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Formulation, Optimization and Characeterization of Gastro Retentive Microspheres of Atorvastatin

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1. ABSTRACT

The average particle size of microspheres, which are discrete spherical particles, ranges between 1 and 1000 m. Preparation of Atorvastatin Gastro Retentive Mucoadhesive microspheres by ionic gelation technique, using various polymers [Sodium alginate, HPMC K100M, Sodium CMC, Ethyl cellulose, Methyl cellulose, Guar gum, Xanthan gum and Carbopol 940]. Promotes mucoadhesive formulations orally primarily aims to solve the stability problem with intestinal fluid and considerably increase the amount of time a medication spends in the GI tract.

In sustained drug delivery, mucoadhesive microsphere carrier systems be composed of biodegradable polymers are used. A significant impact has been made recently in the formulation and development of innovative drug delivery systems by dosage forms that can exact control the release rates and target medications to a specific body region. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. This can be done by creating microspheres with bio adhesive properties and connecting them to microspheres. Oral medication delivery is significantly improved by the use of polymers that stick to the mucin epithelial surface. Both biodegradable and non-biodegradable polymer microspheres have been investigated for sustained release. The degradation products of polymers must not be harmful in order for them to function correctly because they may eventually enter the bloodstream or result in tissue implantation. The microspheres exhibited good Gastro Retentive properties for optimized formulation (F10) in the in vitro wash off test. The muco-adhesive microspheres' gradual, up to 8-hour atorvastatin release was dependent on the coat's makeup. Diffusion control and Higuchi kinetics were used to regulate drug release. Thus, these gastro-retentive microspheres are appropriate for atorvastatin's oral controlled release. In the improved formulation, The FTIR studies ruled out the drug-polymer interaction in the optimized formulation (F10). The SEM results have shown the Size and Surface Morphology of the Atorvastatin Gastro Retentive Microspheres.

KEYWORDS: Gastro retentive microspheres, Atorvastatin, polymers, Ionic gelation technique, Oral drug delivery, FTIR studies, SEM studies.

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2. INTRODUCTION

Microspheres are discrete spherical particles ranging in average particles size from 1 to 1000µm.

A primary object of using mucoadhesive formulations orally would achieve a substantial increase in the length of stay of the drug in GI tract stability problem in the intestinal fluid can be improved. Mucoadhesive microsphere carrier systems are made from the biodegradable polymers in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery system. They have varied applications and are prepared using assorted polymers. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membrane. This can be achieved by coupling Bioadhesion characteristics to microspheres and developing bioadhesive microspheres. Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to high surface to volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site.

To overcome the relativity short GI time and improve localization for oral controlled or sustained release drug delivery systems. The polymers which adhere to the mucin epithelial surface are effective and lead to significant improvement in oral drug delivery based on this three broad categories.

- Polymer that becomes sticky when placed in water and owes their Bioadhesion to sickness.
- Polymers that adhere through non-specific, non-covalent interaction are primarily electrostatic in nature.
- Polymer that binds to specific receptor site on the cell valerate.

Microspheres of biodegradable and non biodegradable polymers have been investigated for sustained release. An important requirement of polymers is that degradation products should be non toxic because such products eventually enter systemic circulation or result in tissue deposition.

Good [1] defined mucoadhesion as the state in which two materials, at least one biological in nature, are held together for an extended period of time by interfacial forces. It is also defined as the ability of a material (synthetic or biological) to adhere to a biological tissue for an extended period of time [2]. In case of mucoadhesion, the biological tissue is the mucous membrane. For mucoadhesion to occur, a succession of phenomena is required. The first stage involves an intimate contact between a mucoadhesive polymer and a membrane, either from good wetting of the mucoadhesive surface or from the swelling of the mucoadhesive. In the second stage, after Contact is established,

penetration of the mucoadhesive into the crevice of the tissue surface or interpenetration of the chains of the mucoadhesive with those of the mucus takes place. Low chemical bonds can then settle [3].

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 mucin-epithelial surface can be conveniently divided into three broad classes:

1. Polymers that become sticky when placed in water and owe their mucoadhesion to stickiness.
2. Polymers that adhere through nonspecific, noncovalent interactions that 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. All three polymer types can be used for drug delivery.

Characteristics of an ideal mucoadhesive polymer

An ideal mucoadhesive polymer has the following characteristics [4]:

1. The polymer and its degradation products should be nontoxic and should be non absorbable from the gastrointestinal tract.
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.

2.1 Mucoadhesive Controlled Delivery [5]

Mucoadhesion is the relatively new and emerging concept in drug delivery. Mucoadhesion keeps the delivery system adhering to the mucus membrane. Transmucosal drug delivery systems show various merits over conventional drug delivery systems.

Mucoadhesion, or the attachment of a natural or synthetic polymer to a biological substrate, is a practical method of drug immobilization and an important new aspect of controlled drug delivery. The subject of mucoadhesion is not new, they has been increased interest in recent years in using mucoadhesive polymers for drug delivery. The study and alteration of the adhesion of bioadhesive materials, as well as the diffusion of various drugs from bioadhesive devices, is of significance.

The motivation of controlled drug release is the necessity to maintain a constant effective drug concentration in the body for an extended time period. For optimal performance drug concentration in the body should be maintained above the effective level and below the toxic level.

A mucoadhesive controlled release device can improve the effectiveness of the drug concentration between the effective and toxic levels, inhibiting the dilution of the drug in the body fluids, and allowing targeting and localization of a drug at a specific site. A drug can be incorporated in to a cross linked polymeric device that would adhere to a mucous substrate in the body. The drug can then diffuse from the device directly in to the tissues.

Mucoadhesion also increase the intimacy and duration of contact between a drug containing polymer and mucous surface. The combined effects of the direct drug absorption and the decrease in excretion rate allow for an increase bioavailability of drug with smaller doses and less frequent administration. Another advantage of using a polymer carrier for drug delivery is prevention of first pass metabolism of certain protein drugs by liver through the introduction of the drug via a root bypassing the digestive tract. A polymeric device also allows for slow, controlled and predictable drug release overtime and reduces the initial drug loading concentration needed. This reduction also decreases the toxicity and waste of expensive drugs as well as improves patient compliance because the drug would not have to be administered as often.

2.2 Physiology Of Mucin [6]

The tissue layer responsible for the formation of the adhesive interface is mucous. Mucous is a translucent and viscid secretion, which forms a thin, continuous gel blanket adherent mucosal epithelial surface. The mean thickness of this layer varies from about 50-450 µm in humans[1].

The principle role of the mucous is to protect and lubricate the epithelial tissue beneath it. In adhesion to lubricating, ocular mucin helps to hydrate, clean and remove cell debris from eye. Those mucin also serve to defend against pathogens. Mucous is primarily composed of approximately 95% water, 1% electrolytes, 0.5-1% proteins and 0.5-1% lipids and glycoproteins[4]. It is important to note that the placement of and adhesive polymer device on the mucosal surface decreases the rate of mucin turnover, which can result in the mucoadhesive remaining on the mucous for up to 15hrs. Therefore, the location of application and any presence of disease should be considered when designing the mucoadhesive device.

2.3 Mechanism of Mucoadhesion [5, 6]

Most of the research has focused on analyzing bioadhesive interactions between polymer hydrogels and on soft tissues. The process involved in the formation of bioadhesive bonds has been described in 3 steps.

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. Bioadhesive 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.

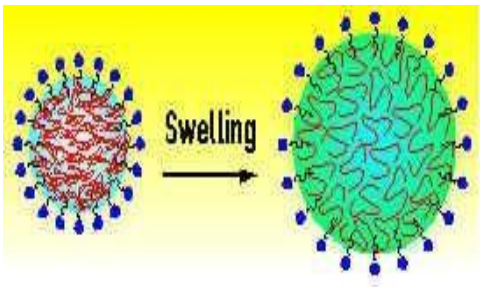

Figure 1: Swelling of the polymer

Step 2: The surface of mucosal membranes are composed of high molecular weight polymers known as glycoproteins. In step 2 of the bioadhesive bond formation, the bioadhesive polymer chains and the mucosal polymer chains intermingle and entangle to form semi permeable adhesive bonds. The strength of these bonds depends on the degree ofpenetration between the two polymer groups. In order to form strong adhesive bonds, one polymer group must be soluble in the other and both polymer types must be of similar chemical structure.

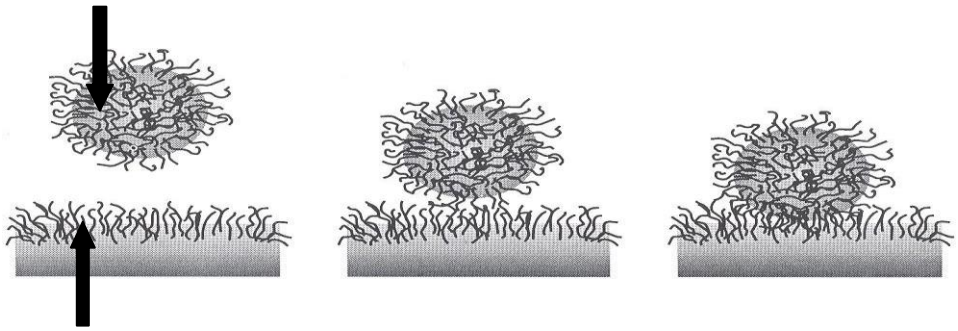

Figure 2: The Interpenetration of polymer chain

Step 3: Formation of weak chemical bonds between entangled chains. 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.

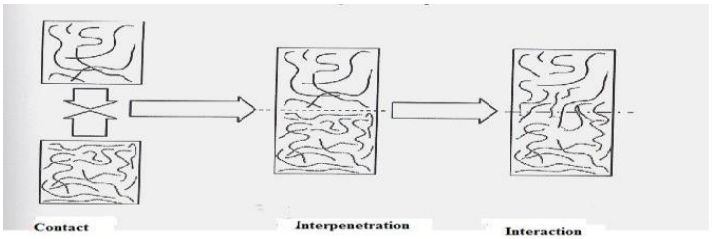

Figure 3: Mechanism of Mucoadhesion

2.4 THEORIES OF BIOADHESION [7]

The theories of polymer-polymer adhesion can be adapted to polymer-tissue adhesion or bio-adhesion by recognizing that bio-adhesion is different only because of the differing properties of the tissue as opposed to those of the polymer.

2.4.1 Electronic theory: According to this theory, electron transfer occurs up on contact of an adhesive polymer with a mucus glycoprotein network because of differences in their electronic structures. This results in formation of an electrical double layer at the interface.

2.4.2 Absorption theory: According to this theory, after an initial contact between two surfaces, the material adheres because of surface forces acting between the atom in the two surfaces. Two types of chemical bonds resulting from these forces.

2.4.2.1 Primary chemical bonds of covalent nature, which are undesirable in bio- adhesion because their high strength may result in permanent bonds.

2.4.2.2 Secondary chemical bonds having many different forces of attraction, including electrostatic forces, Vanderwaal forces and hydrogen bonds.

2.4.3 Wetting theory is predominantly applicable to liquid bio-adhesive systems and analyses adhesive and contact behavior in terms of the ability of a liquid or a paste to spread over a biological system.

2.4.4 Diffusion theory: According to this theory, the polymer chains and the mucus mix to a sufficient depth to create a semi permanent adhesive bond. The exact depth to which the polymer chains penetrate the mucus depends on the diffusion coefficient and the time of contact.

2.4.5 Fracture theory: This theory attempts to relate the difficulty of separation of two surfaces after addition.

$$G = (E\,\varepsilon/L)^{1/2}$$

Where 'E' is the Young's modulus of elasticity. '**ε**' is the fracture energy.

'L' is the critical crack length.

2.5 TYPES OF BIO-ADHESION [4, 8]

2.5.1 Non-Specific Bio-adhesion: Non-specific bio-adhesion with the intestinal membrane occurs through physicochemical interactions. Some natural polymers have the ability to adhere on wet mucosal surfaces by means hydrogen bonding or vander waals forces. With sellable hydrophilic polymers, adhesion is optimal when the mucosal contact is made with the dry polymer[6]. Moreover, the progressive hydration of the polymer leads to formation of hydrogen, which is responsible for the development of a considerable adhesive strength. This concept is quite efficient in moderately flooded cavities of the body, such as nasal and buccal cavities. However, in the GIT particles are directly mixed with liquid material in the stomach, which is likely to strongly decrease the adhesiveness of such polymers because of the premature hydration of the polymer, which takes place before the contact with the mucosal surface. So the various approaches of GI bioadhesion of colloidal particles are based on the use of non-

swellable, hydrophobic polymer such as poly (alkyl cyanoacrylate) or poly (lactic acid).in this case adhesion is mainly due to the inherent tendency of these small particles to develop intimate contacts with large mucosal surfaces.

2.5.2 Specific Bioadhesion: Non-specific Bioadhesion suffers from two major drawbacks,

1. Only a fraction of the dosage form administered is absorbed while remaining part is subjected to direct fecal elimination.
2. Due to the unspecific of the interaction, targeting to specialized area of the mucosa with unmodified particles is unrealistic.

2.6 Mucoadhesive dosage forms [9]

The primary objectives of mucoadhesive dosage forms are to provide intimate contact of the dosage form with the absorbing surface and to increase the residence time of the dosage form at the absorbing surface prolong drug action. Due to mucoadhesion, certain water - soluble polymers become adhesive on hydration and hence can be used for targeting drug to a particular region of the body for extended periods of time. The mucosa lines a number of regions of the body including the gastrointestinal tract, the urogenital tract, the airways, the ear, nose,
and eye. These represent potential sites for attachment of any mucoadhesive system and hence, the mucoadhesive drug delivery system may include the following.

- Buccal delivery system.
- Gastrointestinal delivery system.
- Nasal delivery system.
- Ocular delivery system.
- Vaginal delivery system.
- Rectal delivery system.

2.7 Advantages Of Mucosdhesive Drug Delivery System [10]

- Prolonged residence time at the site of action or absorption ;
- Localization of the drug delivery system at a given target site;
- An increase in the drug concentration gradient due to the intestine contact of the particles with the mucosal surface;
- Direct contact with intestinal cells, which is the step earlier to particle absorption. Mucoadhesion also increases the intimacy and duration of contact between a drug-

containing polymer and a mucous surface. The combined effects of the direct drug prolonged residence time allow for an increased bioavailability of the drug with a smaller dosage and less frequent administration. Bio adhesive systems can prevent the first pass metabolism of certain protein drugs by the liver through the introduction of the drug via route bypassing the digestive tract. Drugs that are absorbed through the mucosal lining of tissues can enter directly in to the blood stream and prevented from enzymatic degradation in the GIT.

2.8 Mucoadhesive Polymers

There are two broad classes of mucoadhesive polymers:

- Hydrophilic polymers and
- Hydro gels.

In the large classes of hydrophilic polymers those containing carboxylic group exhibit the best mucoadhesive properties, poly vinyl pyrrolidine (PVP), methyl cellulose (MC), sodium
carboxy methyl cellulose (SCMC), hydroxyl propyl cellulose (HPC) and other cellulose derivatives. Hydrogels are the class of polymeric biomaterial that exhibit the basic characteristics of an Hydrogels to swell by absorbing water interacting by means of adhesion with the mucus that covers epithelia i.e.

- Anionic group- Carbopol, Polyacrylate and their cross linked modifications
- Cationic group- Chitosan and its derivatives
- Neutral group- Eudragit- NE30D etc.

2.9 Factors Important For Bioadhesion

2.9.1 Polymer related factors

2.9.1.1 Molecular weight: The inter penetration of polymer molecules is favorable for low molecular weight polymers where as entanglements are favored for high molecular weight polymers. The optimum molecular weight for maximum bio adhesion depends on the type of polymers. Their nature dictates the degree of swelling in water in turn determines interpenetration of polymer molecules within the mucus. The bio adhesive force increases with the molecular weight of bio adhesive polymer up to 100,000 and beyond this level there is not much effect.

2.9.1.2 Concentration of active polymer: there is an optimum concentration of polymer corresponding to the best bio adhesion. In highly concentrated systems the adhesive strength drops significantly. In fact, in concentrated solutions the coiled molecules become solvent poor the chains available for interpenetration are not numerous.

2.9.1.3 Flexibility of polymer chain: As water-soluble polymers become cross linked, the mobility of individual polymer chain decreases. As the cross linking density increase, the effective length of the chain which can penetrate into the mucus layer decreases even further and mucoadhesive strength is reduced.

2.9.1.4 Spatial conformation: Despite a high molecular weight of 19,500,000 for dextrans, they have similar adhesive strength to that of PEG with a molecular weight of 200,000.

2.9.2 Environment related factors:

2.9.2.1 pH was found to have a significant effect on the mucoadhesion as observed in studies of polyacrylic polymers cross linked with –COOH groups. pH influences the charge on the surface of both mucus and polymers. Mucus will have a different charge density depending on pH because of differences in dissociation of functional groups on the carbohydrate moiety andamino acids of polypeptide back bone.

2.9.2.2 Applied strength: To place a solid bio adhesive system, poly (acrylic acid / di vinyl benzene) or Carbopol 934, the adhesion strength increases with applied strength. The pressure initially applied to the mucoadhesive tissue contact surface affect the depth of interpenetration.

2.9.2.3 Initial contact time: The initial contact time between muco adhesives and the mucus layers determines the extent of swelling and interpenetration of polymer chains. Along with internal pressure, the initial contact time can dramatically affect the performance of the system.

2.9.2.4 Selection of the model substrate surface: The handling and treatment of biological substrate during the testing of muco adhesives in an important factor, since physical and biological changes may occur in mucus gels or tissues under the experimental conditions.

2.9.2.5 Swelling: This characteristic is related to the polymer itself, and also its environment. Interpenetration of chains is easier as polymer chains or disentangled and free of interactions. Swelling depends both on polymer concentration and on presence of water. When swelling is too great, a decrease in bio adhesion occurs. Such a phenomenon must not occur too early in order to lead to a sufficient action of the bio adhesive system. It allows easy detachment of the bio adhesive system after the discharge of the active ingredient.

2.9.3 Physiological variables [11]

2.9.3.1 Mucin turnover: The mucin turnover is expected to limit the residence time on the muco adhesives on the mucus layer and mucin turnover results in substantial amounts of soluble mucin molecule. These molecules interact with muco adhesives before they have a chance to interact with the mucus layer. Calculated mucin turn over time of 47–200 mins.

2.9.3.2 Disease status: The physico-chemical properties of mucus are known to be to change during disease conditions such as common cold, gastric ulcers, ulcerative colitis, cystic fibrosis, bacterial and fungal infections of the female reproductive tract and inflammatory conditions of the eye.

2.10 Experimental Techniques In Mucoadhesive Drug Delivery

The bio adhesion of these polymers must be evaluated so that polymers can be compared and their relative bio adhesion determined. Bio adhesion tests are fundamental for the development, control of quality, processing and proper use of bio adhesives. The adhesive strength of a bioadhesive bond is subjective, its value being a function of several experimental parameters including contact time, temperature and openness of the polymer network. Numerous and in vitro ex vivo techniques to qualify bio adhesion have been developed in recent years.

2.10.1 Willhelmy plate method: a glass plate is coated with a bioadhesive polymer and immersed in beaker of mucin solution. A microbalance is connected to a plate to measure the dynamic force and plate as the beaker is lowered away from the mucin solution. The force measured is then related to the wettability of the mucin on the

polymer surface and correspond to the adhesive force between the bioadhesive and the mucin. This technique has the advantage of being expensive and rapid, although disadvantage include possible errors resulting from capillary forces, hysteresis and polymer dissolution in the mucin solution.

2.10.2 Ex vivo fluorescence method of measuring bioadhesion in which human epithelial cells are labeled with the fluorescent probes pyrene are fluorescein isothiocynate. These cells are then combined with bio adhesive polymer. When a photo excited moiety combines with an unexcited moiety, an eximer is formed the ratio of eximer to monomers is monitored as function of time in order to assess the affinity of the cells for the mucin. There are some minor problems associated with it, i.e., migration of pyrene from the cells may act to reduce eximer formation, showing an under estimated value for the affinity of the cells for the mucin.

2.10.3 Flow channel technique: In this method bio adhesive spherical polymer particle was placed on mucus surface inside a Plexiglas channel. A laminar flow of air or a viscoelastic solution was directed over the particle while photographs were taken to determine the static and bio adhesive behavior of the particle.

2.10.4 Falling film: In this method measuring the ability of a polymer in a flowing fluid to adhere to mucus. Using this method, small spherical latex particles are coated with a bio adhesive polymer and combined with a buffer solution to create a suspension of particles with a known concentration. The solution with the contained microspheres is then pumped over a rat small intestine that has been cut lengthwise and placed in semi cylindrical trough. The eluted solution and the particles are collected in beaker and the collected particles are counted using an electronic particle counter. The fraction particles that adhered to the mucus during the flow experiment are then related to the bio adhesion of the polymer.

2.10.5 Tensio metric technique: In these techniques, the tensile strength is needed to separate a bio adhesive from tissues is measured. One such technique is that in which an animal tissue is placed on a clamp of a tissue device and brought into contact with a bio adhesive polymer tablet. Swelling of that tablet occurs at the interface over time while it is in contact with the mucus. A vertical force is applied until the tablet and mucus separate and this force is used to calculate the work of addition. If a good bio adhesive material is used, the addition of the mucus to the polymer is stronger than the cohesion of the mucous gel, causing mucin molecule to part from mucous gel, upon separation.

2.10.6 In vivo technique [7]: This is developed based on γ-scintinography. Using this method, a bio adhesive device is labeled with Tc or In , administered to an animal while the residence time of the device in the body is monitored with gamma camera. The length of the time the device spends in the gastric area is related to the mucoadhesive ability of the device. This technique is advantageous because it is noninvasive.

2.11 USE OF MUCOSAL ADHESIVE PREPARATIONS [12]

- The extent of drug absorption is limited by the residence time of drug at the absorption site.
- In ocular drug delivery, less than 2 mins are available for drug absorption after installation of a drug solution into the eye, since it is removed rapidly by solution drainage and hence the ability to extend contact time improves drug bioavailability.

- In oral drug delivery, the drug absorption is limited by the GI transit time of the dosage form.
- In most of the route of drug administration ocular, nasal, buccal, respiratory, gastrointestinal, rectal and vaginal are coated with mucus layer, muco adhesives are expected to increase the residence time.
- Muco adhesives provide intimate contact between a dosage form and the absorbing tissue which may result in high drug concentration in local area.
- Mucoadhesion has high drug flux through the absorbing tissue, further the intimate contact may increase the total permeability of high molecular weight drugs such as peptides and proteins.
- Many drugs are absorbed only from the upper small intestine, localizing oral drug delivery systems in the stomach or in the duodenum would significantly improve the extent of drug absorption.

Table 1: List of Drugs – Mucoadhesive drug delivery systems [12]

S. No.	DOSAGE FORM	DRUGS
1	Microspheres	Clonazipam, Rifampicin+Isoniazid, Ciprofloxacin, Ofloxacin, Metronodazole, Aceclofenac.
2	Granules	Terbinafin.
3	Films	Cinnarizine.
4	Powders	Several basic drugs.
5	Capsules	Fluconazole, Omeprazole.

Table 2: Pharmaceutical applications of bioadhesive microspheres [12]

Drug	Route of administration	Bioadhesive polymers use	Comments/results
Acyclovir	Ocular	Chitosan	Slow release rate increased AUC
Methyl prednisolone	Ocular	Hyaluronic acid	Slow release rates sustained drug concentration in tear fluids.
Gentamicin	Nasal	DSM+LPC	Increased nasal absorption
Insulin	Nasal	DSM+LPC	Efficient delivery of insulin into the systemic circulation via nasal route
Human growth hormone (hGH)	Nasal	DSM+LPC	Rapid and increased absorption
Desmopressin	Nasal	Starch	Addition of LPC causes a five folds increase in Cmax and two folds increase in bioavailability
Haemagglutinin (HA) obtained from influenza A virus	Nasal	HYAFF	With mucosal adjuvant serum lgG antibody response as compared to i.m.immunization
Furosemide	GI	AD-MMS (PGEFs)	Increased bioavailability Higher AUC effective absorption from the absorption window
Amoxicillin	GI	Ethyl cellulose-Carbopol-934P	Greater anti H. pylori activity

Delapril HCL	GI	AD-MMS (PGEFs)	MRT of drug is increased
Glipizide	GI	Chitosan	Prolonged blood glucose reduction
Glipizide	GI	Chitosan-alginate	Prolonged blood glucose reduction
Vancomycin	Colonic	PGEF coated with WithEudragit S 100	Well absorbed even without absorption enhancers
Insulin	Colonic	PGEF coated with Eudragit S 100WithEudragit S 100	Absorbed only in the presence of absorption enhancers, e.g. EDTA salts

3. MATERIALS AND METHODS

Table No 1 : Materials used for the formulation development

S.No	MATERIAL	MANUFACTURING COMPANY	GRADE
1	Atorvastatin	Hetero Pharma, Hyderabad	
2	Carbopol 940P	SD Fine Chemicals Ltd.,Mumbai	Pharmaceutical grade
3	Xanthan gum	SD Fine Chemicals Ltd.,Mumbai	Pharmaceutical grade
4	Ethyl cellulose	SD Fine Chemicals Ltd.,Mumbai	Pharmaceutical grade
5	Guar gum	SD Fine Chemicals Ltd.,Mumbai	Pharmaceutical grade
6	Methyl cellulose	SD Fine Chemicals Ltd.,Mumbai	Pharmaceutical grade
7	Sodium.CMC	SD Fine Chemicals Ltd.,Mumbai	Pharmaceutical grade
8	Sodium alginate	SD Fine Chemicals Ltd.,Mumbai	Pharmaceutical grade
9	Calcium chloride	SD Fine Chemicals Ltd.,Mumbai	Pharmaceutical grade

EQUIPMENTS USED

Table No 2: Equipments used for the process

S.No.	Name of the Equipment	Manufactured by
1	8 Bowl Dissolution apparatus	Electro Lab
2	18 gauge needle with syringe	Dispovan
3	U.V. Spectrophotometer	Labinda
4	Analytical Balance	Adair Dutt Instruments Pvt. Ltd., AD50B
5	Disintegration apparatus	Electro Lab
6	FTIR	Bruker
7	SEM	Cipra Lab

4. DISCUSSION

The objective of the present study was to prepare and evaluate Gastro Retentive microspheres of Atorvastatin. The microspheres were prepared by orifice- ionotropic gelation method using polymers such as HPMC (K 100 M), Carbopol 940P, Sodium CMC, Guar gum, Sodium Alginate, Ethyl Cellulose, Methyl Cellulose, Xanthan gum and 10% Calcium Chloride solution. Totally 15 different formulations of Atorvastatin were prepared by using above the

polymers. Finally the microspheres were evaluated for various characteristics like Drug content, Encapsulation efficiency, Percent Gastro Retentive property and the *In-vitro* drug release studies were evaluated for 8 hrs.

Microspheres of Atorvastatin with a coat consisting of sodium alginate and different Gastro Retentive polymers - Sodium CMC, Methylcellulose, Carbopol 940P, HPMC K100M, Ethyl cellulose, in 1:1, with HPMC K100M, Carbopol 940P, Guar gum, Xanthan gum, Methyl cellulose in 1:2, with Guar gum ,and Xanthan gum 1:3 could be prepared by the orifice-ionic gelation process. The Microspheres were found to be discrete, spherical, free-flowing, and of the mono- lithic matrix type.

The prepared batches of microsphere were evaluated for Micromeritic study such as tapped density, bulk density, Carr's index, Hausner ratio and angle of repose**(Table 19).**
Microspheres with a coat consisting of sodium alginate and a Gastro Retentive polymer exhibited good Gastro Retentive properties in the in vitro wash-off test. **(Table 12&13).** The microencapsulation efficiency was in the range of 57% to 96% being highest for F4 and lowest for F5.
Result of in vitro wash-off test studies indicate that the formulation F10, F13, F14, and F15 having considerable Gastro Retentive property.

Atorvastatin release from the microspheres was studied in phosphate buffer (pH 7.0) for 8 hours. Drug release from the microspheres was slow and depended on the composition of the coat. Drug Release followed zero-order kinetics (R^2 = 0.953). From the all batches F10 (Drug: Sod. Alginate : Methyl cellulose = 1:2:1) batch is considered to be the most promising
formulation batch because among all the batches it shows better extent of drug release 97.11% (8hrs) , good entrapment efficiency (78%), and *in vitro* wash-off test shows good Gastro Retentive property. Atorvastatin release from alginate – Methyl cellulose (F10) was slow and extended over a period of 8 hrs and these microspheres were found suitable for the oral controlled release formulation.

Higuchi plot showed a "R^2" value of 0.980 in the optimized formulation (F10) suggesting that the diffusion plays an important role in the controlled release formulations. The data was fitted to Korsemeyer -Peppas equation and the value of diffusional exponent 'n' (0.86) indicated that the drug release shows non-fickian diffusion.

Observation of all formulation for physical characterization had shown that, all of them comply with the specification of official pharmacopoeias and/or standard references.

The FTIR studies indicated the lack of drug – polymer interactions in the Optimized formulation (F10). **(Table 14, Figure 13, 14,15).**

The SEM results indicated that the shape of Gastro Retentive microspheres were spherical and completely covered with the coat polymer **(Figure 17)**.[13-35]

5. CONCLUSION

The microspheres exhibited good Gastro Retentive properties for optimized formulation (F10) in the in vitro wash off test. Atorvastatin release from these muco-adhesive microspheres was slow and extended over up to 8 hrs and depended on the composition of the coat. Drug release was diffusion controlled and followed Higuchi kinetics. These Gastro Retentive microspheres are thus suitable for oral controlled release of Atorvastatin. The FTIR studies ruled out the drug-polymer interaction in the optimized formulation (F10). The SEM results have shown the Size and Surface Morphology of the Atorvastatin Gastro Retentive Microspheres.

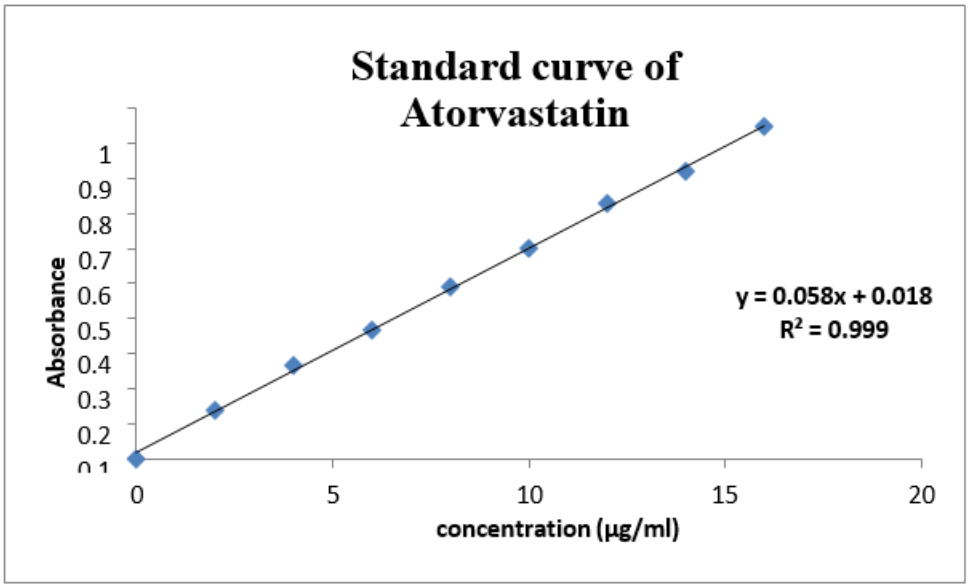

Figure 4: Calibration curve of Atorvastatin in PH 7.0 buffer

Figure 5: The diagrammatic representation of *In vitro* wash off test

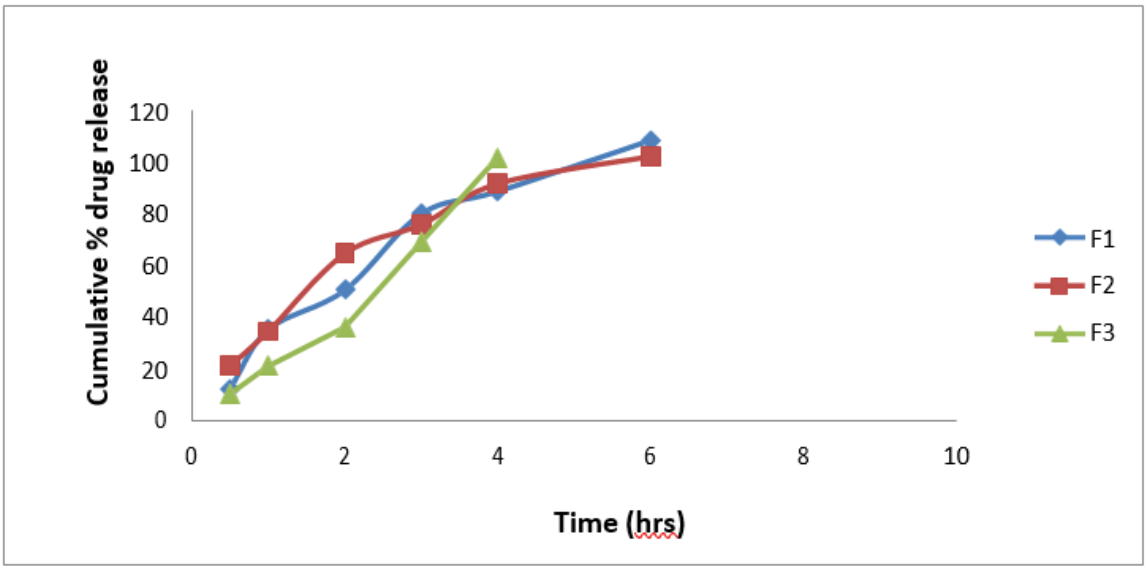

Figure 6: Dissolution profile of Gastro Retentive microspheres of Atorvastatin (F1, F2, F3) formulations.

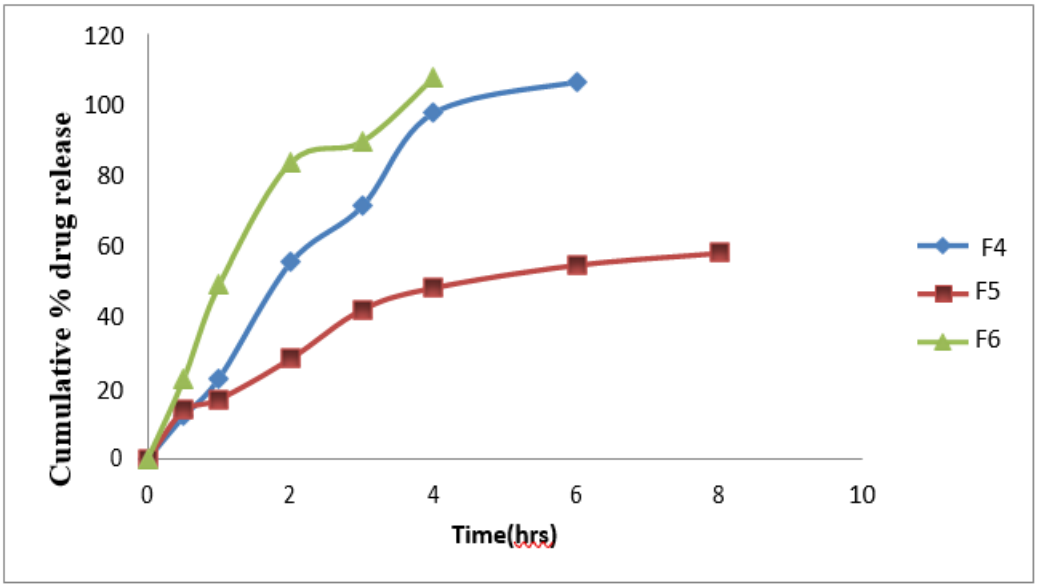

Figure 7 : Dissolution profile of Gastro Retentive microspheres of Atorvastatin (F4, F5, F6)formulations.

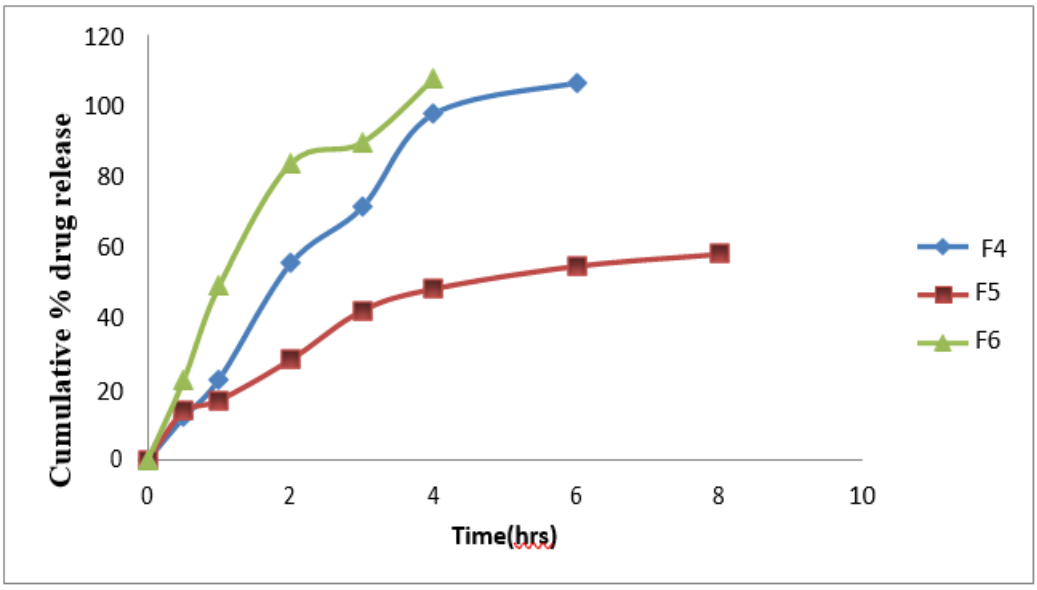

Figure 8: Dissolution profile of Gastro Retentive microspheres of Atorvastatin (F7, F8, F9)formulations.

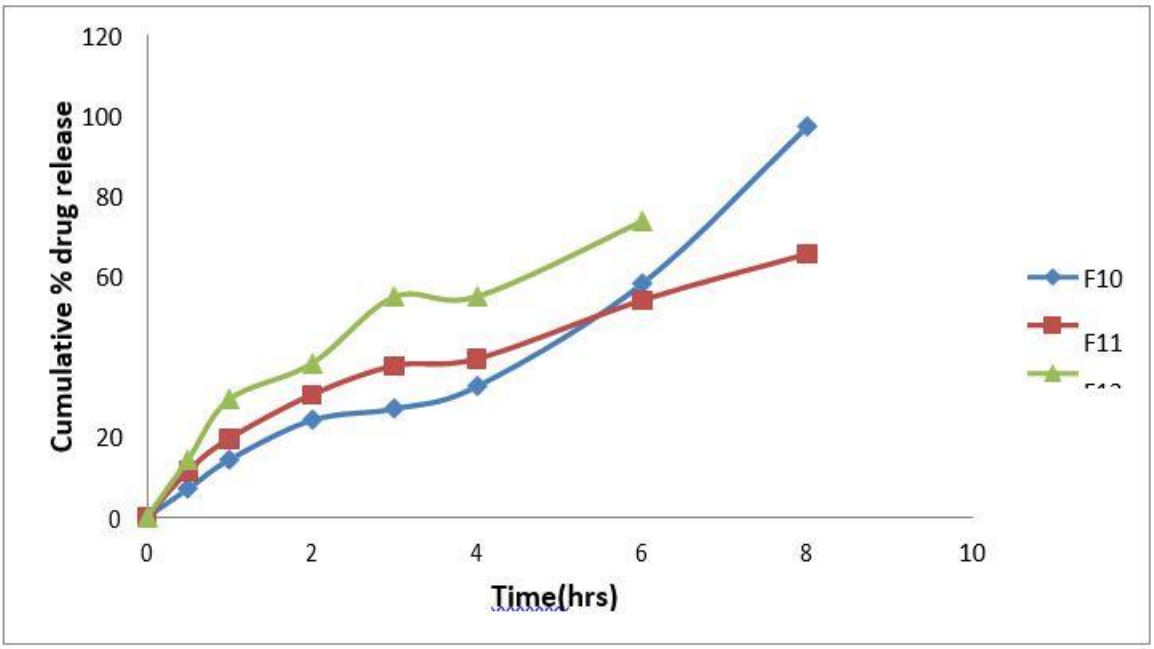

Figure 9: Dissolution profile of Gastro Retentive microspheres of Atorvastatin (F10, F11, F12)formulations.

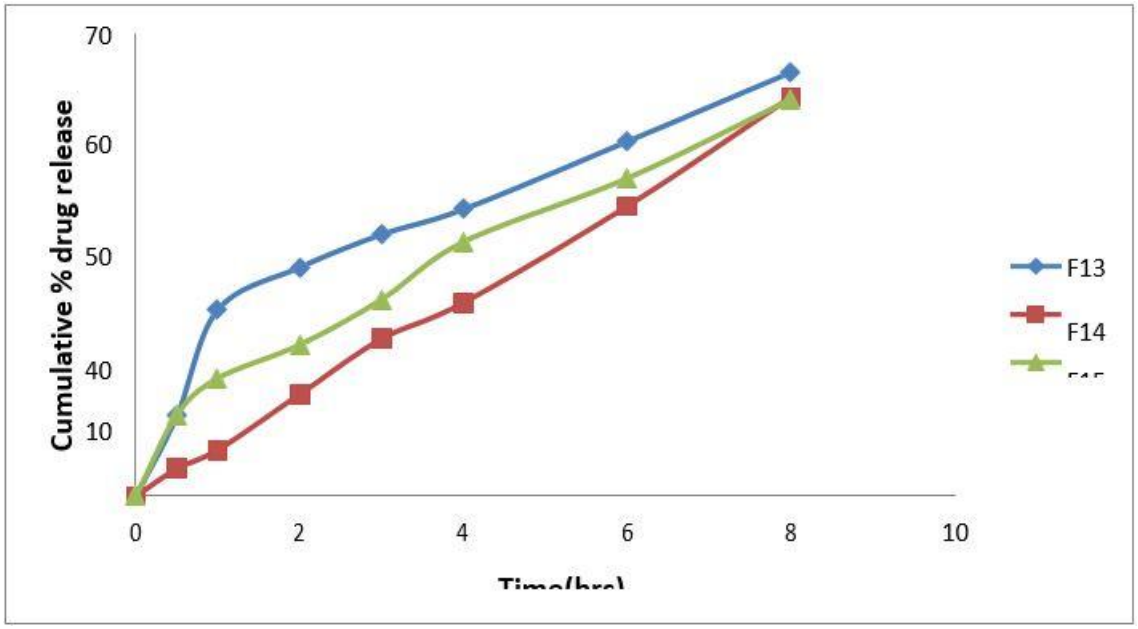

Figure 10: Dissolution profile of Gastro Retentive microspheres of Atorvastatin (F13, F14, F15)formulations.

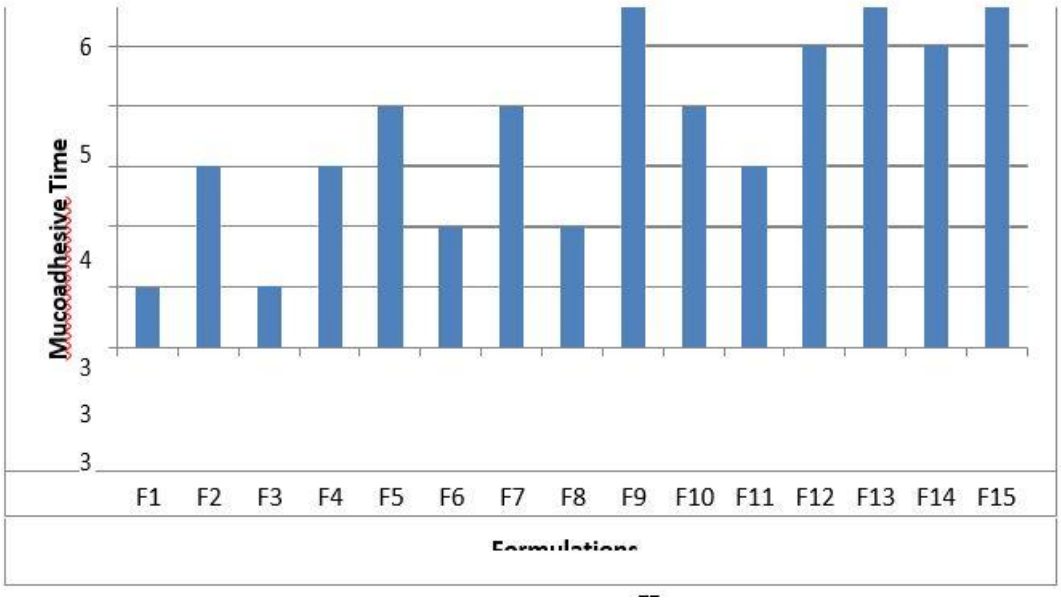

Figure 11: Gastro Retentive Property of different formulations in pH 1.2 HCl buffer.

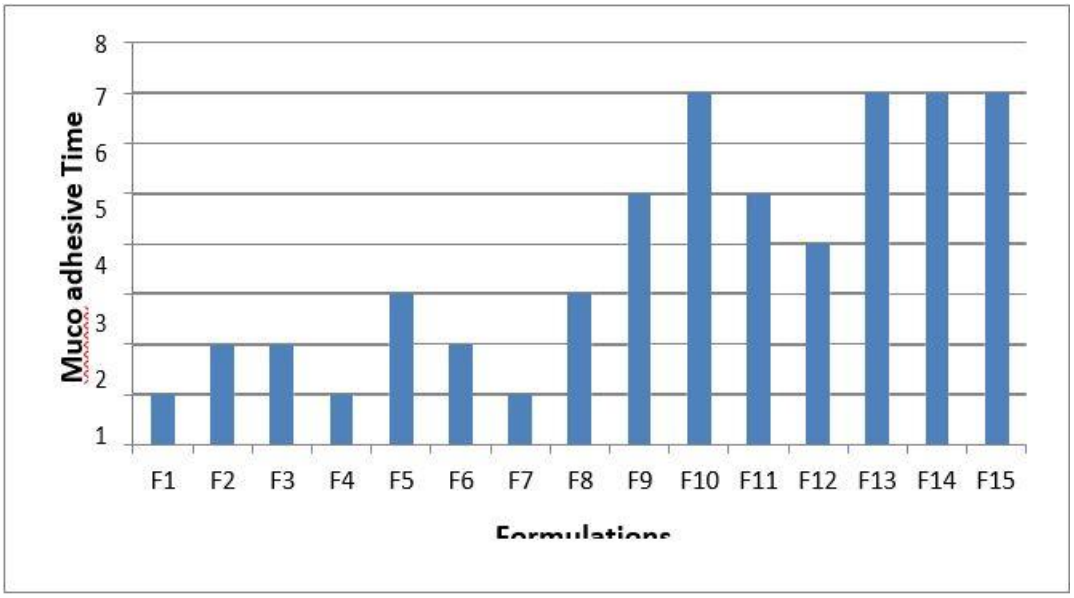

Figure 12: Gastro Retentive Property of different formulations in pH 7.0 Phosphate buffer.

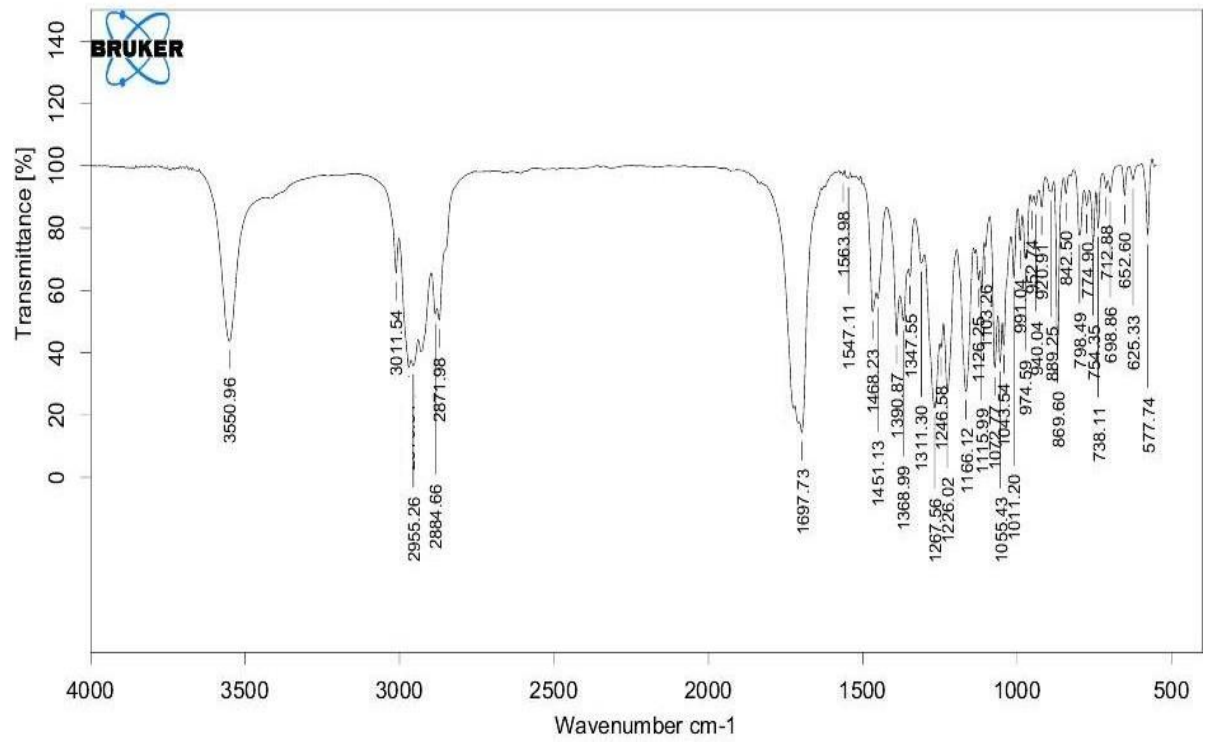

Figure 13: FTIR Spectrum of Atorvastatin

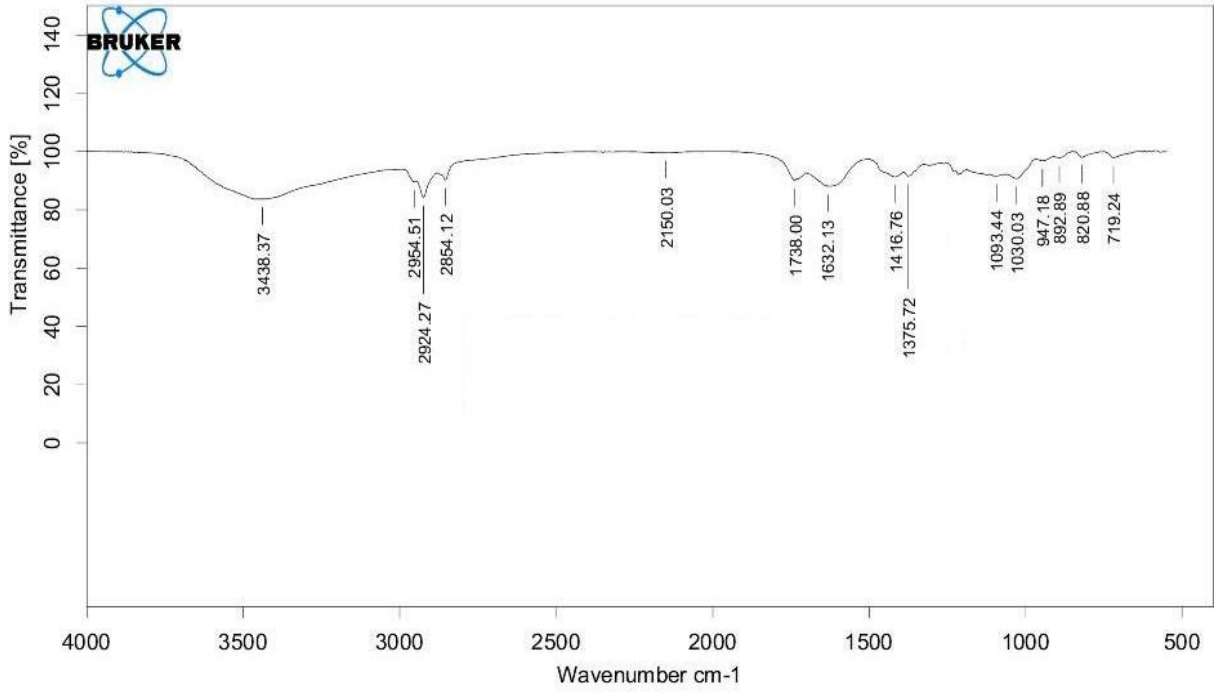

Figure 14: FTIR Spectrum of Sodium Alginate

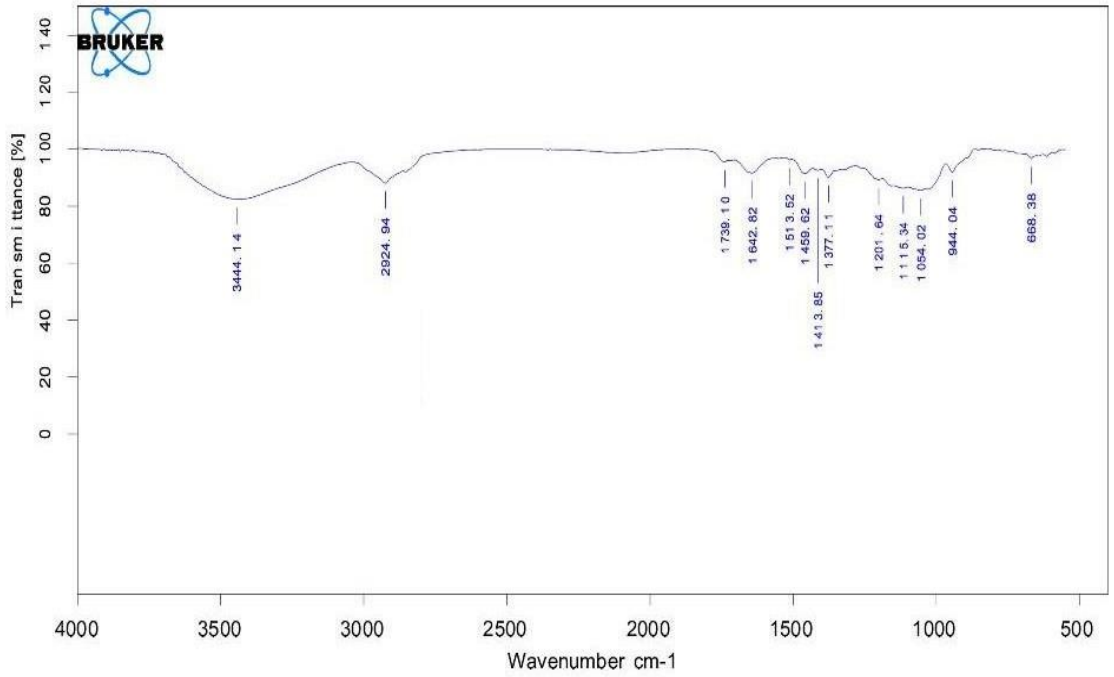

Figure 15: FTIR Spectrum of Methyl Cellulose

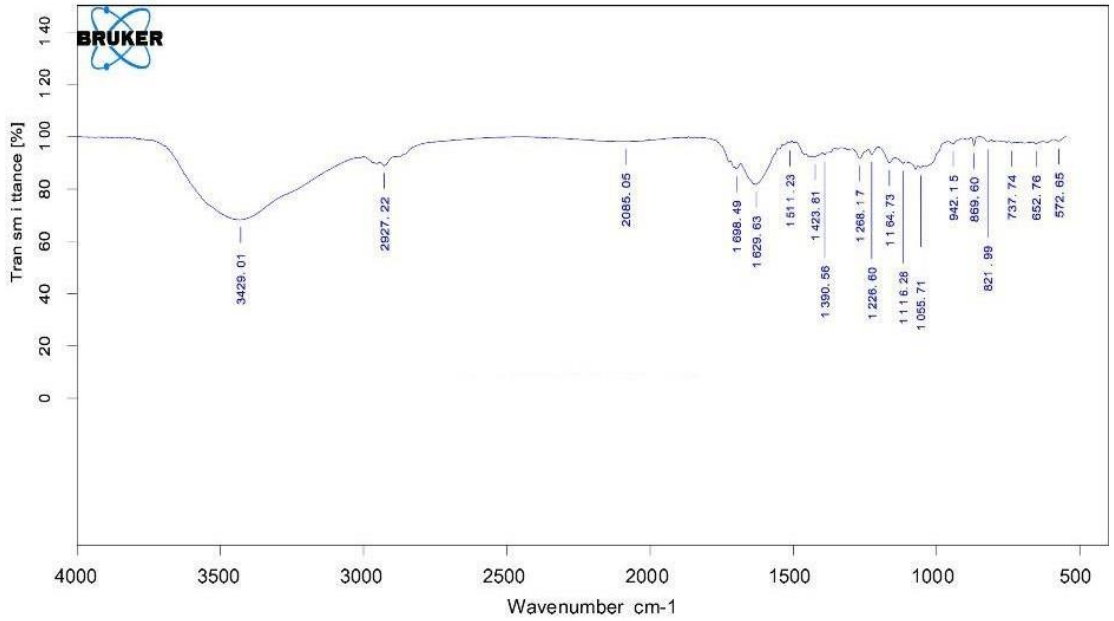

Figure 16: FTIR Spectrum of Optimized Formulation

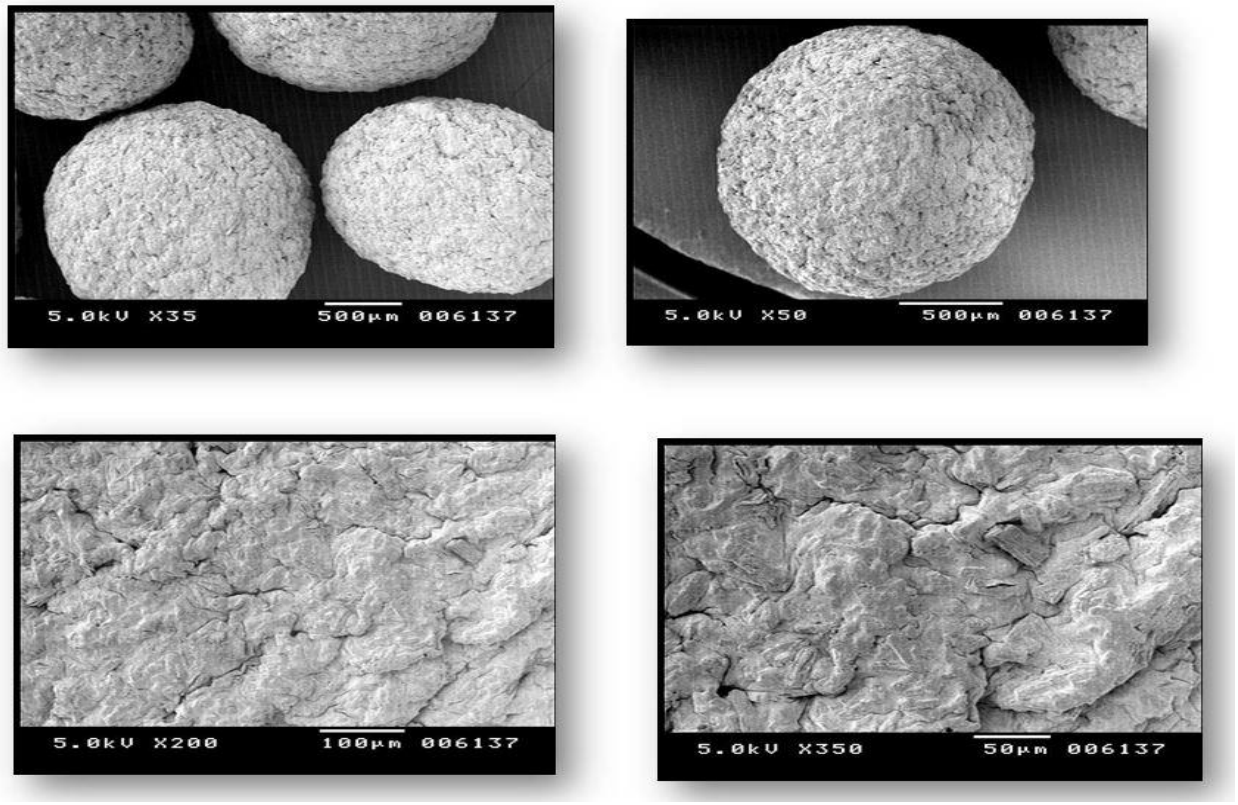

Figure 17: SEM images of Optimized Formulation (F10)

Table 6: Calibration curve of Atorvastatin

Concentration (µg/ml)	Absorbance
2	0.138
4	0.265
6	0.367
8	0.49
10	0.602
12	0.729
14	0.818
16	0.945

Table 7: Significance of Angle of Repose

S.No.	Angle of repose	Flow property
1	<25	Excellent
2	25-30	Good

3	30-40	Passable
4	>40	Poor

Table No 8: Limits for Carr's index and Hausner's ratio

S.No	Carr's Index (or) Compressibility Index:	Hausner's Ratio	Flow Property
1	<10	1.0-1.11	Excellent
2	Nov-15	1.12-1.18	Good
3	16-20	1.19-1.25	Fair
4	21-25	1.26-1.34	Passable
5	26-31	1.35-1.45	Poor
6	32-37	1.46-1.59	Very Poor
7	>38	>1.60	Very, very Poor

Table No 9: Composition of different formulations

Batch code	Coat Composition	Ratio
F1	Drug: Sod. Alginate	01:01
F2	Drug: Sod. Alginate : Carbopol (940)	1:0.9:0.1
F3	Drug: Sod. Alginate : HPMC (K100M)	1:0.9:0.1
F4	Drug: Sod. Alginate : Sod.CMC	1:0.9:0.1
F5	Drug: Sod. Alginate : Ethyl cellulose	1:0.9:0.1
F6	Drug: Sod. Alginate	01:02
F7	Drug: Sod. Alginate : Carbopol (940)	01:02:01
F8	Drug: Sod. Alginate : HPMC (K100M)	01:02:01
F9	Drug: Sod. Alginate : Guar gum	01:02:01
F10	Drug: Sod. Alginate : Methyl cellulose	01:02:01
F11	Drug: Sod. Alginate : Xanthan gum	01:02:01
F12	Drug: Sod. Alginate : Guar gum	01:03:01
F13	Drug: Sod. Alginate : Xanthan gum	01:03:01
F14	Drug: Sod. Alginate : Xanthan gum	03:00.5
F15	Drug: Sod. Alginate : Xanthangum : Guar gum	1:3:1:1

Table 10: Diffusion exponent and diffusion mechanism

Diffusion Exponent	Overall solute diffusion mechanism
0.45	Fickian diffusion
0.45<n<0.89	Anomalous (non-fickian) diffusion
0.89	Case II transport
n>0.89	Super Case II transport

Table 11: Dissolution data of Gastro Retentive Microspheres of Atorvastatin

Time (hrs)	Cumulative Percent Drug Release (n = 3±SD)

	F1	F2	F3
0.5	12.6 ± 2.0	21.42 ±1.00	10.46 ±2.48
1	35.42 ±3.2	34.68 ±1.25	21.27 ±1.2
2	50.55 ±1.21	64.73 ±1.34	36.3 ±7.34
3	80.04 ±1.65	75.91 ±1.9	69.26 ±8.7
4	88.68 ±3.47	91.67 ±1.30	101.8 ±2.8
6	108.4 ±2.02	102.18 ±0.93	---

Table 12: Dissolution Data of Gastro Retentive Microspheres of Atorvastatin

Time (hrs)	Cumulative Percent Drug Release (n = 3±SD)		
	F4	**F5**	**F6**
0.5	12±1.8	13.7±2.2	22.5±0.9
1	22.86±5.52	16.87±0.67	49.28±5.8
2	55.6±5.3	28.37±7.17	83.86±3.06
3	71.46±1.22	42.22±7.65	89.74±1.92
4	97.89±1.48	48.39±4.19	107.82±1.35
6	106.67±1.88	54.78±4.84	----
8	----	58.21±3.84	----

Table 13: Dissolution Data of Gastro Retentive Microspheres of Atorvastatin

Time (hrs)	Cumulative Percent Drug Release (n = 3±SD)		
	F7	**F8**	**F9**
0.5	13.65±4.56	32.79±2.51	12.45±1.58
1	40.27±3.03	42.42±1.59	31.69±4.34
2	56.16±3.67	65.94±1.73	58.89±2.52
3	63.54±5.75	91.39±0.99	72.41±1.87
4	84.24±4.2	102.59±1.56	88.58±5.8
6	105.75±6.76	----	108±1.73

Table 14: Dissolution Data of Gastro Retentive Microspheres of Atorvastatin

Time (hrs)	Cumulative Percent Drug Release (n = 3±SD)		
	F10	**F11**	**F12**
0.5	**7.05**±0.18	11.49±2.52	14.4±0.61
1	**14.26**±0.63	19.54±4.51	29.34±0.62
2	**24.11**±1.25	30.46±7.02	38.26±2.22
3	**26.95**±0.15	37.66±7.59	54.9±3.83
4	**32.5**±4.13	39.39±7.81	54.9±0.67

6	**58.07**±3.16	53.93±1.89	73.65±3.21
8	**97.11**±2.98	65.52±3.44	---

Table 15: Dissolution Data of Gastro Retentive Microspheres of Atorvastatin

Time (hrs)	Cumulative Percent Drug Release (n = 3±SD)		
	F13	**F14**	**F15**
0.5	12.17±3.1	4.15 ±0.83	12.3±1.08
1	28.29±5.19	7.00 ±1.76	17.9±0.609
2	34.69±3.75	15.4 3±1.31	22.96±0.254
3	39.68±1.34	23.8 3±3.88	29.84±2.26
4	43.51±1.97	29.3 1±3.67	38.56±1.82
6	53.79±2.99	43.9 7±4.57	48.22±0.95
8	64.29±7.87	60.5 ±4.68	60.18±3.2

Table 16: Quality Control Parameters of Gastro Retentive Microspheres of Atorvastatin:

S.No	**Batch code**	**Drug Content**		
		Theoretical (percentage)	**Practical (Percentage)**	**Encapsulation efficiency**
1	F1	50	39.7	79.40±0.025
2	F2	50	42.02	84.05±0.027
3	F3	50	39.03	78.07±0.027
4	F4	50	48.33	96.67±0.02
5	F5	50	28.73	57.47±0.012
6	F6	33.33	26.24	78.73±0.013
7	F7	25	19.14	76.57±0.032
8	F8	25	17.47	69.91±0.013
9	F9	25	18.6	74.40±0.017
10	**F10**	**25**	**19.37**	**77.51±0.025**
11	F11	25	18.1	69.64±0.019
12	F12	20	14	70.0±0.014
13	F13	20	13.62	65.75±0.017
14	F14	22.22	16.49	71.46±0.015

15	F15	16.66	10.59	61.18±0.012

Table 17: RELEASE KINETICS: Coefficient Of Correlation (R^2) values of differentbatches of Atorvastatin Gastro Retentive microspheres.

Formulation	Zero Order	First Order	Higuchi's	Peppa's
F1	0.939	0.943	0.984	0.961
F2	0.904	0.964	0.978	0.94
F3	0.98	0.82	0.927	0.969
F4	0.936	0.822	0.976	0.944
F5	0.872	0.929	0.957	0.945
F5	0.872	0.929	0.957	0.945
F6	0.926	0.965	0.967	0.957
F7	0.937	0.933	0.976	0.977
F8	0.951	0.918	0.985	0.992
F9	0.95	0.976	0.996	0.985
F10	**0.953**	**0.913**	**0.98**	**0.926**
F11	0.944	0.986	0.989	0.987
F12	0.987	0.946	0.954	0.961
F13	0.878	0.968	0.967	O.969
F14	0.998	0.996	0.966	0.996
F15	0.965	0.994	0.981	0.98

Table 18: Dissolution Parameters of Atorvastatin Gastro Retentive Microspheres

Formulation	Dissolution Parameters					
	N	**K0(mg/L/hr)**	**K1(hr^{-1})**	**T50(hrs)**	**T75(hrs)**	**T90(hrs)**
F1	0.63	8.64	0.557	2	2.7	4.3
F2	0.59	5	0.61	1.5	3	4
F3	1.14	15.71	0.4	2.5	3.2	3.5
F4	0.88	4.16	0.95	1.8	3.3	4
F5	0.61	2.93	0.09	4.5	--	--
F6	0.56	4.68	0.835	1	1.8	3
F7	0.54	12.06	0.414	1.5	3.5	4.7
F8	0.67	11.2	0.78	1.3	2.4	3
F9	0.68	9.56	0.55	1.3	3.2	4.3
***F10**	**0.86**	**10.86**	**0.13**	**5.3**	**6.8**	**7.5**
F11	0.55	4.14	0.117	5	--	--
F12	0.73	8.92	0.31	2.7	4.2	4.8
F13	0.38	4.85	0.105	5.2	--	--

F14	0.38	7.46	0.09	6.6	--	--
F15	0.59	4.83	0.101	6	--	--

Table : 19: Flow Properties of Different Formulations

Formulation	Angle of Repose	Bulk density(g/ml)	Tapped density(g/ml)	Hausner ratio	Compressibility index
F1	12	0.816	0.816	1	0
F2	14	0.672	0.717	1.06	6.2
F3	11	0.556	0.602	1.08	7.6
F4	12	0.692	0.721	1.04	4.02
F5	15	0.297	0.371	1.24	9.2
F6	13	0.656	0.772	1.17	7.8
F7	16	0.454	0.552	1.21	17.75
F8	19	0.772	0.821	1.06	5.96
F9	14	0.659	0.721	1.09	8.59
***F10**	**19**	**0.604**	**0.679**	**1.12**	**11.04**
F11	18	0.721	0.869	1.2	17.03
F12	16	0.526	0.619	1.17	15.02
F13	17	0.618	0.721	1.16	14.28
F14	15	0.536	0.59	1.1	9.1
F15	19	0.817	0.871	1.06	5.4

Table 20: Percent Gastro Retentive Property of the microspheres of Atorvastatin in p^H 1.2 HCl buffer.[25]

Time (hr)	Percent Gastro Retentive property														
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
0.5	33	41	22	40	54	40	41	50	78	76	54	61	66	84	74
1	**21**	**36**	8	**35**	46	28	32	38	69	68	40	46	58	**71**	66
2	---	**21**	---	**24**	**35**	10	24	**21**	45	52	21	38	42	61	**51**
3	---	**12**	---	**13**	**26**	---	**16**	---	38	43	**10**	28	30	46	**38**
4	---	---	---	---	**14**	---	**4**	---	24	**37**	---	20	26	26	**28**
5	---	---	---	---	---	---	---	---	12	**28**	---	12	18	11	**13**
6	---	---	---	---	---	---	---	---	**5**	**14**	---	----	9	7	**6**
7	---	---	---	---	---	---	---	---	---	---	---	--	---	---	---
8	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

Table 21: Percent Gastro Retentive Property of the microspheres of Atorvastatin in pH 7.0 Phosphate buffer. [25]

Time (hr)	Percent Gastro Retentive property														
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15

0.5	44	51	48	30	56	52	28	54	70	78	56	64	60	80	70
1	20	36	31	**29**	**38**	44	18	42	54	69	42	54	51	70	**61**
2	---	14	27	---	**29**	**13**	---	34	40	60	32	38	47	62	**53**
3	---	---	---	---	**13**	---	---	**12**	28	**55**	26	38	38	51	**49**
4	---	---	---	---	---	---	---	---	18	**43**	**15**	24	29	43	**40**
5	---	---	---	---	---	---	---	---	10	**39**	**8**	---	20	34	**33**
6	---	---	---	---	---	---	---	---	---	**26**	---	----	11	28	**21**
7	---	---	---	---	---	---	---	---	---	**8**	---	---	7	11	**9**
8	---	---	---	---	---	---	---	---	---	--	---	---	---	---	---

Table 22: DATA FOR IR SPECTRA OF ATORVASTATIN

Functional Group	Frequency (cm^{-1})
C-OH Aromatic (stretching)	3550
C=O (stretching) Acid Ester	1011
C-O-C (stretching)	1043

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