as a stabilizing agent for oil-in-water emulsions.
6. Recently, sodium alginate has been used for the aqueous microencapsulation of drugs, in contrast with the more conventional microencapsulation techniques which use organic solvent systems. It has also been used in the formation of nanoparticles

Table1 3.1.Sodium alginate is also used in cosmetics and food products

USE	CONCENTRATION (%)
Pastes and creams	5-10
Stabilizer in emulsions	1-3
Suspending agent	1-5
Tablet binder	1-3
Tablet disintegration	2.5-10

3.2.3 Methylcellulose

(Raymond C,Rowe.,et al., 2003)

Nonproprietary Names:

BP: Methylcellulose

JP: Methylcellulose

PhEur: Methylcellulose

USP: Methylcellulose

Synonyms:

Benecel; Cellacol; Culminal MC; E461; Mapolose; Methocel; methylcellulosum; Metolose; Tylose; Viscol.

Chemical Name and CAS Registry Number:

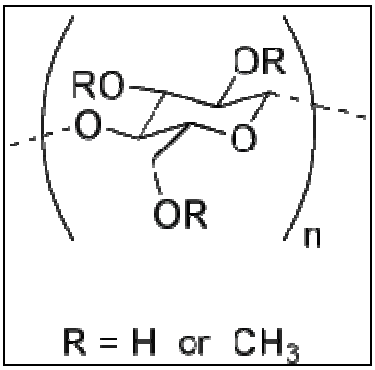
Cellulose methyl ether [9004-67-5]

Empirical Formula and Molecular Weight:

Methylcellulose is long-chain substituted cellulose in which approximately 27–32% of the hydroxyl groups are in the form of the methyl ether. The various grades of methylcellulose have degrees of polymerization in the range 50–1000, with molecular

weights (number average) in the range 10 000–220 000 Da. The degree of substitution of methylcellulose is defined as the average number of methoxyl (CH3O) groups attached to each of the anhydroglucose units along the chain. The degree of substitution also affects the physical properties of methylcellulose, such as its solubility.

Structural Formula:



Description

Methylcellulose occurs as a white, fibrous powder or granules. It is practically odorless and tasteless. It should be labeled to indicate its viscosity type (viscosity of a 1 in 50 solution).

Functional Category

Coating agent, emulsifying agent, suspending agent, tablet and capsule disintegrant, tablet binder, viscosity-increasing agent.

Applications in Pharmaceutical Formulation or Technology

1. Methylcellulose is widely used in oral and topical pharmaceutical formulations
2. In tablet formulations, low- or medium-viscosity grades of methylcellulose are used as binding agents, the methylcellulose being added either as a dry powder or in solution. Highviscosity grades of methylcellulose may also be incorporated in tablet formulations as a disintegrant.

3. Methylcellulose may be added to a tablet formulation to produce sustained-release preparations.
4. Tablet cores may also be spray-coated with either aqueous or organic solutions of highly substituted low-viscosity grades of methylcellulose to mask an unpleasant taste or to modify the release of a drug by controlling the physical nature of the granules.
5. Methylcellulose coats are also used for sealing tablet cores prior to sugar coating.
6. Low-viscosity grades of methylcellulose are used to emulsify olive, peanut, and mineral oils.
7. They are also used as suspending or thickening agents for orally administered liquids, methylcellulose commonly being used in place of sugar-based syrups or other suspension bases.
8. Methylcellulose delays the settling of suspensions and increases the contact time of drugs, such as antacids, in the stomach.
9. High-viscosity grades of methylcellulose are used to thicken topically applied products such as creams and gels.
10. In ophthalmic preparations, a 0.5–1.0% w/v solution of a highly substituted, high-viscosity grade of methylcellulose has been used as a vehicle for eye drops. However, hypromellose-based formulations are now preferred for ophthalmic preparations.
11. Methylcellulose is also used in injectable formulations. Therapeutically, methylcellulose is used as a bulk laxative; it has also been used to aid appetite control in the management of obesity,but there is little evidence supporting its efficacy.

3.2.4 Calcium Chloride

(Raymond C,Rowe.,et al., 2003)

Nonproprietary Names

BP: Calcium Chloride Dihydrate, Calcium Chloride Hexahydrate

JP: Calcium Chloride Hydrate

hEur: Calcium Chloride Dihydrate

Synonyms

Calcium chloridum dihydricum; calcium chloridum hexahydricum.

Chemical Name and CAS Registry Number

Calcium chloride anhydrous [10043-52-4]

Calcium chloride dihydrate [10035-04-8]

Calcium chloride hexahydrate [7774-34-7]

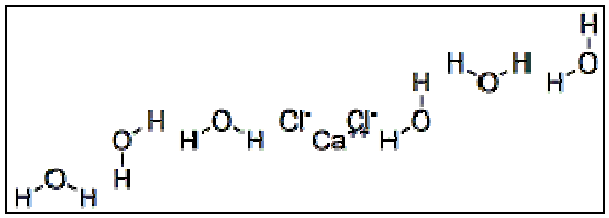
Empirical Formula and Molecular Weight

CaCl2 110.98 (for anhydrous)

CaCl2_2H2O 147.0 (for dihydrate)

CaCl2_6H2O 219.1 (for hexahydrate)

Structural Formula



Functional Category

Antimicrobial preservative; therapeutic agent; water-absorbinggent.

Description

Calcium chloride occurs as a white or colorless crystalline powder, granules, crystalline mass, and is hygroscopic (deliquescent).

Applications in Pharmaceutical Formulation or Technology

The main applications of calcium chloride as an excipient relate to its dehydrating properties and therefore it has been used as an antimicrobial preservative, as a desiccant, and as an astringent in eye lotions.

Therapeutically, calcium chloride injection 10% (as the dehydrate form) is used to treat hypocalcaemia.

Stability and Storage Conditions

Calcium chloride is chemically stable; however, it should be protected from moisture. Store in airtight containers in a cool, dry place.

Incompatibilities

- Calcium chloride is incompatible with soluble carbonates, phosphates, sulfates, and tartrates.
- It reacts violently with bromine trifluoride, and a reaction with zinc releases explosive hydrogen gas.
- It has an exothermic reaction with water, and when heated to decomposition it emits toxic fumes of chlorine.

AIM & OBJECTIVES....

4. AIM AND OBJECTIVES

Amoebiasis is an infection caused by *Entamoeba histolytica*, a single celled protozoan parasite. The current estimate is that *E. histolytica* causes between 34-50 million symptomatic infections leading to the death of 40-100 thousands of people, which makes Amoebiasis second only to malaria as a cause of death resulting from protozoan parasite. The most preferred choice of drugs for Amoebiasis is metronidazole.

. The present study was aimed to develop and evaluate mucoadhesive microspheres of Metronidazole for better treatment of Amoebiasis and other protozoal infections. metronidazole could prevent unwanted systemic side effects and subsequently a lower dose of the drug may be sufficient to treat protozoal infections.

Objectives:

The development of efficient orally delivered mucoadhesive drug delivery system includes advantages like:

- Maximized absorption rate is mainly due to intimate contact of drug with the mucus membrane to improve and enhance bioavailability of drugs.
- Drug protection is improved by polymer encapsulation and longer gut transit time is obtained, resulting in extended periods for absorption.
- Multiple dosing is avoided and there by counteracts the side effects.
- The main objective is mainly to develop mucoadhesive microspheres of Metronidazole by orifice-ionic gelation process using mucoadhesive polymers release of the drug for extended period of time.
- Formulate and evaluate the microspheres of Metronidazole.
- To study the effect of different polymers and different ratios of polymers employed.
- Performing the stability studies as per ICH guidelines.

PLAN OF WORK....

5. PLAN OF WORK

- **Literature survey**.
- **Materials and equipments.**
- **Pre formulation studies.**
  - **Characterization of Drug.**
    - Appearance.
    - Melting Point Determination.
    - Solubility Study.
    - UV Spectroscopy (λ_{max}).
    - IR Spectroscopy.
    - Loss on drying.
  - **Drug - Polymers Interaction Studies.**
    - Fourier transforms Infra-Red (FTIR) Spectroscopy Study.
    - Differential Scanning Calorimetry (DSC) Analysis.
  - **Preparation of Metronidazole mucoadhesive microspheres.**
  - **Evaluation of Metronidazole mucoadhesive microspheres.**
    - # Percentage yield
    - # Particle Size analysis
    - # Drug content estimation and Encapsulation efficiency
    - # Percentage moisture content
    - # Scanning electron microscopy
    - # *In -vitro* wash - off test for Mucoadhesion
    - # *In -vitro* drug release studies.

- **Results and Discussion.**
- **Summary and Conclusion.**
- **Future Prospects.**
- **Bibliography.**

MATERIALS & EQUIPMENTS....

6. MATERIALS AND EQUIPMENTS

6.1. List of Materials used with Sources

Table 6.1: List of Materials and their Suppliers

S.No.	Name of Material	Supplied by
1	Metronidazole	Bindu pharmaceuticals, Hyderabad.
2	Sodium alginate	Bindu pharmaceuticals, Hyderabad.
3	Hydroxy propyl methyl cellulose	Loba chemie pvt ltd,Mumbai
4	Methyle cellulose	Loba chemie pvt ltd,Mumbai
5	Calcium chloride	Qualigens fine chemicals, Mumbai

6.2. List of Equipments used with model:

Table 6.2 : List of equipments with their make

S.No.	Name of the equipment	Make
1	Electronic balance	Shimadzu, Japan
2	UV-Visible spectrophotometer	Shimadzu, Japan
3	Standared coating pan	Ganson-india
4	FTIR Spectrophotometer	Shimadzu
5	DSC test apparatus	Mettler Teldo
6	Dissolution test apparatus	Vigo Scientifics, Mumbai
7	Digital pH meter	Elico Scientifics, Mumbai
8	Hot air oven	Precision scientific co., Chennai
9	Humidity chamber	Lab tech, Ambala
10	Tap density apparatus	Indo labs, Chennai
11	Melting point test apparatus	Precision scientific co., Chennai
12	Optical microscope	Mettler Toledo
13	SEM	Merlin-FE-SEM

EXPERIMENTAL WORK....

7. EXPERIMENTAL WORK

7.1. PREFORMULATION STUDY

Before formulating a product, the physical and chemical properties of a drug substance have undergone some pre-formulation testing. It is the first step in rational development of dosage form.

7.1.1. Identification of drug

7.1.1. a) Identification by FTIR spectroscopy *(Skoog D.A., et al., 1996; IP,2007)*

Metronidazole discs were prepared by pressing the Metronidazole with potassium bromide and the spectra in between 4000 to 500 cm^{-1} was obtained under the operational conditions. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum represented in Table 8.1 and shown in Figure 8.1.

7.1.1. b) Identificationby melting point *(Moffat A C. et al.,2004)*

Melting point of the drug was determined by capillary tube method.

7.1.2. Physicochemical parameters

7.1.2. a) Organoleptic properties *(Lachman L.,et al.,1991)*

The color, odor and taste of the drug were recorded using descriptive terminology.

7.1.2. b) Solubility study ***(****Moffat A C ., et al.,2004)*

It is important to know about solubility characteristic of a drug in aqueous system, since they must possess some limited aqueous solubility to elicit a therapeutic response. The solubility of drug was recorded by using various descriptive terminologies. The solubility profile was represented in Table 8.2.

7.1.3. Analytical methods

7.1.3. a) Determination of λ max *(Swamy P.V., et al., 2007; USP,2009)*

The absorption maximum of the standard solution was scanned between 200-400 nm regions on UV-Visible spectrophotometer. The absorption maximum obtained with the substance being examined corresponds in position and relative intensity to those in the reference spectrum was shown in Figure 8.2.

7.1.3. b) Development of standard curve of Metronidazole

(IP,1997;USP,2009)

1} Preparation of (pH 1.2).

8.5ml concentrated Hydrochloric acid and 0.8gm of Sodium chloride were dissolved in 1000ml of distilled water.

Principle

Metronidazole showed maximum absorbance at 276 nm in pH 1.2 and obeyed Beer's law at the concentration range between 5-30 mcg/ml.

Procedure

Stock solution

Weighed quantity of Metronidazole (50mg) was dissolved in pH 1.2 and the volume was made up to 50ml with the same medium.

1000 mcg/ml (Standard stock solution - I**)**

1ml of SS I was then made up to 10ml with the same medium.

100 mcg/ml (Standard stock solution- II**)**

Aliquots of 0.5,1,1.5,2,2.5,3 ml of SSS II was pipetted into 10ml volumetric flasks and the volume was made upto 10ml with pH1.2. The absorbance was measured at 276 nm against reagent blank (pH 1.2).

2} Preparation of Phosphate Buffer (pH 7.4).

50ml of 0.2M potassium dihydrogen phosphate and 39.1 ml of 0.2 M NaOH were taken in a 200ml volumetric flask and the volume was made up with water.

Instrument

Elico UV-Visible Spectrophotometer SL 159.

Principle

Metronidazole showed maximum absorbance at 319 nm in Phosphate buffer pH 7.4 and obeyed Beer's law at the concentration range between 5-30 mcg/ml.

Procedure

Stock solution

Weighed quantity of Metronidazole (50mg) was dissolved in Phosphate buffer pH 7.4 and the volume made up to 50ml with Phosphate buffer pH 7.4.

1000 mcg/ml (Standard stock solution- I**).**

1ml of SS I was then made up to 10ml with the same medium

100 mcg/ml (Standard stock solution- II**).**

Aliquots of 0.5, 1, 1.5, 2, 2.5, 3 ml of SSS II was pipetted into 10ml volumetric flasks and the volume was made up to 10ml with Phosphate buffer pH 7.4. The absorbance was measured at 319 nm against reagent blank (Phosphate buffer pH7.4).

7.1.3. c) Determination of Percentage purity of Drug *(IP, 2007)*

Accurately weighed 100 mg of Metronidazole was dissolved in little quantity of methanol to get the concentration of 1mg/ml. The solution was pipetted out of about 0.5 ml to 3 ml and volume was made up with distilled water. From the above stock solution, the concentration and absorbance was observed. The absorbance was measured at 276 nm against the blank using by UV-Visible spectrophotometer. The percentage purity of drug was calculated by using calibration curve method(least square method). The data of percentage purity was represented in Table 8.5.

7.1.4. DRUG EXCIPIENT INTERACTION STUDIES

7.1.4. a) Determination of drug-polymer compatibility ***(****Aulton M.E.,et al.,2002)*

The proper design and formulation of a dosage form requires consideration of the physical, chemical and biological characteristics of all drug substances and excipients.

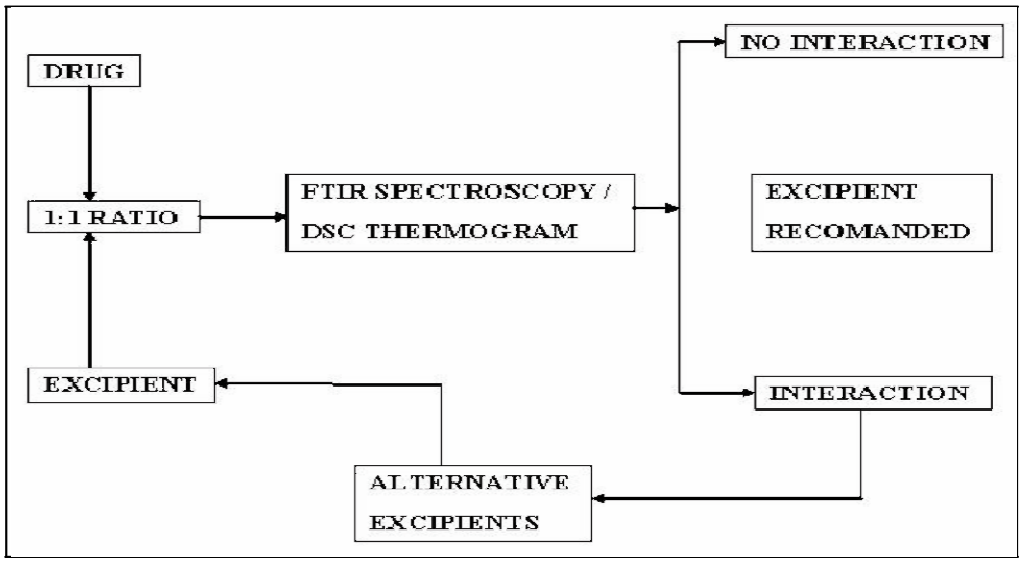


Figure 7.1: Schematic representation of compatibility studies

7.1.4. b) Fourier transform Infra-Red (FTIR) spectroscopy *(IP, 2007)*

FTIR study was carried out to check compatibility of drug with polymers. Fourier transform Infrared Spectrophotometer was determined by using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of Metronidazole and potassium bromide was run followed by Metronidazole with various polymers by using FTIR spectrophotometer. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum was represented in Table 8.6 and shown in Figure 8.4, 8.5, 8.6 and 8.7.

7.1.4. c) Differential scanning calorimetry (DSC) **(***AultonM.E., et al.,2002)*

Any possible drug polymer interaction can be studied by thermal analysis. The DSC study was performed on pure Metronidazole, Metronidazole with HPMC, Metronidazole with Methyl cellulose and Metronidazole with sodium alginate. The 2 mg of sample were heated in a hermetically sealed aluminum pans in the temperature range of 25-300ºC at heating rate of 10ºC /min under nitrogen flow of 30ml/min. The results of DSC analysis were represented in Table 8.7and showed in Figure 8.8, 8.9, 8.10 and 8.11.

7.2 METHOD OF PREPARATION OF METRONIDAZOLE MICROSPHERES (*Sambathkumar.r., et al.,2011)*

7.2 Table 7.1: Formulation of metronidazole microspheres

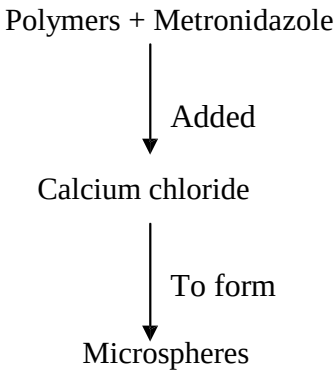
S.No.	Batch.No.	Drug (g)	Sodium alginate (%)	HPMC (%)	Methyle cellulose
1	F1	1	1.5	-	-
2	F2	1	2.5	-	-
;3	F3	1	3.5	-	-
4	F4	1	1.5	2	-
5	F5	1	2.5	2	-
6	F6	1	3.5	2	-
7	F7	1	1.5	-	1
8	F8	1	2.5	-	1
9	F9	1	3.5	-	1

7.2.1. Orifice ionic gelation method (syringes method) *(Swamy P.V., et al., 2007)*

In this technique cross linking was done with calcium chloride solution to release the drug in a controlled manner. Microspheres were prepared by using the Orife ionic gelation technique in which Sodium alginate, HPMC, Methylcellulose in different ratios as mentioned above was added to 32ml of water with continuous stirring to form homogenous solution Then sonicating was done for 20 minutes the drug Metronidazole was then added to the above solution to form a clear solution. The drug polymer mixture was poured in1.5% calcium chloride solution using 22# gauge

needle by stirring at 50rpm. The microspheres thus formed were allowed for 30 min Then the calcium chloride solution was decained and The formed mucoadhesive microspheres washed with distilled water and air dried over night at room temperature

The flow chart of preparation of Metronidazole microspheres



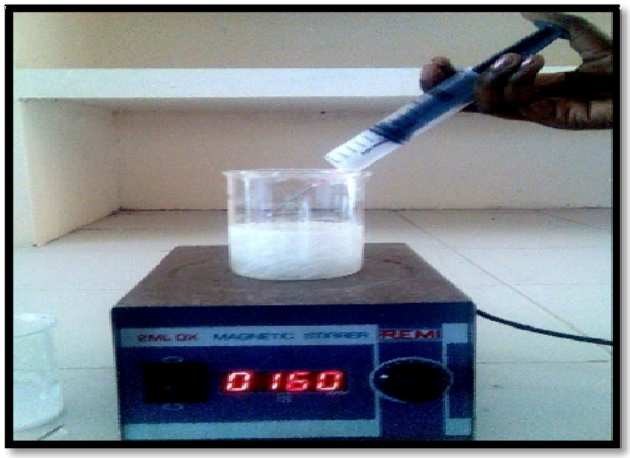


Figure.7.2: Preparation of microspheres using 22# syringe needle in magnetic stirrer

7.3. EVALUATION AND CHARACTERIZATION OF MICROSPHERES

(Bhabani Nayak S., et al., 2009)

Appropriate assessment of a dispersed system requires characterization of both chemical and physical stabilities. Physical properties are very important with respect to the performance of dispersed systems.

7.3.1Particle Size Determination

(Swamy P.V., et al.,2007)

Particle size distribution for the microspheres were measured by sieving method analysis, using set of standard sieves was weighed. Particles having size range between 50 and 1500 μm are estimated by sieving method. This method directly gives weight distribution. The sieving method is a useful application in dosage form development of tablets and spheres

7.3.2. Percentage Yield *(BhabaniNayak S., et al., 2009)*

The total amount of microspheres obtained were weighed and evaluated for percentage yield.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \text{ X } 100$$

7.3.3. Drug content estimation and Entrapment efficiency

(Swamy P.V., et al., 2007)

Metronidazole microspheres (100mg) from each batch were initially stirred in 3 ml sodium citrate solution (1%w/v) until complete dissolution. A quantity of 7 ml of methanol was added to above solution to solubilise the Metronidazole. The filtrate was assayed for drug content by measuring the absorbance at 276 nm after suitable dilution by UV-Visible spectrophotometer and Encapsulation efficiency was calculated using the formula,

$$\text{Encapsulation efficiency} = \frac{\text{Estimated \% drug content in microspheres}}{\text{Theoretical \% drug content in microspheres}} \times 100$$

7.3.4. Percentage moisture content: *(Bhabani Nayak S., et al., 2009)*

The Metronidazole loaded microspheres was evaluated to determine the percentage moisture content which sharing an idea about its hydrophilic nature. The microspheres weighed (w_1) initially kept in desiccator containing Calcium chloride at 37º C for 24 hours. The final weight (w_2) was noted when no further change in weight of sample was observed.

$$\text{Moisture Percentage} = \frac{W_1 - W_2}{W_2} \times 100$$

7.3.5. Scanning electron microscopy (SEM): *(Swamy P.V,.et al.,2007)*

The microspheres were observed under a Scanning Electron Microscopy. They were mounted directly onto SEM sample stub using double-sided sticking tape and coated with gold film with ion spillter with gold target with resolution 3 nm (30 KV HV Mode),10 nm (30 KV HV Mode), 40 nm (30 LV Mode) and a vacuum system is fitted to it.

7.3.6. *In -vitro* wash off test for mucoadhesion:

(BhabaniNayak S., et al., 2009)

The mucoadhesive property of the metronidazole microspheres was evaluated by an in -vitro adhesion testing method known as the wash – off method. Freshly excised pieces of intestinal mucosa (4×5 cm) from sheep were mounted onto glass slides (3×1inch) with poly cyanoacrylate glue. Two glass slides were connected with a suitable each wet rinsed tissue specimen, and immediately thereafter the support were hung onto the arm of a USP tablet disintegrating test machine. When the

disintegrating test machine was operated, the tissue specimen was given a slow, regular up and down movement in the test fluid (400ml) at 37ºC contained in a 1000 ml vessel of the machine. At the end of 1 hr and at hourly interval up to 8 hr, the machine was stopped and the number of microsphere still adhering to the tissue was counted. The test was performed in stomach (pH1.2).

7.3.7 .*In- vitro* drug release studies

(USP, 2009; Swamy P.V.,et al., 2007;Chowdary K. P. R., et al., 2004)

In-vitro drug release study was carried out in USP dissolution test apparatus. A quantity of microspheres equivalent to 100 mg of Metronidazole microspheres was kept in basket type apparatus and immersed in 900ml of phospate buffer (pH 1.2) in 1000 ml dissolution flask and temperature was maintained at 37 ± 0.5ºC throughout the study. At predetermined time intervals 2 ml of samples was withdrawn by means of a syringe fitted with prefilter and same was replaced into the dissolution flask containing pH 1.2.

The absorbance of sample was measured at 276 nm after required dilution with the fresh medium (pH.1.2).All the studies were conducted in triplicate.

7.3.8. Kinetics of *In-vitro* drug release

(Swamy P.V., et al. 2007; Bhabani Nayak S.,et al.,2009)

In-vitro drug released data was subjected to *in- vitro* kinetic models such as zero *order*

first order, Higuchi and Korsemeyer- Peppas

Zero order: $C = K_0 t$

Where K_0 - is the zero-order rate constant expressed in units of concentration/time

t -is the time in hrs

First order: $Log\ C = LogC_0 - Kt / 2.303$

Where C_0 - is the initial concentration of drug,

K - is the first order constant

t - is the time in hrs.

Higuchi: $Qt = Kt^{1/2}$

Where Q_t - is the amount of the release drug in time t,

K- is the kinetic constant and t- is time in hrs

KorsmeyerPeppas: $Mt/ M\infty = Kt$

Where M_t - represents amount of the released drug at time t,

M_∞- is the overall amount of the drug (whole dose) released after 12 hrs

K- is the diffusional characteristic of drug/ polymer system constant

n- is a diffusional exponent that characterizes the mechanism of release of drug.

Table 7.2: Diffusion exponent and solute release mechanism

Diffusion exponent (n)	Overall solute diffusion mechanism
< 0.5	Quasi-Fickian diffusion
0.5	Fickian diffusion
0.5 < n < 1.0	Anomalous (non-Fickian) diffusion
1.0	Case-II transport
> 1.0	Super case-II transport

7.4. STABILITY STUDY

(Manavalan R. and Ramasamy S., 2004)

In any rational drug design or evaluation of dosage forms, the stability of the active component was a major criterion in determining their acceptance or rejection.

Objective of the study

The purpose of stability testing was to provide the evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enabling recommended storage conditions, re-test periods and shelf-lives. The International Conference on Harmonization (ICH) Guidelines titled "Stability testing of New Drug Substances and Products describes the stability test requirements for drug registration application in the European Union, Japan and the States of America.

ICH specifies the length of study and storage conditions

Long-Term Testing: Room temperature; 25˚ C ±2 C at 60% RH ±5% for 12 months

Accelerated Testing: Accelerated temperature; 40˚ C ±2 C at 75% RH ±5% for 6 months

In present study the optimized formulation F9 was exposure up to 3 months Stability studies at accelerated condition (40˚C ±2 C at 75% RH ±5%RH) to find out the effect of aging on drug content and *In-vitro* drug release.

Procedure

The formulation (F9) was stored at accelerated condition in aluminum foils for 3 months. The samples were withdrawn after end of 1st month, 2nd month and 3rd month. The samples were analyzed for its drug content and i*n vitro* drug release.

Results and discussion

8. RESULTS AND DISCUSSION

8.1. PREFORMULATION PARAMETERS

8.1.1. Identification of drug

8.1.1. a) Identification by FTIR spectroscopy

The FTIR spectrum of Metronidazole was shown in Figure 8.1 and the interpretations of IR frequencies were represented in Table 8.1.

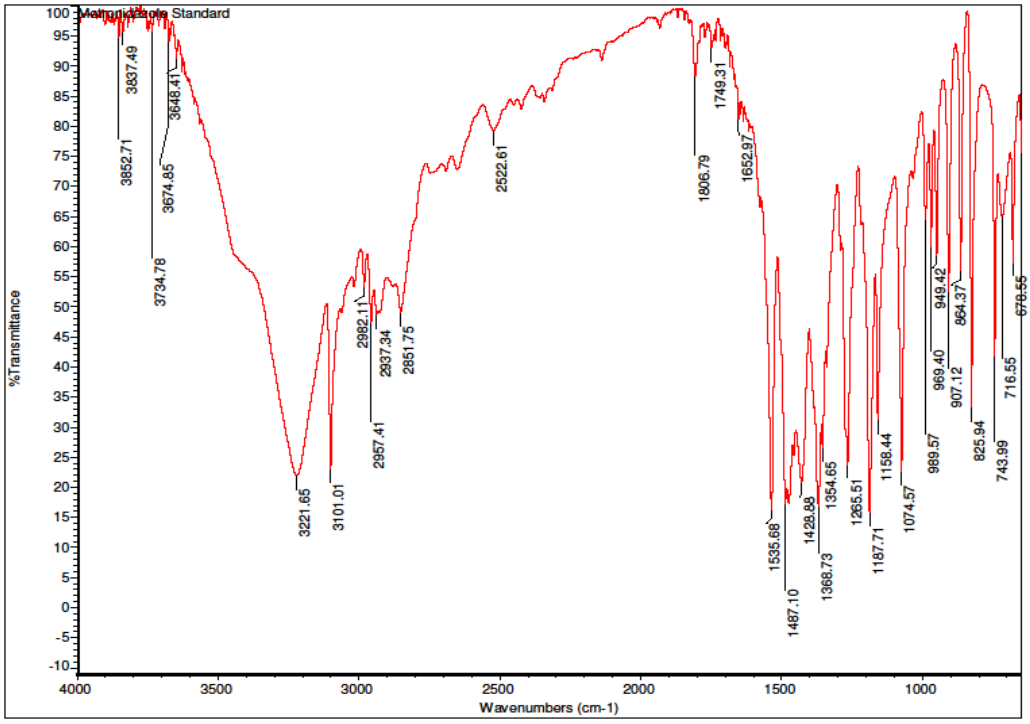


Figure 8.1: FTIR spectrum of metronidazole

Interpretation of FTIR Spectrum

Major functional groups present in metronidazole showed characteristic peaks in FTIR spectrum. The major peaks were identical to functional group of metronidazole Hence, the sample was confirmed as metronidazole.

Table8.1: Characteristic frequencies in FTIR spectrum of metronidazole

Inference	Wave no.(cm^{-1})
O-H stretching	3648
N-H stretching	3221-3101
C-H stretching	2982-2937
C-O stretching	1354-1265
C-H bending(In plane)	1428-1368
C-C stretching	1187-907

8.1.1. b) Melting point

Melting point values of metronidazole sample was found to be in range of 160±1.66^{0}C, the reported melting point for metronidazole was. 159-163°C Hence, experimental values were same as official values.

8.1.2. Physicochemical parameters of drug

8.1.2. A) Organoleptic properties

Odour: Characteristic

Colour: Yellow colour

Nature: Crystalline powder

8.1.2. B) Solubility study

Table 8.2: Solubility of metronidazole in various solvents

Name of solvent	Standard Parts of solvent required for part of solute	Solubility
Distilled water	From 30 to 100	Slightly Soluble
Acetone	From 30 to 100	Slightly Soluble
Methanol	From 1 to 30	Highly soluble
Alcohol	From 1 to 30	Highly Soluble
Isopropyl alcohol	More than 1000	Freely soluble
Acetone	More than 10000	Partially insoluble

8.1.3. Loss on drying

The percentage loss on drying after 3 hours was represented in Table 8.3.

Table 8.3: Percentage loss on drying for Metronidazole

S. No.	Percentage LOD	Average percentage LOD
1	0.3	0.533
2	0.6	
3	0.7	

The sample passes test for loss on drying as per the limit specified (N.M.T.1%)

8.1.3. Analytical methods

8.1.3. a) Determination of λ max

The absorption maximum for metronidazole was found at 276nm

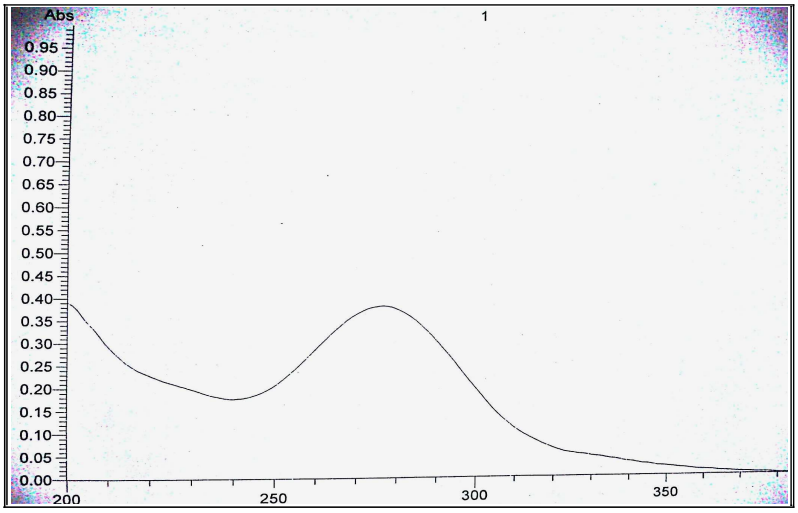


Figure 8.2: λ max observed for metronidazole in pH 1.2

8.1.3. B) Preparation of standard graph of metronidazole

UV absorption spectrum of metronidazole in pH showed λ max at 276nm in figure 8.2. Absorbance obtained for various concentrations of metronidazole in pH 1.2 were represented in Table 8.4.The graph of absorbance vs. concentration for metronidazole was found to be linear in the concentration of 5-30 μg/ml. The drug obeys Beer-Lambert's law in the range of 5-30 μg/ml. was shown in figure 8.3

Table 8.4: Data of concentration vs absorbance for metronidazole in pH 1.2

S.No.	Concentration (µg/ml)	Absorbance (nm)
1	0	0.000
2	5	0.087
3	10	0.194
4	15	0.292
5	20	0.390
6	25	0.486
7	30	0.579

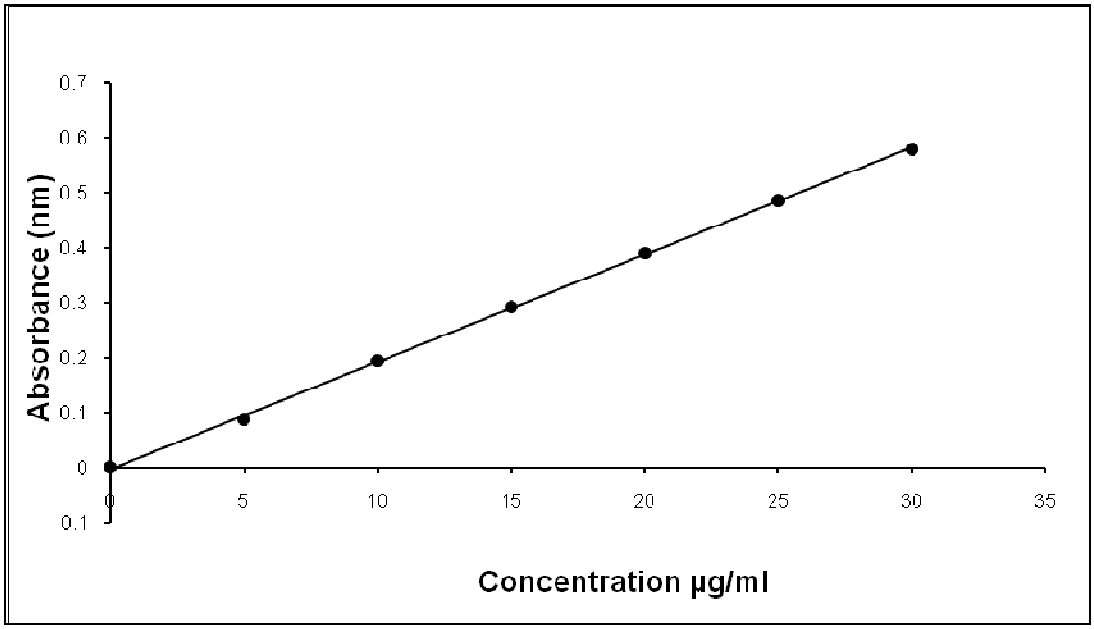


Figure 8.3: Standard curve for metronidazole in pH 1.2

Table 8.5: Data for calibration curve parameters

S. No.	Parameters	Values
1	Correlation coefficient (r)	0.9998
2	Slope	0.05124
3	Intercept	0.154

8.1.3C) Determination of λmax and Preparation of Calibration Curve of Metronidazole by using pH 7.4:

UV absorption spectrum of Metronidazole in pH 7.4 showed λmax at 319 nm. in figure 8.4. Absorbance obtained for various concentrations of Metronidazole was found to be linear in the concentration range of 5 – 30 µg /ml. The Metronidazole absorbance in Phosphate buffer pH 7.4 was given in Table 8.6. The graph of absorbance concentration for drug obeys Beer- Lambert's law in the range of 5 – 30 µg /ml. was shown in figure 8.5

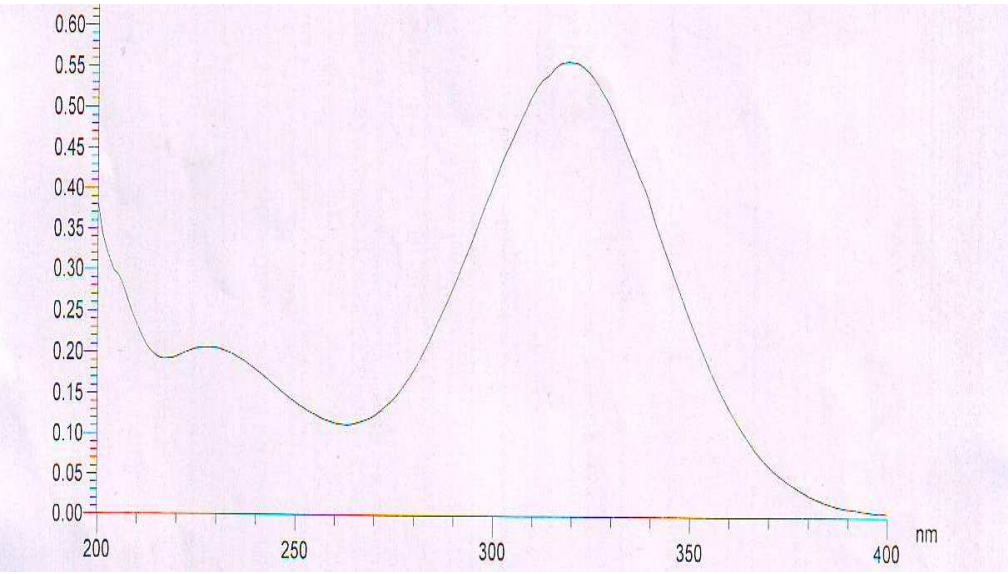


Fig. 8.4: Absorption maximum of Metronidazole in Phosphate buffer pH 7.4

Table 8.6: Data of concentration vs absorbance for metronidazole in pH 7.4

S.No.	Concentration (µg/ml)	Absorbance nm
1	0	0.000
2	5	0.12
3	10	0.24
4	15	0.409
5	20	0.561
6	25	0.708
7	30	0.849

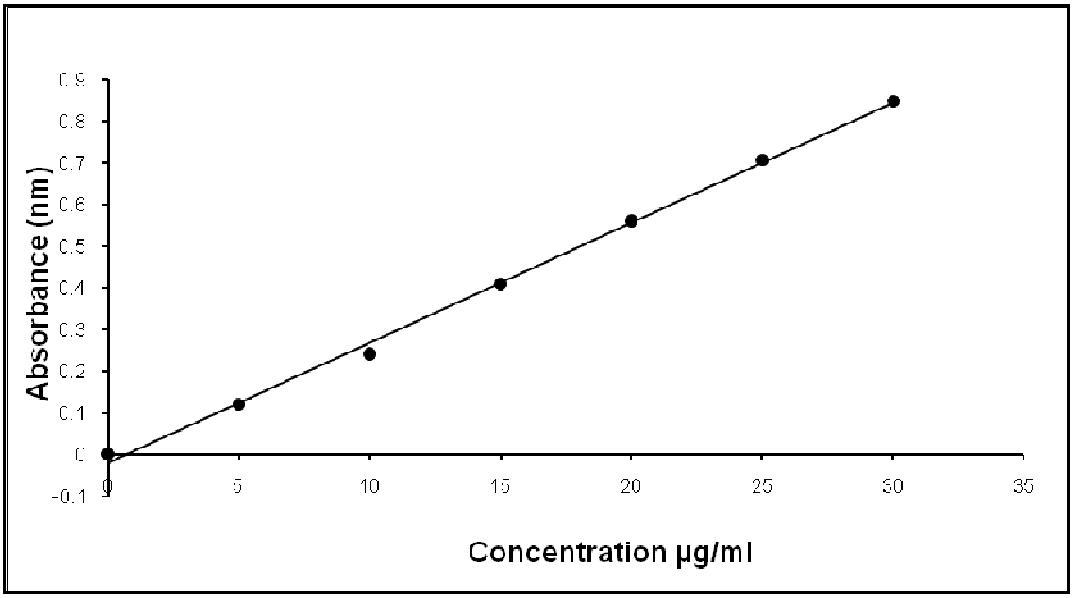


Figure.8.5: Standard curve for metronidazole in pH 7.4

Table 8.7: Data for calibration curve parameters

S. No.	Parameters	Values
1	Correlation coefficient (r)	0.9989
2	Slope	0.034
3	Intercept	0.754

8.1.3. D) Percentage purity of drug

The percentage purity of drug was calculated by using calibration graph method (least square method).The average percentage purity was represented in Table 8.8.

Table 8.8: Data of percentage purity of drug

S. No.	Percentage purity (%)	Avg. percentage purity (%)
1	99.87	100.11±0.240
2	100.12	
3	100.35	

All values are expressed as mean± S.D., n=3

The percentage purity for Metronidazole in IP 2007 is not less than 99.0 % and not More than 101.0 % of the stated amount of Metronidazole. The Average percentage purity was represented in Table 8.8.

8.1.4.A) Determination of compatibility for drug with polymer

FTIR spectroscopy.

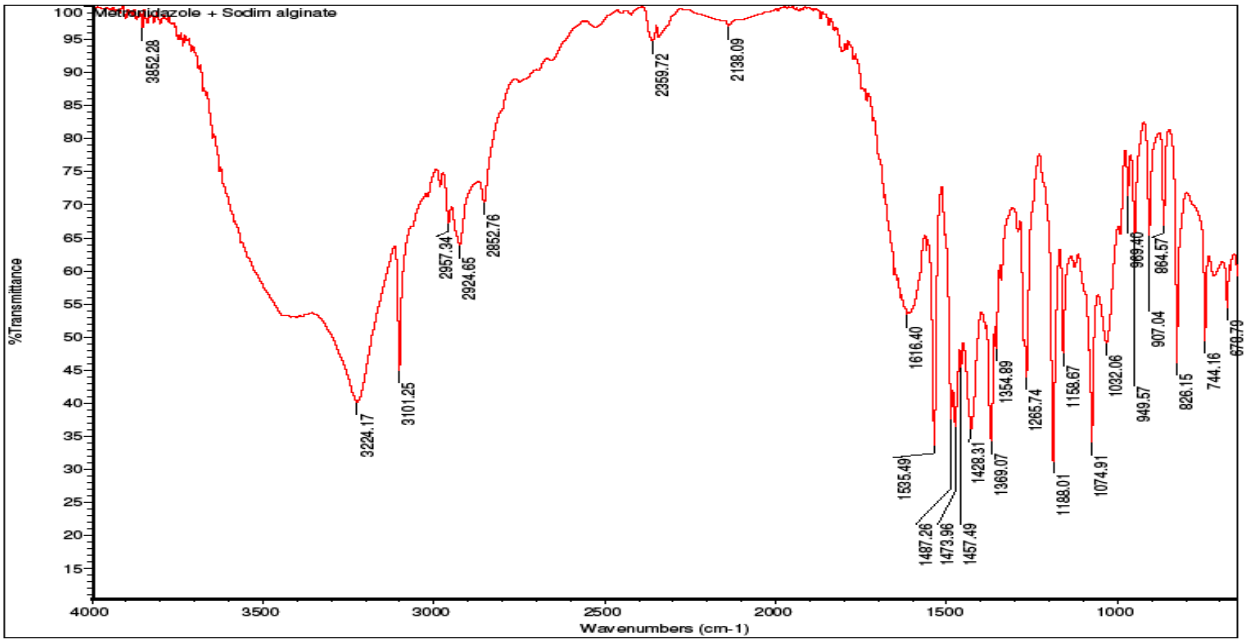


Fig. 8.6: FT-IR spectra of Metronidazole with Sodium alginate

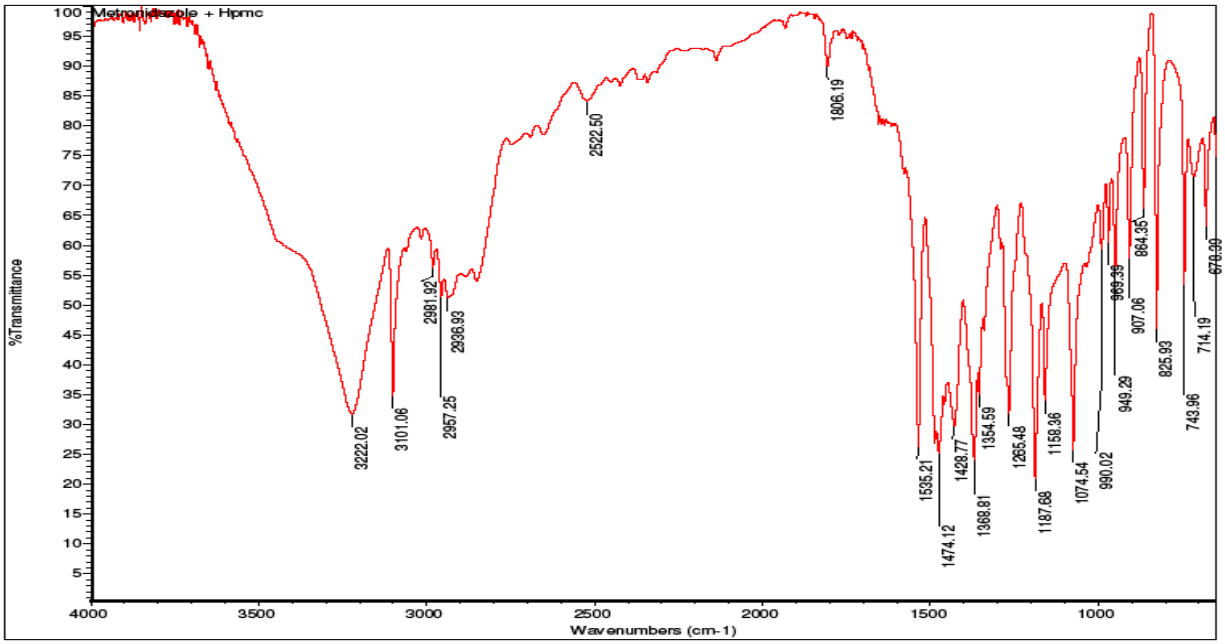


Fig. 8.7: FT-IR spectra of Metronidazole with HPMC

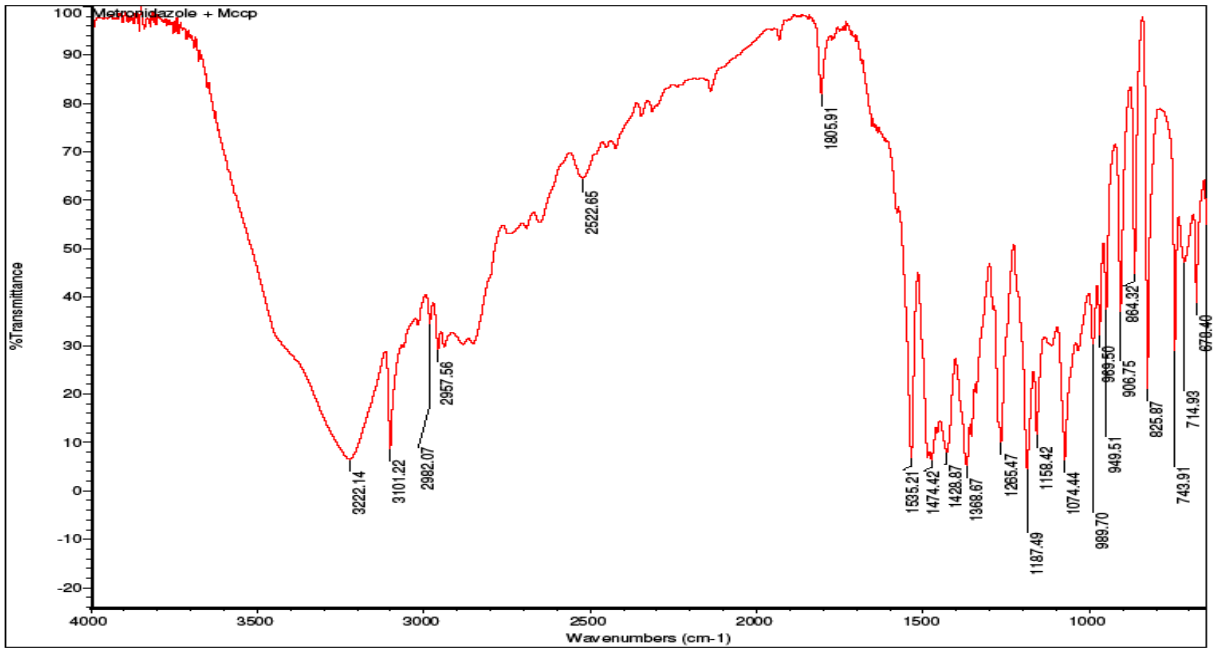


Fig. 8.8:FT-IR spectra of Metronidazole with Methylcellulose:

Table 8.9: The major peak observed in FTIR spectrum of metronidazole and Metronidazole with different polymers.

Wave No. (cm^{-1})	Functional group	Peak observed (Yes/No)			
		Metronidazole	Metronidazole with Sodiumalginate	Metronidazole with HPMC	Metronidazole with Methyle cellulose
3648	O-H Stretching	**Yes**	**Yes**	**Yes**	**Yes**
3221-3101	N-H Stretching	**Yes**	**Yes**	**Yes**	**Yes**
2982-2937	C-H Stretching	**Yes**	**Yes**	**Yes**	**Yes**
1354-1265	C-O stretching	**Yes**	**Yes**	**Yes**	**Yes**
1428-1368	C-H bending(in plane)	**Yes**	**Yes**	**Yes**	**Yes**
1340	C-C stretching	**Yes**	**Yes**	**Yes**	**Yes**

The major peaks of Metronidazole spectrum were compared to Metronidazole with polymers spectrum. There was no interaction between Metronidazole and polymers. The data was represented in Table 8.9 and shown in Figure 8.6, 8.7, and 8.8.

8.1.4. B) DSC thermal analysis:

The interactions between Metronidazole and polymers were determined by DSC studies and results were represented in Table 8.7 and shown Figure 8.9, 8.10, 8.11 and 8.12.

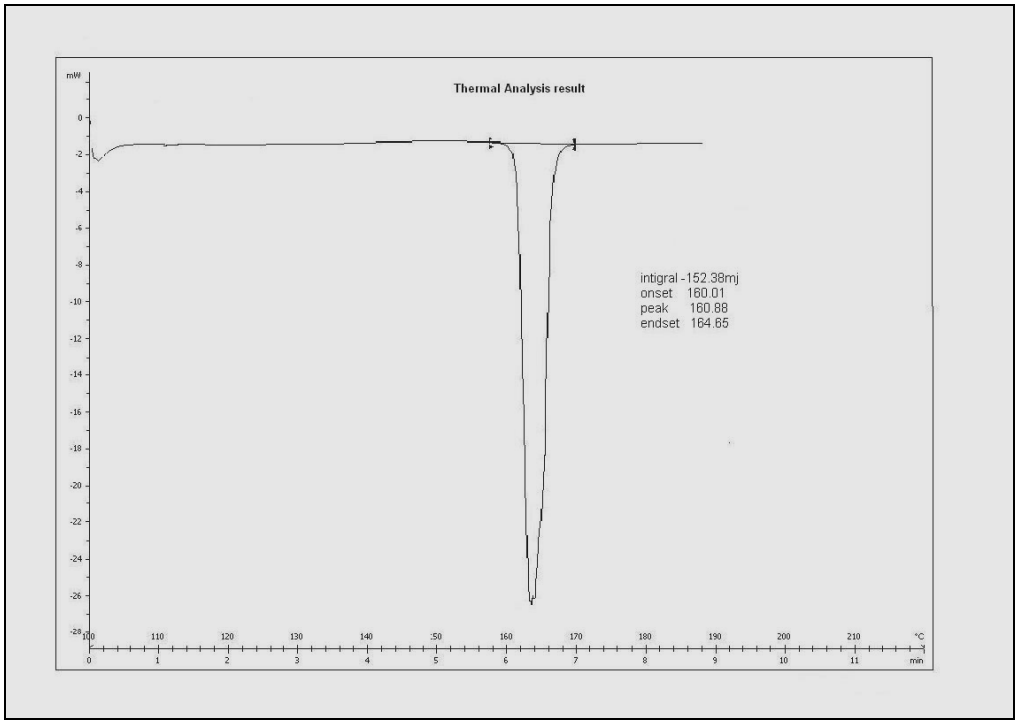


Figure 8.9: DSC thermogram for Metronidazole

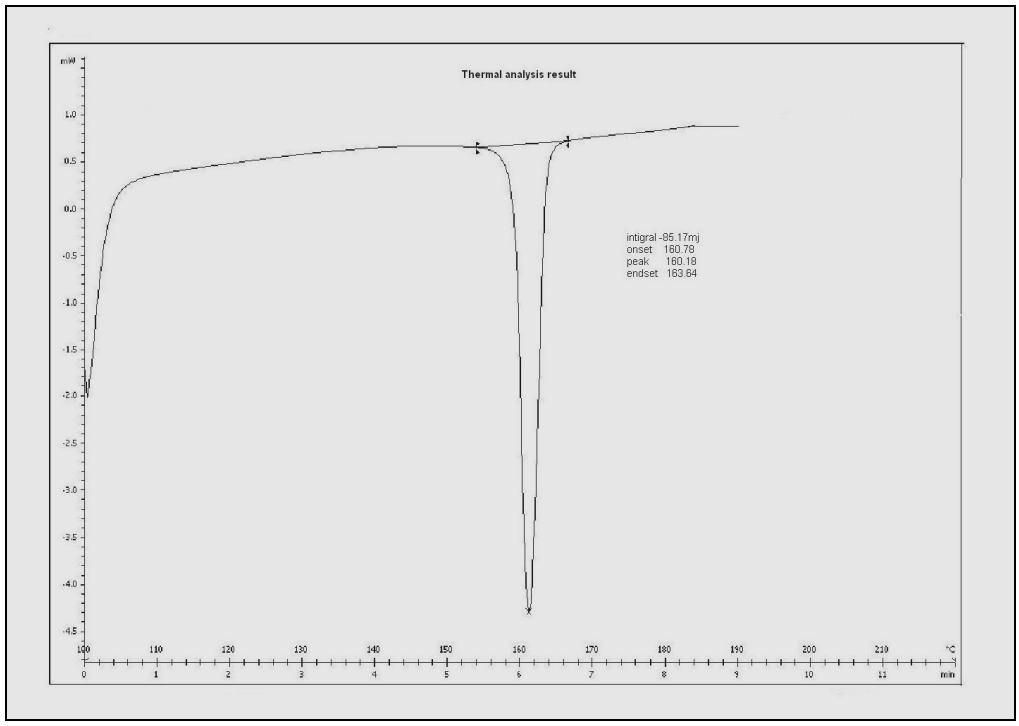


Figure 8.10: DSC thermogram for Metronidazole with Sodium alginate

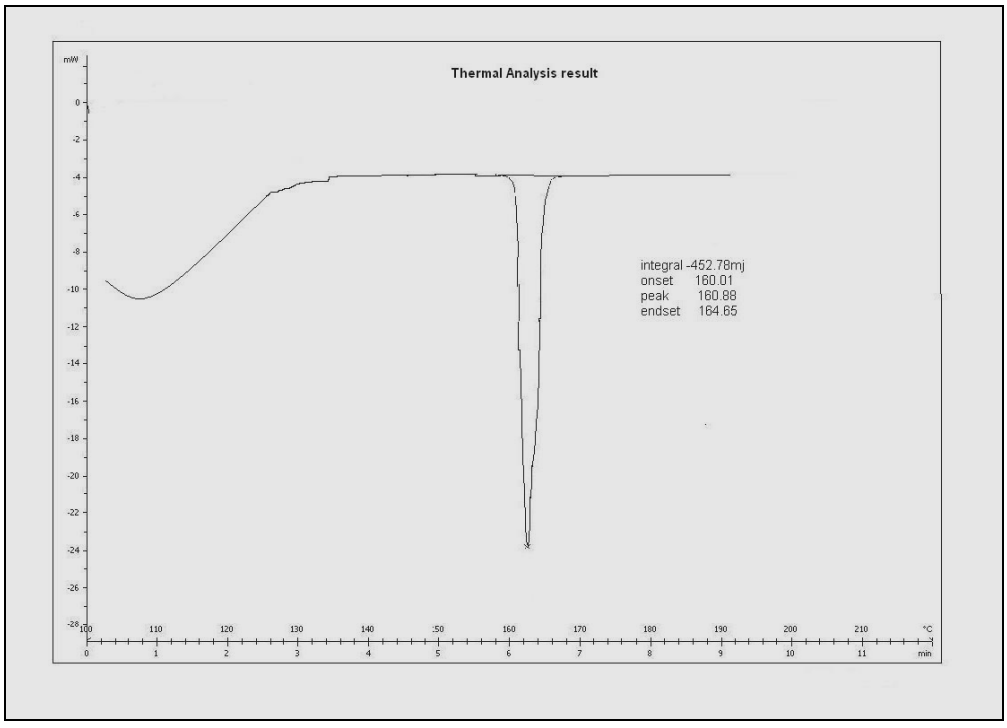


Figure 8.11: DSC thermogram for Metronidazole with HPMC

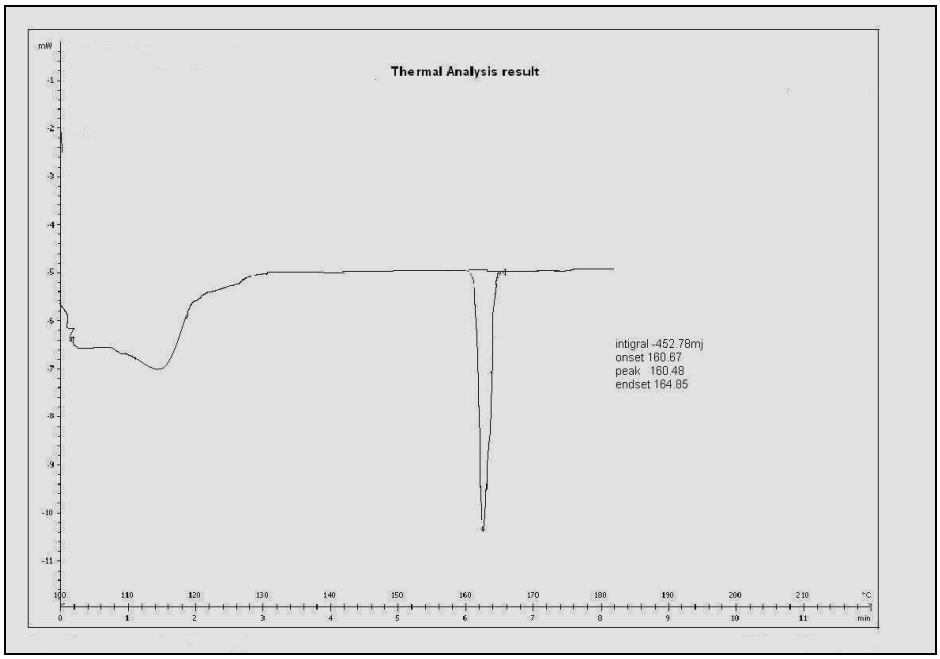


Figure.8.12: DSC thermogram for Metronidazole with methyle cellulose

Table 8.10: Various DSC Thermogram parameter

S.NO	DSC Graphs	Peak (ºC)	Onset temperature (ºC)	Endset temperature (ºC)
1	Metronidazole	160.88	160.01	164.65
2	Metronidazole+ Sodium alginate	161.20	160.98	163.48
3	Metronidazole+ HPMC	160.18	160.78	163.64
4	Metronidazole+ Methyl cellulose	160.48	160.67	164.85

PREPARED MICROSPHERES BY ORIFICE-IONIC GELATION METHOD:

The microspheres prepared by the orifice-ionic gelation method were shown in figure 8.13

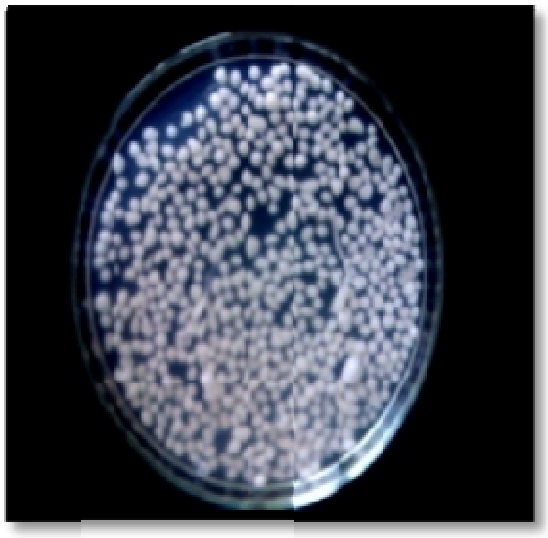


Figure 8.13: Prepared microspheres by orifice –ionic gelation method

8.2. EVALUATION OF METRONIDAZOLE LOADED MUCOADHESIVE MICROSPHERES

- Percentage yield
- Size analysis
- Drug content estimation and Encapsulation efficiency
- Percentage moisture content
- Scanning electron microscopy
- *In vitro* wash –off test for Mucoadhesion
- *In vitro* drug release studies

8.2.1. Percentage yield

The total amount of microspheres obtained were weighed and evaluated for percentage yield was represented in Table 8.11 and shown in figure 8.14. .From this, formulation F9 showed maximum percentage yield among the formulations were prepared.

Table 8.11: Percentage yield of all microspheres formulations

S. No	Formulation Code	Percentage yield (%)
1.	F1	75.19
2	F2	77.40
3	F3	73.20
4	F4	82.20
5	F5	82.00
6	F6	71.60
7	F7	65.60
8	F8	87.95
9	F9	88.65

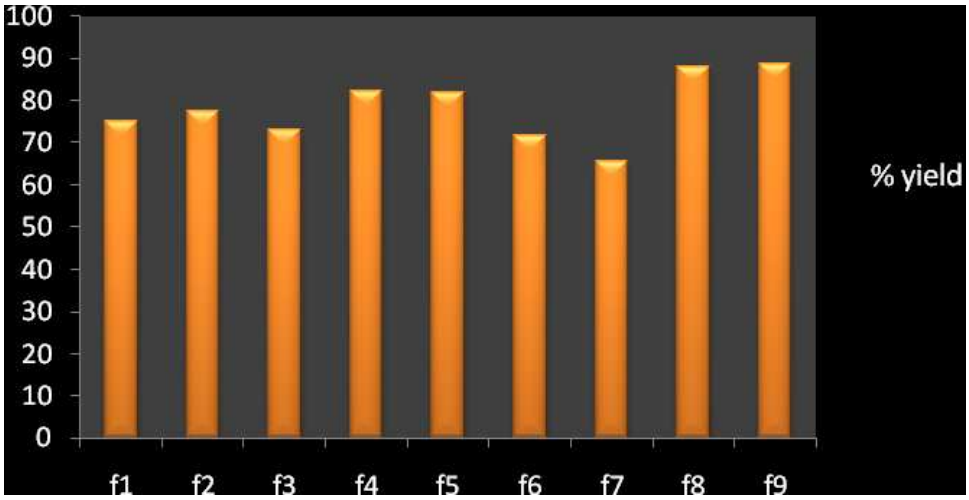


Figure 8.14:Graphical representation of percentage yield

8.2.2. Particle size determinations

Average particle size of microspheres was determined for all the formulations by sieving method analysis by using standard sieves. All the values were represented in Table 8.12. From the values, the formulation f9 had given the less average particle size compared to all othr formulations.

Table 8.12: Average particle size of microspheres

S. No	Formulations	Average particle size (µm)
1	F1	649.38±1.14
2	F2	693.18±0.44
3	F3	720.64±2.54
4	F4	738.28±0.81
5	F5	760.58±1.36
6	F6	794.56±1.94
7	F7	660.85±2.77
8	F8	690.48±0.34
9	F9	642.38±1.14

8.2.3. Drug content estimation and Entrpment efficiency

Metronidazole microspheres (100 mg) from each batch were initially stirred in 3 ml sodium citrate solution (1%w/v) until it completely dissolved .7ml of methanol was added to above solution to gel solubilize calcium alginate and further solubilize Metronidazole. The filtrate was assayed for drug content by measuring the absorbance at 276 nm after suitable dilution. Entrapment efficiency was calculated using the formula. The drug content of microspheres were calculated for all the formulations (F1 to F9) and

represented in Table 8.13 also shown in Figure 8.15. The formulation F9 was showed maximum drug content among the formulations were prepared

$$\text{Encapsulation efficiency} = \frac{\text{Estimated \% drug content in microspheres}}{\text{Theoretical \% drug content in microspheres}} \times 100$$

Table 8.13: Drug content and Entrapment efficiency

S. No	Formulations	Mean drug content (%) ± S.D*	Entrapment efficiency (%)
1	F1	42.32±0.62	70.69
2	F2	36.34±1.46	71.96
3	F3	38.82±1.56	80.75
4	F4	40.62±1.38	83.66
5	F5	41.89±0.49	78.32
6	F6	39.64±2.26	84.68
7	F7	34.06±0.72	70.92
8	F8	32.92±1.86	77.62
9	F9	46.96±0.29	87.21

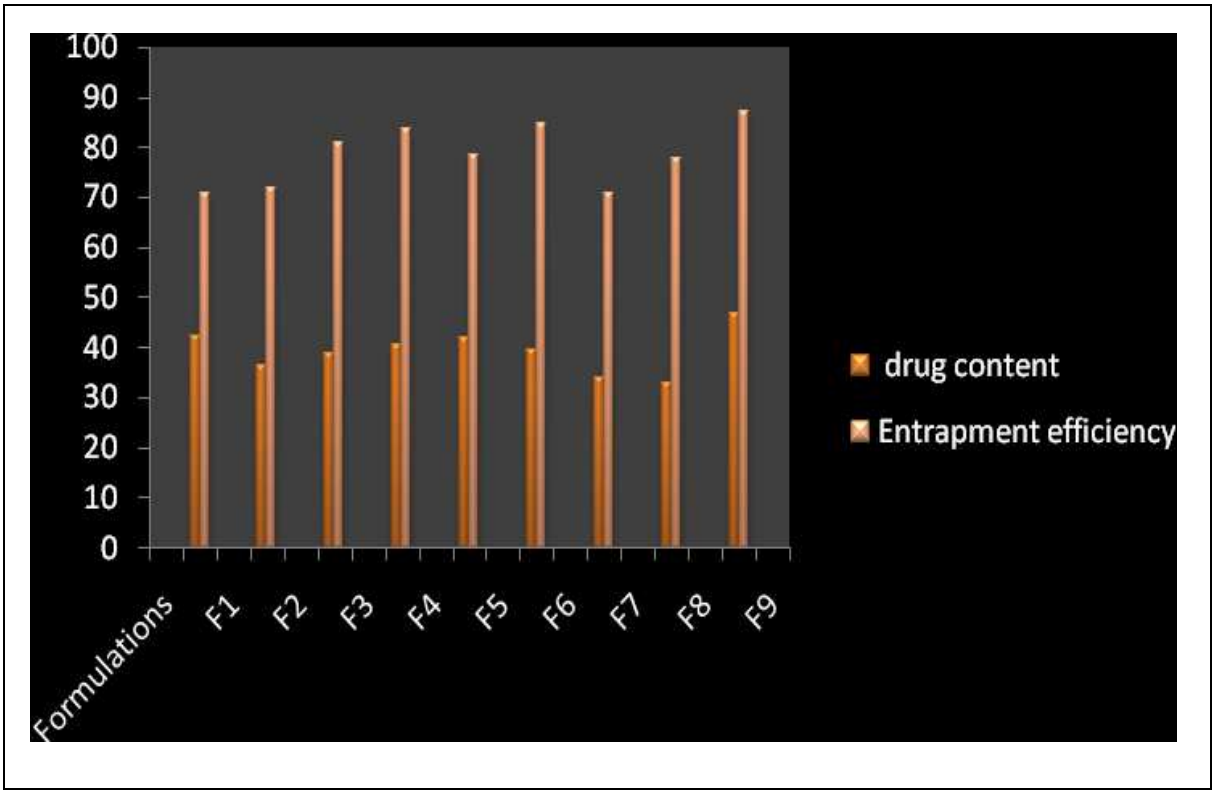


Figure 8.15: Graphical representation of drug content and entrapment efficiency

8.2.4. Percentage moisture content

The percentage moisture content was calculated for all the formulations (F1 to F9) by using desiccator containing Calcium chloride at 37ºC for 24 hrs. The Final weight was determined and compared to initial weight .The values were represented in Table 8.13.

$$\text{Percentage Moisture content} = \frac{w_1 - w_2}{w2} \times 100$$

Table 8.14: Percentage moisture content of microspheres

S. No	Formulations	Percentage moisture content (% ± S.D)
1	F1	8.723±0.144
2	F2	7.637±0.078
3	F3	5.876±0.080
4	F4	4.158±0.121
5	F5	3.529±0.050
6	F6	3.069±0.132
7	F7	2.516±0.040
8	F8	1.944±0.130
9	F9	1.116±0.138

By comparing all the values of all formulations, formulation F9 was found to be the best one. The formulation F9 showed less moisture content. The order was F9<F8<F6<F7<F5<F4<F3<F2<F1.

8.2.5. Scanning electron microscopy (SEM)

The microspheres were observed under a scanning electron microscopy. The resolution of SEM instrument was 3 nm (30 KV HV Mode), 10 nm (30 KV HV Mode), 40 nm (30 LV Mode) and a vacuum system is fitted to it. The shape of the Metronidazole microspheres was evidenced from the Scanning Electron Microscopy was found to be spherical and uniformly distributed and was shown in Figure 8.16.

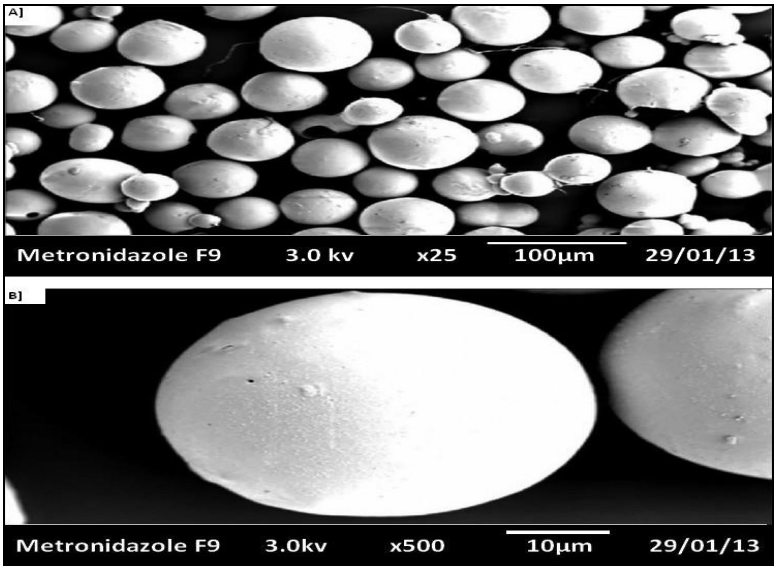


Figure 8.16: Scanning electron microscopy of Metronidazole loaded sodium alginate and methyle cellulose

8.2.6. *In vitro* wash off test for Mucoadhesion

The wash off test for Mucoadhesion for all formulations (F1 to F9) were represented in Table 8.15

Table 8.15: Data of *in- vitro* wash off test to assess mucoadhesive properties of microspheres

Formulations	**1 hr**	**2 hr**	**4 hr**	**6 hr**	**8 hr**
F1	96.07	88.79	68.34	56.45	40.08
F2	94.74	84.21	78.92	60.96	41.16
F3	98.19	80.32	76.49	66.46	46.36
F4	98.52	86.41	72.37	58.37	44.29
F5	96.63	82.62	76.59	54.38	52.56
F6	97.42	90.35	82.72	66.74	54.84
F7	98.27	88.53	82.49	64.36	44.19
F8	96.86	90.81	78.67	58.49	42.28
F9	99.84	97.68	92.48	84.39	62.18

Microspheres with a coat consisting of alginate and a mucoadhesive polymer exhibited good mucoadhesive properties in the *in- vitro* wash off test. The wash off test was faster at a pH 1.2 (stomach pH). It was observed that the pH of the medium was critical for the degree of hydration, solubility and mucoadhesion of the polymers.

The results of the wash off test indicated that the formulation F9 had very good mucoadhesive properties with more than 62.18% retention for 8 hrs in pH 1.2.

8.2.7. *In vitro* drug release studies

Table.8.16: Data of *in vitro* drug released profile of Metronidazole loaded mucoadhesive microspheres*

S. No	Time in hours	Formulations								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	1	13.89±0.11	10.68± 1.54	17.42 ±1.04	11.58 ±1.54	15.46 ±1.56	12.65 ±0.45	18.35± 2.78	16.93±0.45	19.84±1.54
2	2	23.36±1.23	16.98± 1.89	21.54±2.66	17.29±0.61	19.75±1.05	20.67±0.15	25.90±1.96	22.76±0.55	26.72±1.45
3	3	29.07±1.54	25.37±0.54	27.65±0.20	21.45±0.02	23.67±1.51	26.74±1.63	30.05±0.12	28.23±0.56	31.36±1.52
4	4	36.64±1.56	32.46±1.65	31.56±1.53	28.87±0.91	29.56±1.55	33.45±1.55	39.45±0.56	35.98±0.22	38.43±0.01
5	5	42.34±0.57	45.36±0.22	39.78±1.01	35.47±0.78	34.78±0.45	39.87±1.23	44.89±1.56	41.87±1.51	46.64±0.12
6	6	49.07±0.23	47.48±0.55	48.97±0.50	41.68±0.51	40.56±0.61	43.75±1.25	52.98±3.45	48.24±1.02	51.28±0.65
7	7	53.03±1.54	50.67±0.23	51.87±1.21	48.89±0.05	46.78±0.49	49.56±0.61	59.56±1.56	52.76±2.55	57.14±0.54
8	8	61.96±1.45	56.89±1.55	58.16±0.54	52.98±0.07	54.81±0.61	53.23±0.74	64.87±1.26	59.56±2.16	66.49±1.55
9	9	66.76±0.77	61.87±0.54	64.89±0.26	59.89± 0.07	63.46±1.54	65.56±0.07	69.90±0.16	67.87±2.55	72.38±0.65
10	10	69.26 ±0.54	65.08±1.54	71.08±0.50	63.47±0.52	67.89±0.91	70.45±0.21	74.13±1.51	76.12±1.16	81.76±0.55

*All values are expressed as mean ± S.D. n=3

All *in vitro* drug release values were represented in Table 8.16 and shown in figures 8.17 to 8.26.

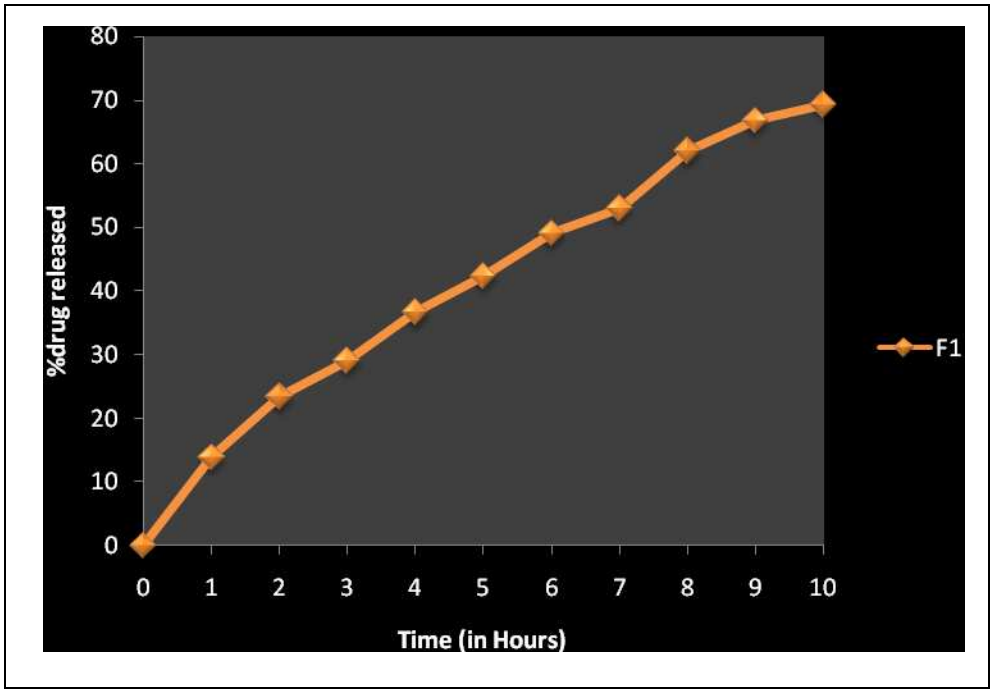


Figure 8.17 : *In vitro* drug released curve of formulation F1

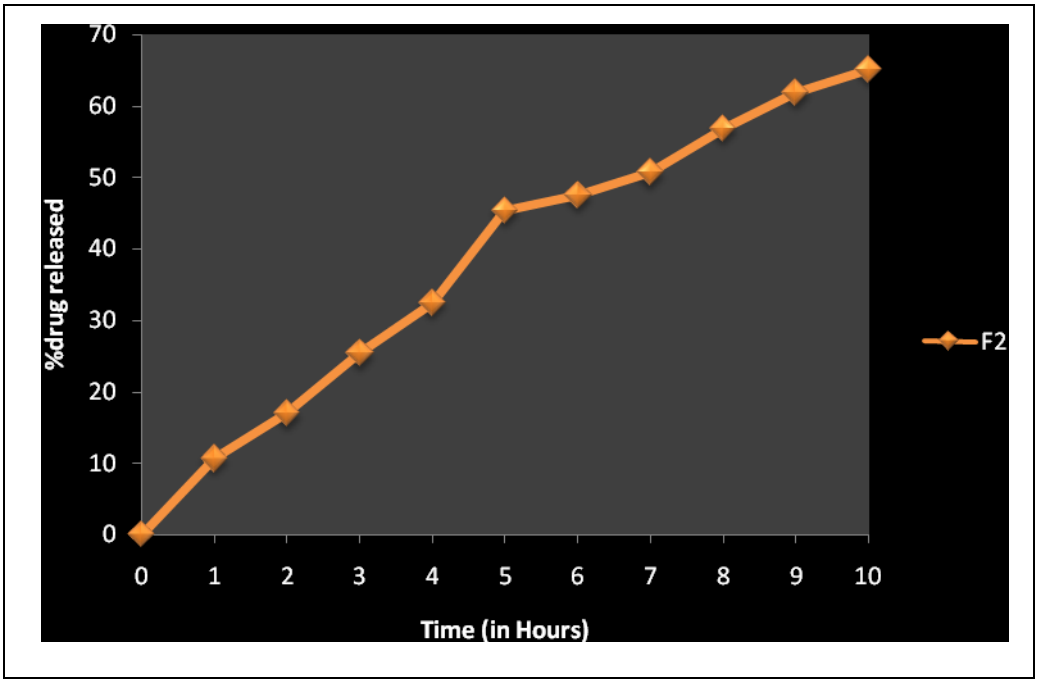


Figure 8.18 : *In vitro* drug released curve of formulation F2

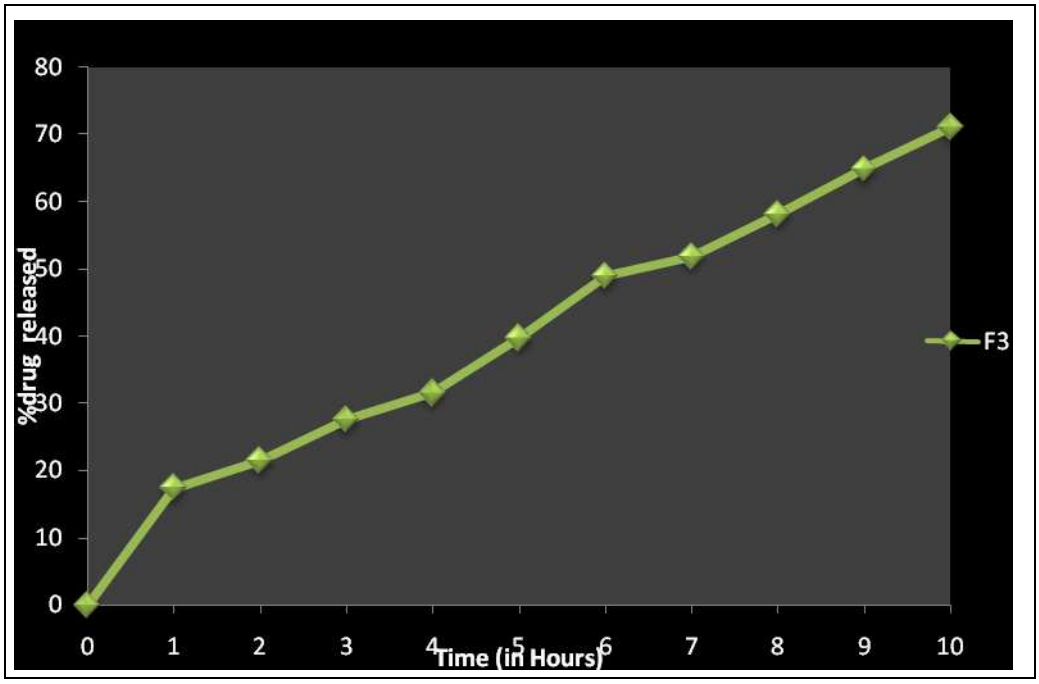


Figure 8.19: *In vitro* drug released curve of formulation F3

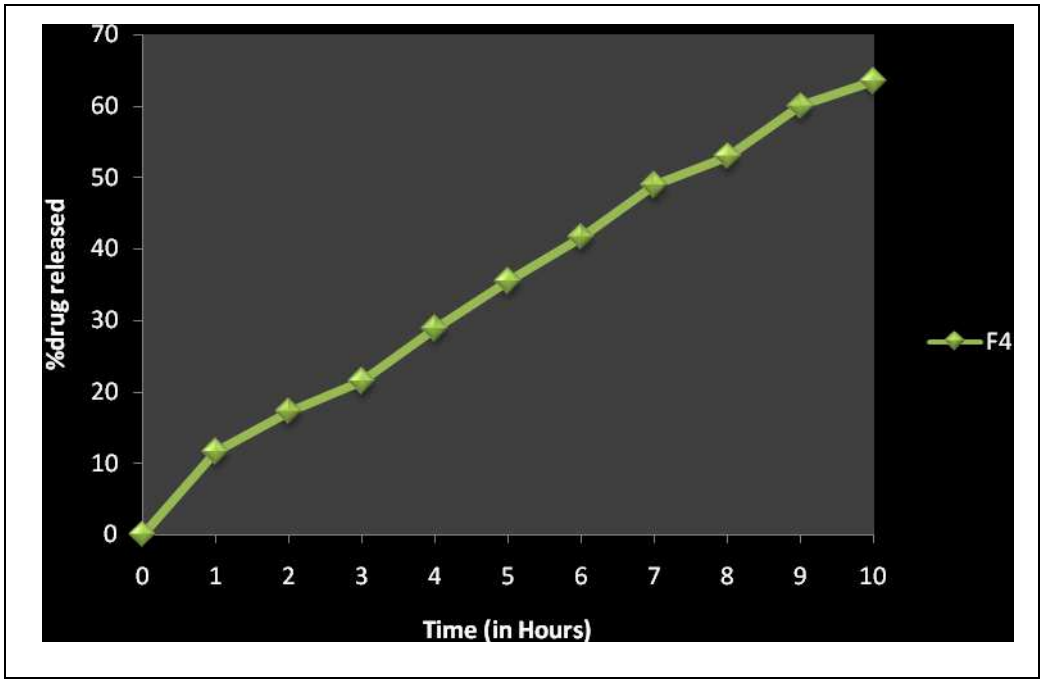


Figure 8.20 : *In vitro* drug released curve of formulation F4

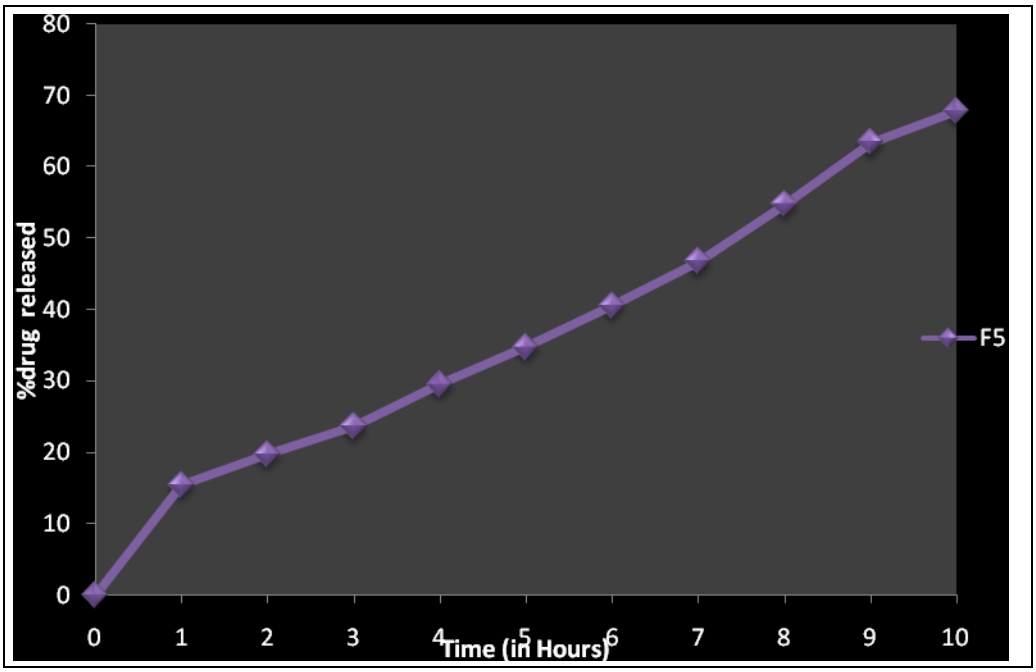


Figure 8.21 : *In vitro* drug released curve of formulation F5

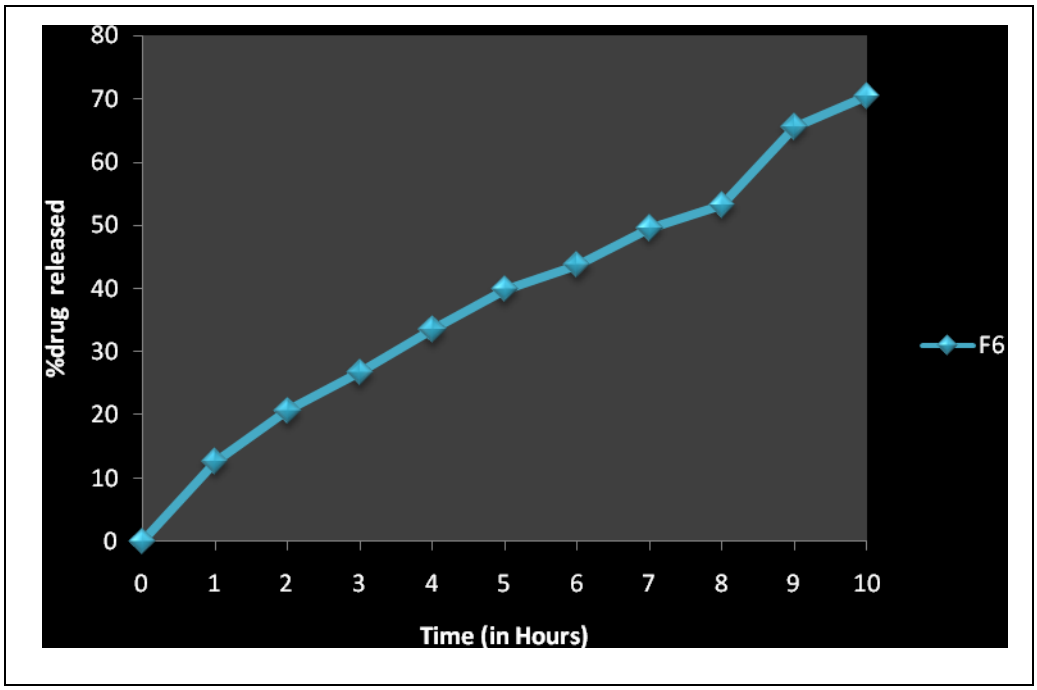


Figure 8.22 : *In vitro* drug released curve of formulation F6

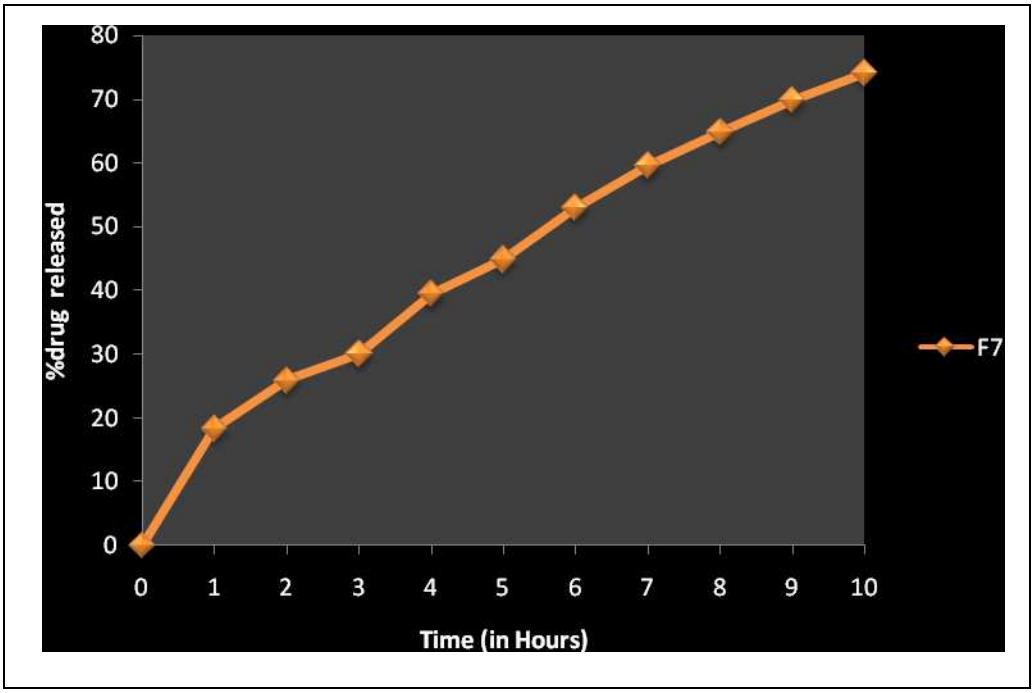


Figure 8.23 : *In vitro* drug released curve of formulation F7

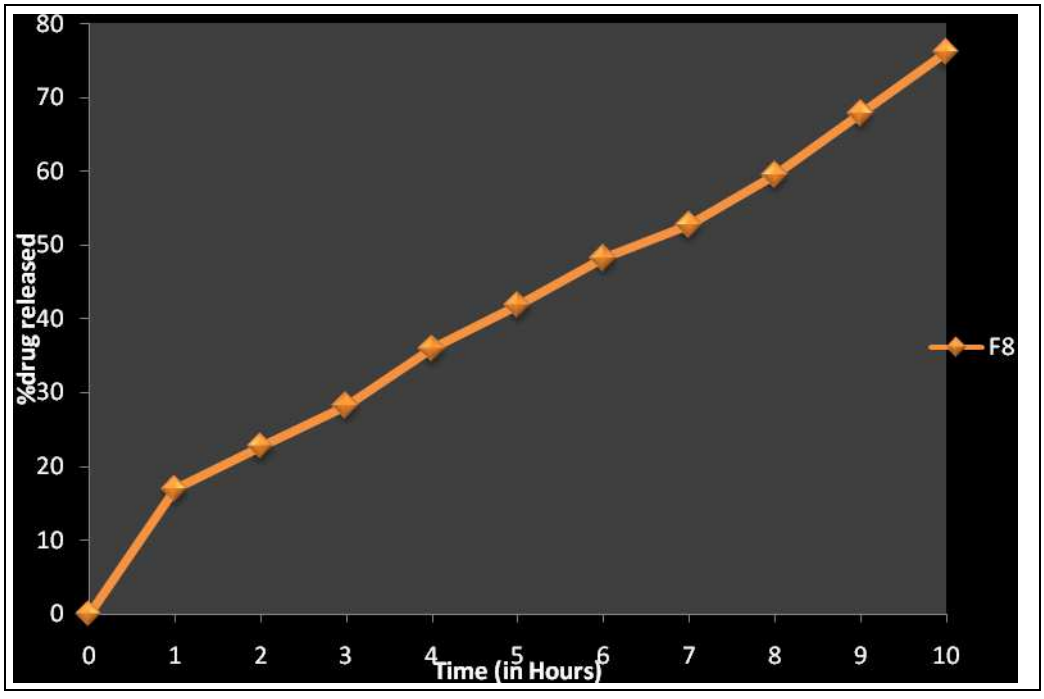


Figure 8.24 : *In vitro* drug released curve of formulation F8

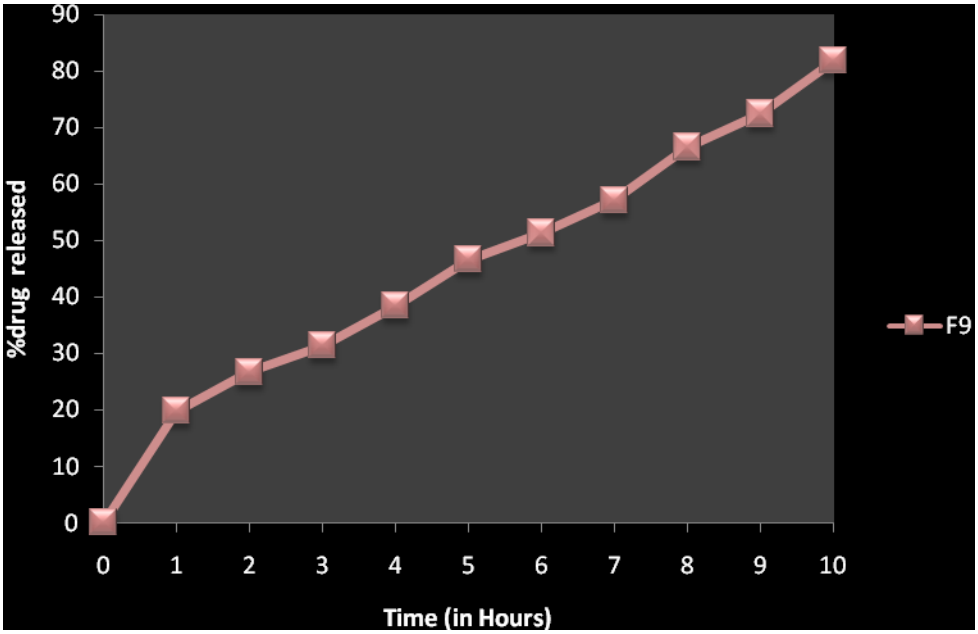


Figure 8.25 : *In vitro* drug released curve of formulation F9

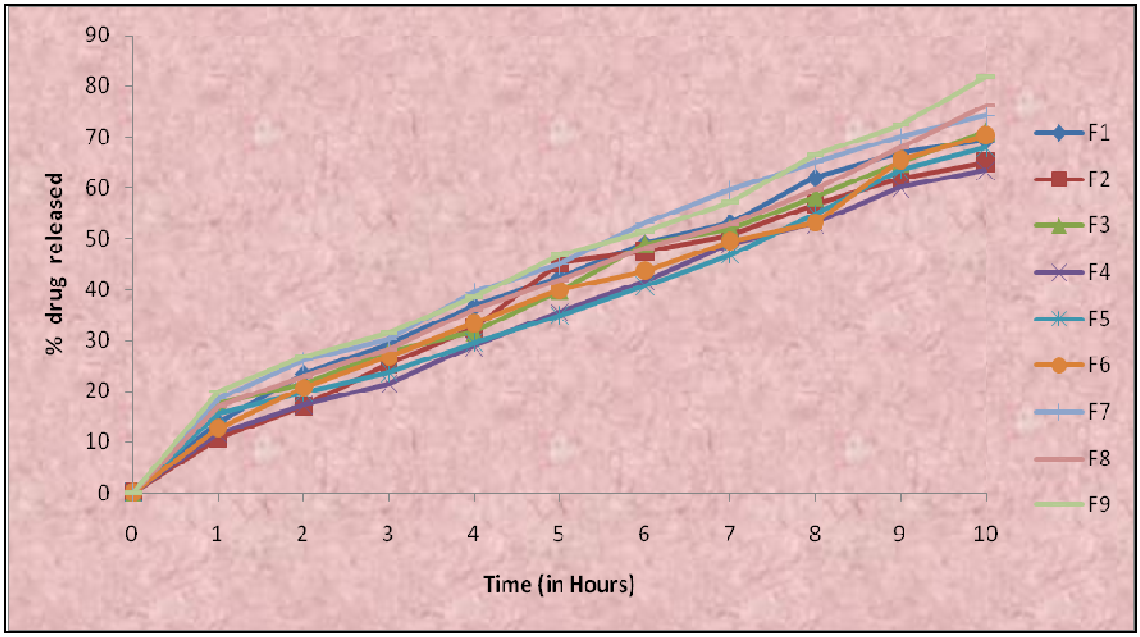


Figure 8.26 : plot for Comparision of *in- vitro* drug released for formulations F1 to F9

8.2.8. Kinetics of Drug release

The kinetics of *In-vitro* drug release was determined by applying the drug released data to various kinetic models such as zero order, first order, Higuchi and Korsmeyer- Peppas. The result obtained was represented in Table 8.17 and shown in Figure 8.27, 8.28, 8.29, 8.30, 8.31, 8.32, 8.33, 8.34 and 8.35.

Table 8.17: *In vitro* drug released kinetics studies of all formulations

Formulation code	Zero order R^2	First order R^2	Higuchi R^2	Korresmayer Peppas		Best fit model
				R^2	n	
F1	0.9621	0.9501	0.9723	0.9908	0.432	Peppas
F2	0.9650	0.9705	0.9663	0.9909	0.456	Peppas
F3	0.9900	0.9471	0.9530	0.9985	0.402	Peppas
F4	0.9503	0.8439	0.9782	0.9908	0.485	Peppas
F5	0.9592	0.8811	0.9729	0.9903	0.365	Peppas
F6	0.9622	0.9307	0.9684	0.9903	0.462	Peppas
F7	0.9540	0.8611	0.9770	0.9901	0.398	Peppas
F8	0.9597	0.9162	0.9756	0.9907	0.441	Peppas
F9	0.9647	0.9609	0.9663	0.9907	0.356	Peppas

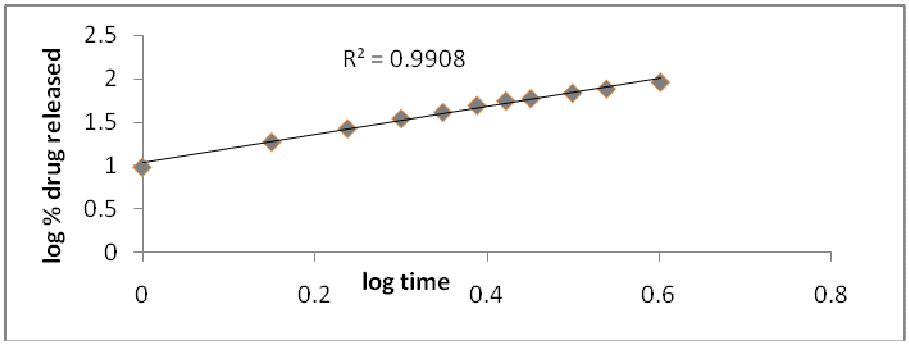


Figure 8.27: Best fit model (korsemeyer peppas) of formulation F1

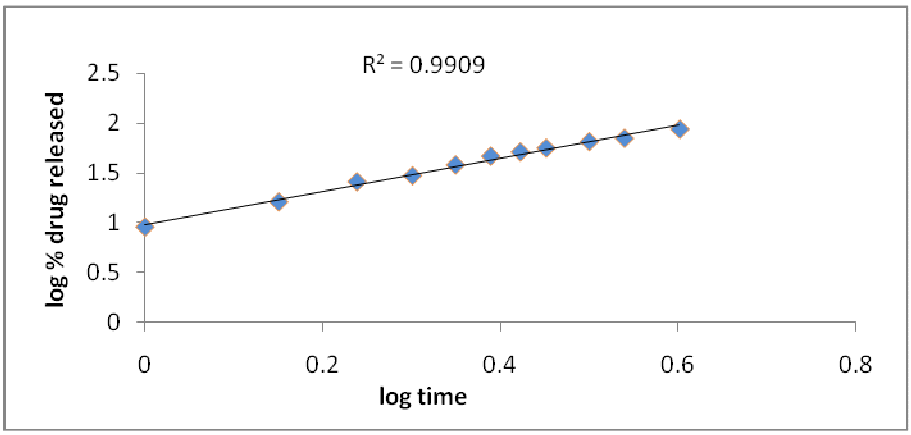


Figure 8.28: Best fit model (korsemeyer peppas) of formulation F2

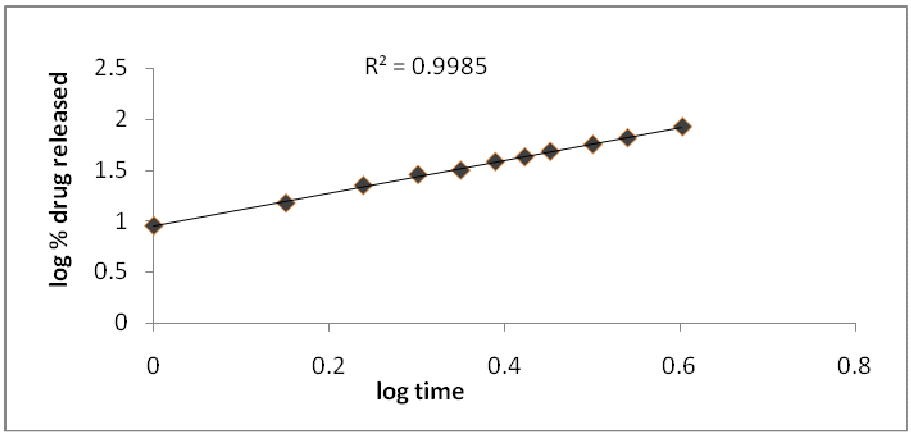


Figure 8.29: Best fit model (korsemeyer peppas) of formulation F3

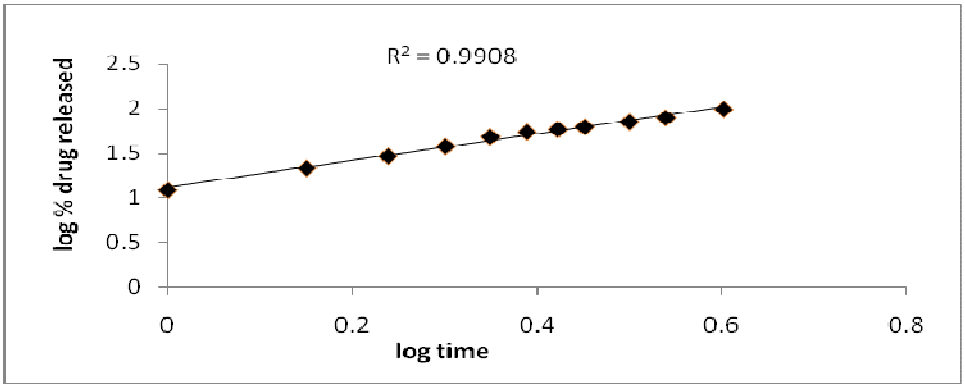


Figure 8.30: Best fit model (korsemeyer peppas) of formulation F4

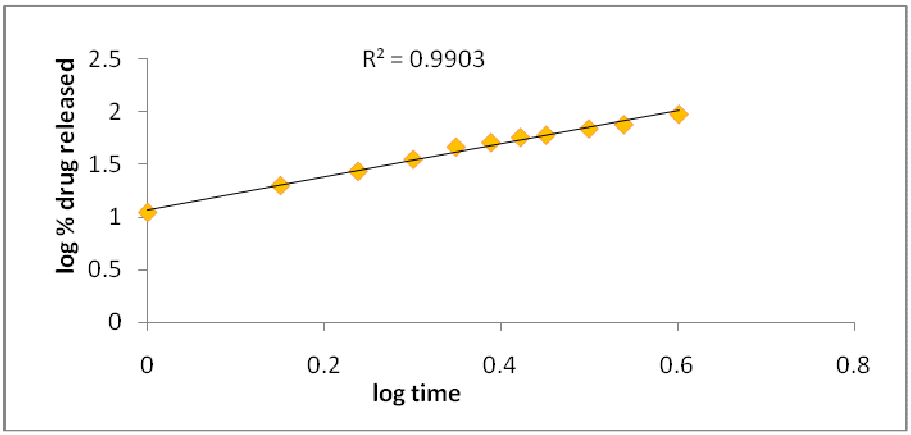


Figure 8.31: Best fit model (korsemeyer peppas) of formulation F5

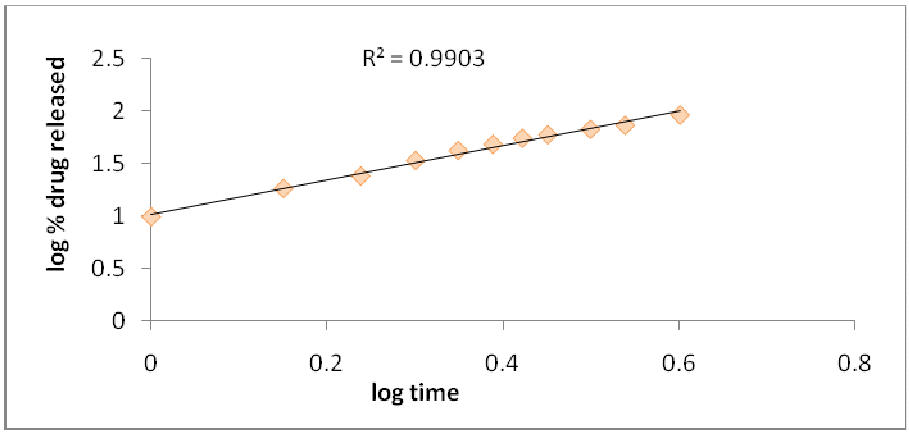


Figure 8.32: Best fit model (korsemeyer peppas) of formulation F6

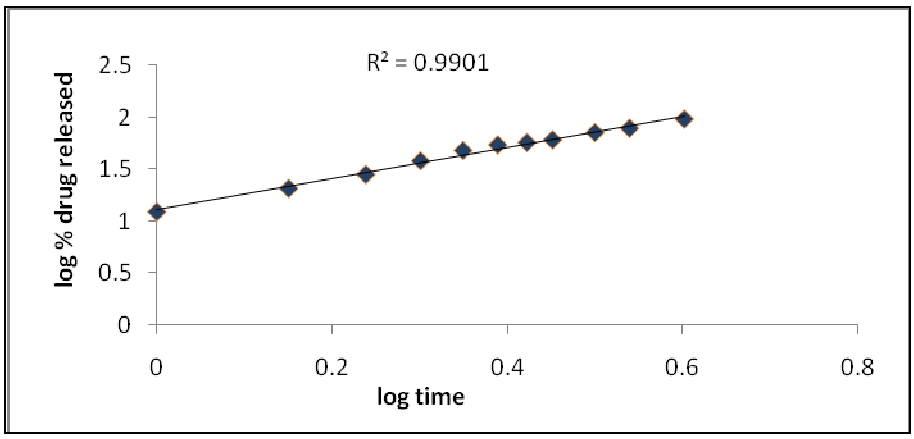


Figure 8.33: Best fit model (korsemeyer peppas) of formulation F7

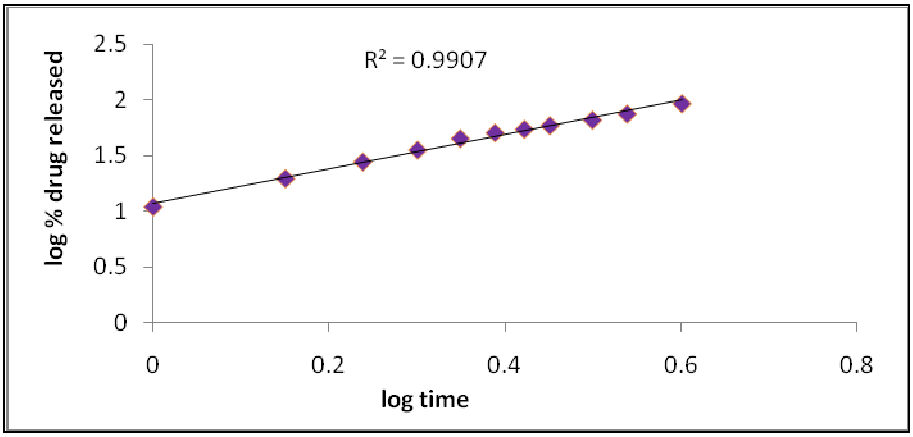


Figure 8.34: Best fit model (korsemeyer peppas) of formulation F8

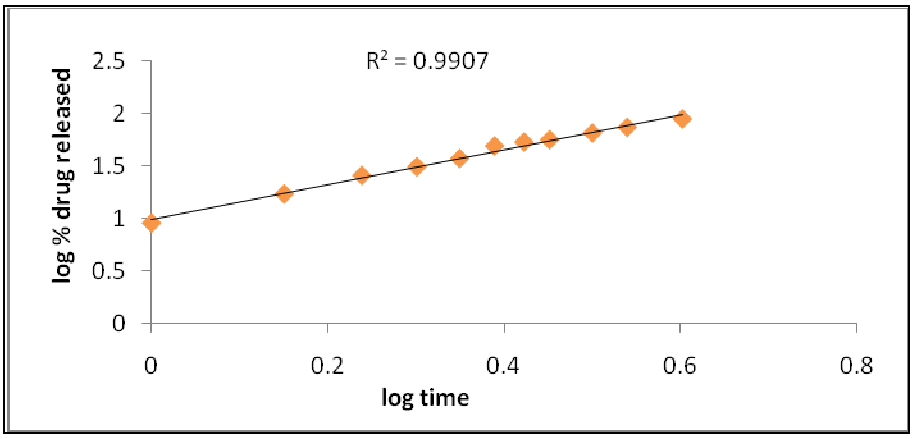


Figure 8.35: Best fit model (korsemeyer peppas) of formulation F9

8.3. STABILITY STUDIES

The formulation F9 was observed after specified period stability studies as per ICH guidelines .The formulations was monitored for drug content and *In-Vitro* drug released profile and results were represented in Table 8.17 and percentage drug released profile was shown

T**able 8.18:** Data of stability studies of formulation (F9)

Characteristics	**Initials***	**1 month***	**2 month***	**3 month***
Drug content (%)	46.96±0.29	46.54±0.18	46.34±0.38	45.99±0.07
In-vitro drug released	81.76	81.26	81.12	81.04

*All the values are expressed as mean± S.D., n=3

Accelerated Testing: Accelerated temperature: 40° C ±2° C at 75% RH±5% for 3 Months

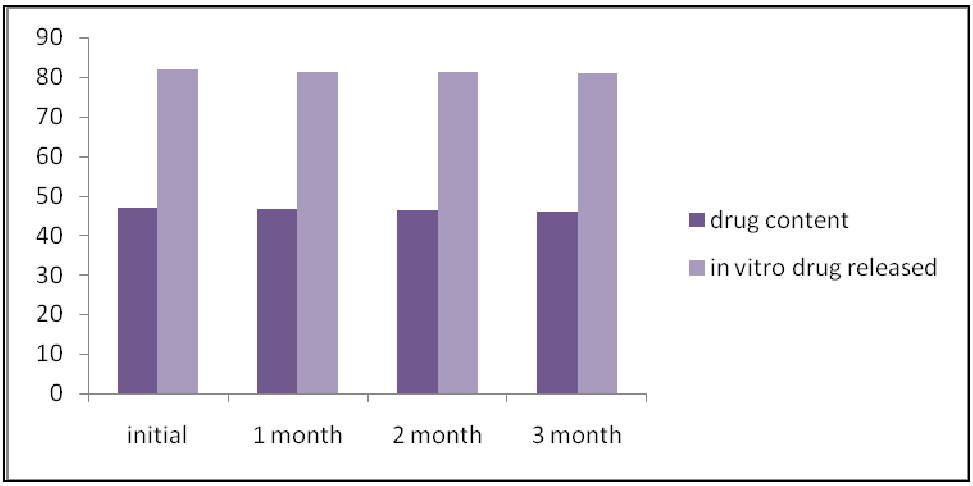


Figure8.36:Graphical representation of stability study

SUMMARY AND CONCLUSION

9. SUMMARY AND CONCLUSION

The goal of any drug delivery system was to provide the therapeutic amount of drug to the proper site in the body also to achieve and maintain the desired drug concentration in blood. Improving the therapeutic efficacy of existing drugs has been tried by different technologies. One of the effective technologies exiting in recent years of pharmacy is Microspheres.

Mucoadhesive drug delivery system was developed in pharmacy field and drug retention for a prolonged time has been achieved. Hence, it was made an effective attempt to formulate the mucoadhesive microspheres by using Metronidazole as the model drug it possess the mean half life of six hours and bioavailability was found to be only 60%. Hence, it was chosen as the good candidate for the mucoadhesive microspheres in order to improve the bioavailability and prolong period of drug released.

The identification of the drug was done by the FTIR spectroscopy analysis and drug polymers interaction was studied by DSC studies. It was concluded that no interaction was found between the Metronidazole and polymers.

Mucoadhesive microspheres of Metronidazole were prepared by Orifice- ionic gelation method. Metronidazole mucoadhesive microspheres were composed of sodium alginate alone and in combination with HPMC and methyle cellulose. For first three formulations F1, F2 and F3 sodiumalginate alone and F4, F5 and F6 were composed sodium alginate and HPMC and F7, F8 and F9 were composed of sodium alginate and methyle cellulose. All the formulations were evaluated for the particle size, percentage yield, drug content and encapsulation efficiency, percentage moisture content, SEM analysis, *in vitro* wash off test, *in vitro* drug released and stability

studies.

The higher drug content and encapsulation efficiency was observed as the concentration of alginate increased. This may be attributed to the greater availability of active calcium-binding sites in the polymeric chains and consequently the greater degree of cross linking as the quantity of sodium alginate increased resulting in the formation of nonporous microspheres. The drug loading efficiency greatly improved when alginate was blended with methyle cellulose at 1% level.

The wash of test were carried out in pH 1.2 Mucoadhesive property of formulation F9 consisting of sodium alginate3.5% along with methylcellulose 1% exhibited good mucoadhesive property.

The particle size analysis was carried out by sieving method The particle size ranges from 642.38±1.14µm to 794.56±1.94µm. The percentage yield were found to be in range of 65.60% to 88.65% and The percentage moisture content were found to be in range of 1.116±0.318% to 8.723±0.144%.

The *in vitro* drug release studies were carried out in the pH 1.2. The microspheres were prepared by ionic orifice gelation technique using calcium chloride as cross-linking agent. The microspheres cross-linked with calcium showed delay in disintegration and consequently a slow release of drug was obtained. To retared the drug released from the microspheres, HPMC and methyle cellulose were blended with the sodium alginate matrix.

This kind of release was the characteristics of swelling-controlled system in which the rate of solvent uptake into a polymer was largely determined by the rate of swelling and relax-action of the polymer chains. It was assumed that the drug molecules diffused out through a dissolving gel-like layer formed around the drug during the dissolving process.

On comparing the major criteria in evaluation such as drug content, encapsulation efficiency, *in vitro* wash off test and *in vitro* drug release characteristics, the **formulation F9** was selected as the best formulation, as it showed the drug content as 46.96% and encapsulation efficiency was 87.21%, showed a good mucoadhesive nature in the *in vitro* wash off test was nearly 62.18% up to 8 hrs and *in vitro* drug released 81.76% up to 10 hrs. Based on all the above evaluation parameters it was concluded that the formulation F9 was found to be best formulation among the formulations F1 to F9 were prepared. The mechanism of drug released was calculated by applying the kinetic models and it was concluded that the formulations F9 follows the Korsmeyer – Peppas model and it undergoes Fickian diffusion mechanism ($n \geq 0.5$).

According to the stability studies, the formulation F9 was found to be stable up to 3 months of storage period in drug content and *in vitro* drug released profile.

The **formulations F9** was concluded best formulation among the formulations were prepared.

FUTERE PROSPECTUS

10. FUTURE PROSPECTUS

In this present work, Physio-Chemical characterization and *in vitro* evaluation of Metronidazole Mucoadhesive microspheres were performed.

The following work had to perform in future:

The microspheres can be also formulated by using other different mucoadhesive polymers.

The mucoadhesive microspheres can also be formulated for advanced drug delivery other than oral administration.

In vivo and *in vitro* correlation studies had yet to be performed and the results has to be determined .From the correlation results, it can serve as the model for humans and gain a better understanding of drug absorption and its dependence *in vitro* drugrelease.

Bibliography

11.BIBLIOGRAPHY

1. Akanksha garud., et al. Metronidazole microcpasules with a coat consisting of alginate and natural cationic polymers, *Journal of Pharmacy Research,* **2010**, 271-2996.
2. Amit Alexandar., Ajazuddin., Tripathi D. K., Swarna and JyothiMaurya. Mechanism responsible for mucoadheson delivery review, *International journal of Biological and Chemical Sciences*, **2011**, 2(1), 323-367.
3. Anonymous, IndianPharmacopoeia. The controller of publication, Govt. of India, Ministry of Health and Family Welfare, New Delhi. Vol I and II, **2007**,134, 143-162,475-480, 143-162, 685-688.
4. Anonymous, The United States Pharmacopoeia, XXVII, NF XXII. 27thedn, Vol-3, The United States Pharmacopoeial Convention Inc, Rockvilley, **2009**, 3842-3843.
5. Aulton M.E. Eds. Pharmaceutics, The science of dosage form design, 2ndedn, Churchill Livingstone, New York, **2002**, 270-278.
6. habaniNayak S. HarshadParma., Sunil BakliwalNayan and Gujarathi SuniPawar. Different methods of formulation and evaluationof mucoadhesive microspheres, *Acta Pharmaceutica Sinica*, **2009**, 1(3), 1157-1168.
7. Bhatt.J H., et al. Controlled release of Metronidazole mucoadhesive microspheres, *International Journal of Pharma Tech Research*, **2009**, 2(4), 2575-2595.
8. Chein Y. W. Novel drug delivery systems, 1st edn, Informa healthcare, New York, **2009,**50, 139-199.
9. Chowdary K. P. R. Mucoadhesive microsphere for controlled drug delivery, **2004**,1717-1724.
10. Devraj., et al. oral dosage form proposed for the attainment of timed release drug delivery of Metronidazole, *Journal of Pharmacy Research,* **2010**, 211-219

11. Gupta A K., Garg SK., Pal SK., Development and characterization of SRM microspheres of repaglinide, *Journal of Drug Delivery and Therapeutics,* **2011**, 71-77.

12. Jain N. K., Progress in controlled and novel drug delivery system, 1st edn.CBS Publisher and distributers, New Delhi, **2004**, 353-376.

13. Khar R. K. and Vyas S. P. Controlled drug delivery and advances, 1st edn. CBS Publishers and Distributors, New Delhi, **2002**, 174-176, 257-263.

14. Lachman L. Lieberman H. A and Kanig J. L. The theory and practice of industrial pharmacy, 3rd edn, Varghese Publishing House, Mumbai, **1991**, 67-71, 183-184.

15. Madhavi Boddupalli B., Naga Madhumudium and David Banji., Formulation and Evaluation of Mucoadhesive Microspheres of Venlafaxine Hydrochloride, *Journal of Pharmacy Research,* **2010**, 2597-2600.

16. Mahendra singh and Vinayvijay., Formulation and characterization of mucoadhesive microspheres of verapamil Hydrochloride as a model drug, *International Journal of Pharmaceutical Sciences,* **2011**, 2(12), 3260-3268.

17. Malay Das K., Divya P and Maurya. Evaluation of diltiazem hydrochloride loaded mucoadhesives microspheres prepared by emulsification internal gelation method, *Acta Poloniae Pharmaceutica-Drug Research*, **2008**, 65(2), 249-259.

18. Manavalan R. and Ramasamy S. Physical Pharmaceutics, Accelerated Stability Testing, 1st edn, Vignesh Publisher, Chennai, **2004**, 288-299.

19. Moffat A. C. Osselton M. D. and Widdop B. In Clarke's analysis of drugs poisons, 3rd edn, Pharmaceutical Press. **2004**. Vol-2, 1692-1693.

20. Nagda Chirag., Chotai Narendra., Patel Sandip., Patel Upendra and AhirKeyur. Design and characterization of bioadhesive microspheres prepared by double eulsion solvent evaporation method, *Acta Polonica.* **2009**, 261-270.

21. Nishanth kumar N., Walikhindi A.K. and Jamkandi V.J. Formulation and Evaluation of gliclazide Microspheres, *International Journal of Pharmacy and Life Sciences*, **2011**, Vol-1, 191-196.

22. Ofokansi K. C and Adikwu M. U. Formulation and evaluation of microsphere based on gelatin mucin admixtures for the rectal delivery of ceftriaxone sodium, *Tropical Journal of Pharmaceutical Research,* **2007**, 6(4), 825-832.

23. Patil P. B., Gawali V. U., Patil H. N., Hardikar S. R and Bhosale A. V. Parparation and evaluation of mucoadhesive microspheres of atenolol and propanolol, *International Journal of Pharma Tech Research*, **2009**, 1(3), 639-643.

24. Punitha S and Ganga S., Pornasak Sriamornsak., Nathya Wattankon and Hirofumi Takeuchi. Study on the mucoadhesive mechanism of pectin by atomic force microscopic and mucin – particle method, *Carbohydrate Polymer*, **2010**, 79, 54-56.

25. Raymond C, Rowe., Paul J. Sheskey and Sian C. Owen.Hand book of Pharmaceutical Excipients. 4th edn, Pharmaceutical Press, London, **2003**, 89, 132, 297, 323.

26. Rajeshwar Kamal Kant Arya., Ripudam Singh and Vijay Juyal. Mucoadhesive microspheres of famotidine - preparation and characterization and invitro evaluation, *International Journal of Pharma Sciences*, **2010**, 2(6), 1575-1580.

27. Ram Chand Dhakar., Sunil Prajapathi K., Anish Gupta K., Girish Yadav and Kand Dangi K. Rosiglitazone maleate microspheres for extended drug release - formulation and evaluation, *International Journal of Pharma Research and Development*, **2010**, 56-65.

28. Sambathkumar R., Venkateswaramurthy S and Perumal P., Formulation of clarithromycin loaded mucoadhesive microspheres by emulsification -internal gelation technique for anti-helicobactor pyloritherapy, *International Journal of Pharmaceutical Sciences,* **2011**, 3(2), 175-187.

29. Saravanakumar K ,Patel and Desai., Formulation and Optimization of naproxan sodium Microspheres Employing Central Composite Design, *Indian Journal of Pharmaceutical Sciences*, **2011**, 408-413.

30. Senthil A., Naranaswamy., Bajit I., GalgeDeepak S and Bhosale Rahul S. Mucoadhesive microspheres, *International Journal of Research In Ayurvedha Pharmacy*, **2011**, 9(1), 55-62.

31. Shiv Shankar Hardenia., Ankit., Jain., Ritesh Patel and Anu Kaushal. Formulation and evaluation of mucoadhesive microspheres of ciprofloxacin, *Journal of Advanced Pharmacy Education Research*, **2011**, 1(4), 214-224.

32. Shoba Rani R. Hiremath. Textbook of Industrial Pharmacy, Orient Longman Private Ltd New Delhi, **2008,** 117-128.

33. Skoog D. A. Holler FandNieman S. Principles of instrumental analysis, 5th edn,Thomson Asia Pvt. Ltd, Singapore, **1996**, 410-413.

34. Swamy P.V., HadaAmit., Shrisand S. B., Hiremath S. N and Raju., Preparation and *in- vitro* evaluation of mucoadhesive microcapsules of atenolol, *Indian Journal of Pharmaceutical Education and Research*, **2007**, 41(4), 358-364.

35. Veena Belgamwar., Viral Shah and Surana S.J., Formulaton and evaluation of oral mucoadhesive multiparticulate system containing metoprololtarate, An in vitro-in vivo characterization, *Current Drug Delivery*, **2009**, 113-221.

36. Venkateswaramurthy N., Sampathkumar R., Vijaybaskararn MandPerumal P., Formulation and invitro evaluation of furazolidone mucoadhesive microspheres, *International Journal of Pharmacy and Pharmaceutical Sciences*, **2010**, 104-120.

37. Vyas S. P. and Khar R. K., Controlled drug delivery and advances, 1stedn.CBS Publishers and Distributors, New Delhi, **2002**, 174-176, 257-263.

38. Yadav N., Bhiseb S.B. and sandeb T.A., Synthesis and Characterization of Sustained Release Atenolol Microspheres by Solvent Evaporation Technique, *Journal of Pharmaceutical Science and Technology*, **2011**, 559-562.