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MUCOADHESIVE MICROSPHERES AN OVERVIEW

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ABSTRACT

Drug development technologies constituting innovations at the formulation end in the pharmaceutical industry has received a lot of attention in past two decades. Drug delivery as an opportunity to extend product life cycles has indeed proved its place in the market with significant advantages of therapeutic gains as well as commercial success. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etc. which modulates the release and absorption characteristics of the drug. Mucoadhesion is a topic of current interest in the design of drug delivery systems. Mucoadhesive microspheres exhibit a prolonged residence time at the site of application or absorption and facilitate an intimate contact with the underlying absorption surface and thus contribute to improved and/or better therapeutic performance of drugs. Hence, uptake and consequently bioavailability of the drug is increased and frequency of dosing reduced with the result that patient compliance is improved. In recent years such Mucoadhesive microspheres have been developed for oral, buccal, nasal, ocular, rectal and vaginal for either systemic or local effects. This review article aims to provide various aspects of mucoadhesion, theories of mucoadhesion and the polymers which will shows the excellent mucoadhesive properties. It also contains a number of available methods of preparation of microspheres and its evaluation including in vitro-wash off test for to determine the mucoadhesive property of prepared microspheres.

Keyword: Mucoadhesion, Mucoadhesive polymers, Microspheres, Controlled drug delivery

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INTRODUCTION

For many decades, medication of an acute disease or a chronic disease has been accomplished by delivering drugs to the patients via various pharmaceutical dosage forms like tablets, capsules, pills, creams, ointments, liquids, aerosols, injectables and suppositories as carriers. To achieve and then to maintain the concentration of drug administered within the therapeutically effective range needed for medication, it is often necessary to take this type of drug delivery systems several times in a day. This results in a fluctuated drug level and consequently undesirable toxicity and poor efficiency. This factor as well as other factors such as repetitive dosing and unpredictable absorption leads to the concept of controlled drug delivery systems [1-2]. The word new or novel in the relation to drug delivery system is a search for something out of necessity. An appropriately designed sustained or controlled release drug delivery system can be major advance toward solving the problem associated with the existing drug delivery system[3-4].

The objective of controlled release drug delivery includes two important aspects namely spatial placement and temporal delivery of drug.

- Spatial placement relates to targeting a drug to a specific organ or tissue, while
- Temporal delivery refers to controlling the rate of drug delivery to the target tissue[5].

Oral controlled release dosage forms have been developed over the past three decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation. However, this approach is be dilled with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the gastrointestinal tract (GIT) due to variable motility and relatively brief gastric emptying time (GET) in humans which normally averages 2-3 h through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose[6].

The objective in designing a controlled release system is to deliver the drug at a rate necessary to achieve and maintain a constant drug blood level. This rate should be similar to that achieved by continuous intravenous infusion where a drug is provided to the patient at a rate just equal to its rate of elimination. This implies that the rate of delivery must be independent of the amount of drug remaining in the dosage form and constant over time, i.e release from the dosage form should follow zero-order kinetics[7].

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years 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 systems[8-10]. Microspheres have varied applications and are prepared using assorted polymers[11]. However; the success of these microspheres is limited owing to their short residence time at the site of absorption. So, various attempt have been made to increase the bioavailability as well as prolong the gastric residence time of dosage form in the stomach resulted in development of bio adhesive drug delivery system which will provide an intimate contact of the drug delivery system with the absorbing membranes[12-15]. This can be achieved by coupling mucoadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site[16-19]. Gastric mucoadhesive drug delivery offers a number of applications for drugs having poor bioavailability because of narrow absorption window in the upper part of gastrointestinal tract. It retains the dosage form at the site of absorption and thus enhances the bioavailability[20].

Mucoadhesion / Bio adhesion

Mucoadhesive drug delivery system are the systems which utilizes the property of bio adhesion of certain polymers which become adhesive on hydration and can be used for targeting a drug to a particular region of the body for extended periods of time. The term "mucoadhesion" was coined for the adhesion of the polymers with the surface of the mucosal layer[21]. Bio adhesions are a phenomenon in which two materials at least one of which is biological and are held together by means of interfacial forces [22]. In biological systems, bio adhesion can be classified into 3 types:

1. Adhesion between two biological phases, for example, platelet aggregation and wound healing
2. Adhesion of a biological phase to an artificial substrate, for example, cell adhesion to culture dishes and bio film formation on prosthetic devices and inserts
3. Adhesion of an artificial material to a biological substrate, for example, adhesion of synthetic hydrogels to soft tissues and adhesion of sealants to dental enamel[23]

For drug delivery purposes, the term bio adhesion implies attachment of a drug carrier system to a specified biological location. The biological surface can be epithelial tissue or the mucus coat on the surface of a tissue. If adhesive attachment is to a mucus coat, the phenomenon is referred to as mucoadhesion / mucoadhesion as the interaction between a mucin surface and a synthetic

or natural polymer[24] . In bio adhesion, the polymer is attached to the biological membrane.

Advantages of mucoadhesive systems: Mucoadhesive systems have three distinct advantages when compared to conventional dosage forms.

1. Readily localized in the region applied to improve and enhance the bioavailability of drugs. E.g. testosterone & its esters, vasopressin, dopamine, insulin and gentamycin etc.
2. Facilitate intimate contact of the formulation with underlying absorption surface. This allows modification of tissue permeability for absorption of macromolecules. e.g. peptides and proteins.
3. Prolong residence time of the dosage form at the site of application and absorption to permit once or twice a day dosing[25].

Mechanism of mucoadhesion: A complete understanding of how and why certain macromolecules attach to a mucus surface is not yet available, but a few steps involved in the process are generally accepted, at least for solid systems. Several theories have been proposed to explain the fundamental mechanism of adhesion[26]. A general mechanism of mucoadhesion drug Delivery system is show in Figure 1.

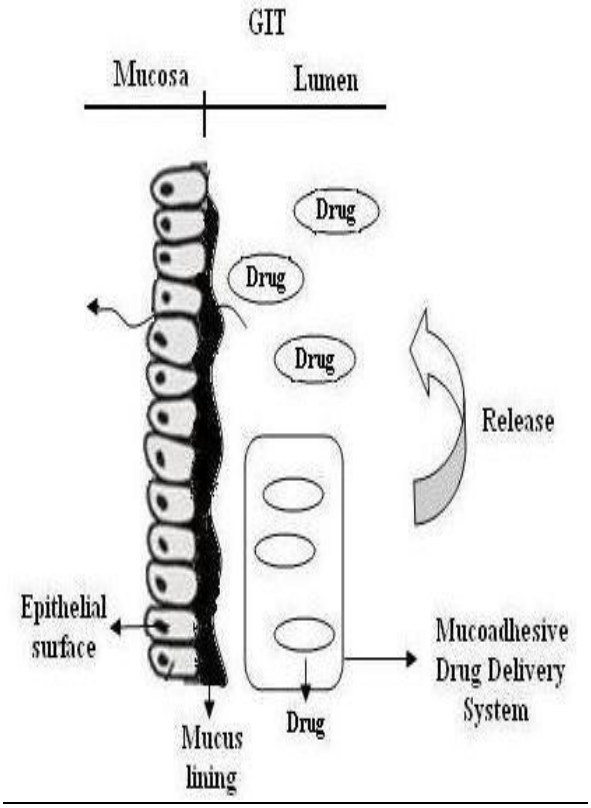

Figure 1 Mechanism of Mucoadhesion

Theory of mucoadhesion:

The phenomena of bioadhesion occur by a complex mechanism. Six theories have been proposed, which will explain the mechanism of bioadhesion. The theories are as follows[27-31].

Electronic theory: Involves the formation of an electric double layer at the mucoadhesive interface by the transfer of electrons between the mucoadhesive polymer and the mucin glycoprotein network. For example: Interaction between positively charged polymers chitosan and negatively charged mucosal surface which becomes adhesive on hydration and provides an intimate contact between a dosage form and absorbing tissue.

Wetting Theory: States that if the contact angle of liquids on the substrate surface is lower, then there is a greater affinity for the liquid to the substrate surface. If two such substrate surfaces are brought in contact with each other in the presence of the liquid, the liquid may act as an adhesive amongst the substrate surfaces.

Adsorption Theory: According to this theory, after an initial contact between two surfaces, the material adheres because of surface force acting between the atoms in two surfaces. Two types of chemical bonds resulting from these forces can be distinguished as primary chemical bonds of covalent nature and Secondary chemical bonds having many different forces of attraction likes electrostatic forces, Vander Walls forces, hydrogen and hydrophobic bonds.

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 chain penetrates the mucus depends on the diffusion coefficient and the time of contact. The diffusion coefficient in terms depends on the value of molecular weight between cross linking and decreases significantly as the cross linking density increases.

Mechanical Theory: Explains the diffusion of the liquid adhesives into the micro-cracks and irregularities present on the substrate surface thereby forming an interlocked structure which gives rise to adhesion.

Cohesive Theory: Proposes that the phenomena of bio adhesion are mainly due to the intermolecular interactions amongst like-molecules. Based on the above theories, the process of bio adhesion can be broadly classified into two categories,

- Chemical: Electronic and adsorption theories
- Physical: Wetting, diffusion and cohesive theory.

The process of adhesion may be divided into two stages. During the first stage (also Known as contact stage), wetting of mucoadhesive polymer and mucous membrane occurs followed by the consolidation stage, where the physicochemical interactions take place.

POLYMERS USED FOR MUCOADHESIVE SYSTEM:

Mucoadhesive delivery systems are being explored for the localization of the active agents to a particular location / site. Polymers have played an important role in designing such systems so as to increase the residence time of the active agent at the desired location. Mucoadhesive polymers are water-soluble and water insoluble polymers, which are swellable networks, joined 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. An ideal polymer for a bio adhesive drug delivery system should have the following characters is as follows[32-33].

- Polymer and its degradation products should be nontoxic, non absorbable &nonirritant.
- It should preferably form a strong non covalent bond with the mucus or epithelial cell Surface & adhere quickly to moist tissue and possess some site specificity.
- It should allow easy incorporation of the drug and offer no hindrance to its release.
- Polymer must not decompose on storage or during the shelf life of the dosage form.
- Cost of the polymer should not be high so that prepared dosage form remains competitive.

Polymers that adhere to biological surfaces can be divided into three broad categories[34-35],

1. Polymers that adhere through non specific, non covalent interactions which are primarily electrostatic in nature
2. Polymers possessing hydrophilic functional groups that hydrogen bond with
3. Similar groups on biological substrates
4. Polymers that bind to specific receptor sites on the cell or mucus surface.

Hydrophilic polymers: Are soluble in water & swell when put into an aqueous media with subsequent dissolution of the matrix. The polyelectrolyte's have greater mucoadhesive property when compared with neutral polymers[36].

Anionic polyelectrolyte's: Have been extensively used for designing mucoadhesive delivery systems due to their ability to exhibit strong hydrogen bonding with the mucin present in the mucosal layer. E.g. poly (acrylic acid) and carboxy methyl cellulose[37-38].

Cationic Polyelectrolyte's: Used for developing mucoadhesive polymer due to its good biocompatibility and biodegradable properties. E.g. Chitosan, which will undergoes electrostatic interactions with the negatively charged mucin chains thereby exhibiting mucoadhesive property[39.]

Non-Ionic Polymers: Used for mucoadhesive properties. This hydrophilic polymers form viscous solutions when dissolved in water and hence may also be used as viscosity Modifying/enhancing agents in the development of various delivery systems to increase the bioavailability of the active agents. E.g. poloxamer, hydroxyl propyl methyl cellulose, methyl Cellulose, poly (vinyl alcohol) and poly (vinyl pyrrolidone) [36].

Hydrogels: It can be defined as three-dimensionally cross linked polymer chains which have the ability to hold water within its porous structure. The water holding capacity of the hydrogels is mainly due to the presence of hydrophilic functional groups like hydroxyl, amino and carboxyl groups. Hydrogels prepared by the condensation reaction of poly acrylic acid and sucrose, indicate an increase in the mucoadhesive property with the increase in the cross linking density and was attributed to increase in the poly acrylic acid chain density per unit area[40].

Thiolated polymers: Presence of free thiol groups in the polymeric skeleton helps in the formation of disulphide bonds with that of the cysteine-rich sub-domains present in mucin which can substantially improve the mucoadhesive properties of the polymers e.g. chitosan–iminothiolane, poly(acrylic acid)–cysteine, poly(acrylic acid)–homocysteine, chitosan thioglycolic acid, chitosan–thioethylamidine, alginate–cysteine, poly(methacrylic acid)–cysteine and sodium carboxy methyl cellulose–cysteine[41-45].

Table 1 Polymer and their mucoadhesion properties

Polymers	Bioadhesive property
Carboxy methyl cellulose	+++*
Carbopol 934	+++
Polycarbophil	+++
Tragacanth	+++
Poly(acrylic acid /divinyl benzene)	+++
Sodium alginate	+++
Hydroxy ethyl cellulose	+++
Gum Karaya	++
Gelatin	++
Guargum	++
Thermally modified starch	+
Pectin	+
Polyvinyl pyrrolidone	+
Acacia	+
Polyethylene glycol	+
Psyllium amberlite-200 resin	+
Hydroxy propyl cellulose	+
Chitosan	+
Hydroxy ethyl methacrylate	+

+++*Very High, ++High, +Moderate.

Lectin-based polymers: Lectins are proteins which have ability to reversibly bind with specific sugar carbohydrate residues and are found in both animal and plant kingdom. the specific affinity of lectins towards sugar or carbohydrate residues provides them with specific cyto-adhesive property and is being explored to develop targeted delivery systems. Lectins extracted from legumes have been widely explored for targeted delivery system. E.g. lectins extracted from *Ulex europaeus* I and *Lens culinarius* Various polymers and their mucoadhesive properties shown in table 1[46-49].

METHODS OF PREPARATION OF MUCOADHESIVE MICROSPHERES:

Mucoadhesive microspheres can be prepared using any of the following techniques.

Air suspension:

This process consists of the dispersing of solid particles of core materials in a supporting air stream and the spray coating of the air suspended particles.

Coacervation:

This process consists of mainly three steps carried out under continuous agitation. Formulation of three immiscible chemical phases, deposition of coating, rigidization of the coating. Three immiscible phases include a liquid manufacturing vehicle, a core material phase and a coating material phase. The core material is dispersed in a solution of the polymer, the solvent for the polymer being the liquid manufacturing vehicle phase. Microspheres can be prepared by changing the temperature of the polymer solution, By adding salt, Using a non solvent, and also by the addition of an incompatible polymer to the polymer solution and polymer-polymer interaction[5].

Spray drying:

In spray drying, the polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporate instantaneously leading the formation of the microspheres in a size range 1-100 µm. Microparticles are separated from the hot air by means of the cyclone separator while the trace of solvent is removed by vacuum drying. One of the major advantages of process is feasibility of operation under aseptic conditions. This process is rapid and this leads to the formation of porous micro particles[51].

Pan coating:

In this process, the coating material is applied as solution or as atomized spray to the desired solid core material in the coating pan. Warm air is passed over the coated materials to remove the coating solvent.

Solvent evaporation:

This process is carried out in a liquid manufacturing vehicle. The microcapsule coating is dispersed in a volatile solvent which is immiscible with the liquid manufacturing vehicle phase. A core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation the core material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size microcapsule. The mixture is then heated if necessary to evaporate the solvent for the polymer of the core material is disperse in the polymer solution, polymer shrinks around the core. If the core material is dissolved in the coating polymer solution, matrix – type microcapsules are formed. The solvent evaporation technique is shown in Figure 2. The core materials may be either water soluble or water in soluble materials. Solvent evaporation involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous[52].

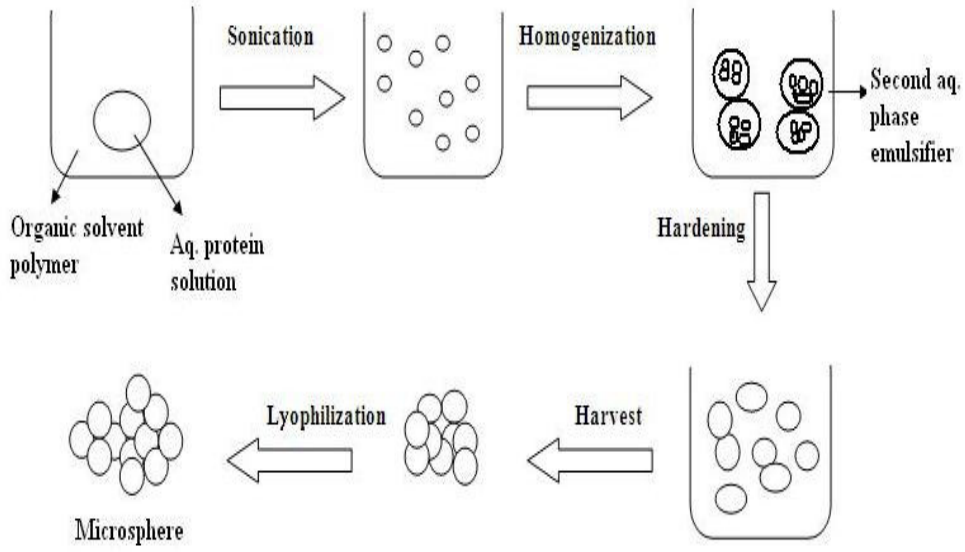

Figure 2 Steps involved in solvent evaporation method

Polymerization:

This method involves the reaction of monomeric sub units located at the interface existing between a core material substance and a continuous phase in which the core material is dispersed. The continuous or core material supporting phase is usually a liquid or a gas and

therefore the polymerization reaction occurs at a liquid-liquid, liquid –gas, solid liquid or solid-gas interface[53].

Wet inversion technique:

Chitosan solution in acetic acid was dropped in to an aqueous solution of counter ion sodium tripolyposphate through a nozzle. Microspheres formed were allowed to stand for 1 hr and cross linked with 5% ethylene glycol diglysidyl ether. Microspheres were then washed and freeze dried. Changing the pH of the coagulation medium could modify the pore structure of CS microspheres[54].

Hot melt microencapsulation:

The polymer is first melted and then mixed with solid particles of the drug that have been sieved to less than 50 μm. The mixture is suspended in a non-miscible solvent (like silicone oil), continuously stirred, and heated to 5 °C above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microspheres are washed by decantation with petroleum ether. The primary objective for developing this method is to develop a microencapsulation process suitable for the water labile polymers, e.g. poly anhydrides. Microspheres with diameter of 1-1000 μm can be obtained and the size distribution can be easily controlled by altering the stirring rate. The only disadvantage of this method is moderate temperature to which the drug is exposed[55].

Solvent removal:

It is a non-aqueous method of microencapsulation, particularly suitable for water labile polymers such as the ploy anhydrides. In this method, drug is dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent like methylene chloride. This mixture is then suspended in silicone oil containing span 85 and methylene chloride. After pouring the polymer solution into silicone oil, petroleum ether is added and stirred until solvent is extracted into the oil solution. The resulting microspheres can then be dried in vacuum [56].

Preparation of microspheres by thermal cross-linking:

Citric acid, as a cross-linking agent was added to 30 mL of an aqueous acetic acid solution of chitosan (2.5% w/v) maintaining a constant molar ratio between chitosan and citric acid (6.90×10^{-3} mol chitosan : 1 mol citric acid). The chitosan cross-linker solution was cooled to 0 °C and then added to 25 mL of corn oil previously maintained at 0 °C, with stirring for 2 minutes. This emulsion was then added to 175 mL of corn oil maintained at 120 °C, and cross-linking was

performed in a glass beaker under vigorous stirring (1000 rpm) for 40 minutes. The microspheres obtained were filtered and then washed with diethyl ether, dried, and sieved[57].

Preparation of microspheres by glutaraldehyde cross linking:

A 2.5% (w/v) chitosan solution in aqueous acetic acid was prepared. This dispersed phase was added to continuous phase (125 mL) consisting of light liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing 0.5% (w/v) Span 85 to form a water in oil (w / o) emulsion. Stirring was continued at 2000 rpm using a 3- blade propeller stirrer. A drop-by-drop solution of a measured quantity (2.5 mL each) of aqueous glutaraldehyde (25% v/v) was added at 15, 30, 45, and 60 minutes. Stirring was continued for 2.5 hours and separated by filtration under vacuum and washed, first with petroleum ether (60 °C- 80 °C) and then with distilled water to remove the adhered liquid paraffin and glutaraldehyde, respectively. The microspheres were then finally dried in vacuum desiccators[58].

Preparation of microspheres by Tri polyphosphate:

Chitosan solution of 2.5% w/v concentration was prepared. Microspheres were formed by dropping the bubble-free dispersion of chitosan through a disposable syringe (10 mL) onto a gently agitated (magnetic stirrer) 5% or 10% w/v Tri polyphosphate solution. Chitosan microspheres were separated after 2 hours by filtration and rinsed with distilled water, and then they were air dried[59-60].

Hydrogel Microspheres:

Microspheres made of gel-type polymers, such as alginate, are produced by dissolving the polymer in an aqueous solution, suspending the active ingredient in the mixture and extruding through a precision device, producing micro droplets which fall into a hardening bath that is slowly stirred. The hardening bath usually contains calcium chloride solution, whereby the divalent calcium ions crosslink the polymer forming gelled microspheres. The method involves an “all-aqueous” system and avoids residual solvents in microspheres[61].

Orificeionic gelation method:

It involves reaction between sodium alginate and polycationic ions like calcium to produce a hydrogel network of calcium alginate. Sodium alginate and the mucoadhesive polymer were dispersed in purified water (25 mL) to form a homogeneous polymer mixture. The active pharmaceutical ingredient were added to the polymer premix and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added through a lab-scale developed spray device with a air compressor into calcium chloride (10% w/v) solution. The

addition was done with continuous stirring; the added droplets were retained in the calcium chloride solution for 15 minutes to complete the curing reaction and to produce rigid spherical microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of microspheres and then air-dried[62].

EVALUATION OF MUCOADHESIVE MICROSPHERES:-

Particle size, shape & surface morphology analysis: The size distributions in terms of average diameter of the microspheres were determined by an optical microscope method. A compound microscope fitted with a calibrated ocular micrometer and a stage micrometer slide was used to count at least 100 particles. Scanning electron microscope was performed to characterize the surface morphology of the formed microspheres .The parameter of SEM were an acceleration voltage of 20 kv, a chamber pressure of 0.6 mmHg and an original magnification of X 80 [63].

Swelling Index:

Swelling index was determined by measuring the extent of swelling of microspheres in the given buffer. To ensure the complete equilibrium, exactly weighed amount of microspheres were allowed to swell in given buffer. The excess surface adhered liquid drops were removed by blotting and the swollen microspheres were weighed by using microbalance. The microspheres then dried in an oven at 60 °C for 5 hr until there was no change in the dried mass of sample. The swelling index of the microsphere was calculated by using the formula[65-66].

- % Swelling index = (mass of swollen microspheres - Mass of dried Microspheres) / Mass of dried microspheres x 100.

In vitro wash-off test:

A 1 cm x 1 cm piece of rat stomach mucosa was tied onto a glass slide (3 inch x 1 inch) using a thread. Microsphere was spread onto the wet, rinsed, tissue specimen and the prepared slide was hung onto one of the groves of the USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that that the tissue specimen regular up and down movements in a beaker containing the simulated gastric fluid. At the end of every time interval, the number of microsphere still adhering on to the tissue was counted and there adhesive strength was determined[67].

***In Vitro* drug release:**

To carry out In Vitro drug release, accurately weighed 50 mg of loaded microspheres were dispersed in dissolution fluid in a beaker and maintained at 37±2 °C under continuous stirring at

100 rpm. At selected time intervals 5 mL samples were withdrawn through a hypodermic syringe fitted with a 0.4 μm Millipore filter and replaced with the same volume of pre-warmed fresh buffer solution to maintain a constant volume of the receptor compartment. The samples were analyzed spectrophotometrically. The released drug content was determined from the standard calibration curve of given drug[64].

***In vitro* diffusion studies:**

In Vitro diffusion studies were performed using in vitro nasal diffusion cell[68]. The receptor chamber was filled with buffer maintained at 37 ± 2 °C. Accurately weighed microspheres equivalent to 10 mg were spread on sheep nasal mucosa. At selected time intervals 0.5 mL of diffusion samples were withdrawn through a hypodermic syringe and replaced with the same volume of pre warmed fresh buffer solution to maintain a constant volume of the receptor compartment. The samples were analyzed spectrophotometrically.

Stability studies of microsphere:

The preparation was divided into 3 sets and was stored at 4°C (refrigerator), room temperature and 40 °C (thermostatic oven). After 15, 30 and 60 days drug content of all the formulation was determined spectrophotometrically[69].

Drug polymer interaction (FTIR) study:

Infra red spectroscopy can be performed by Fourier transformed infrared spectrophotometer. The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000- 600 cm^{-1}. FTIR study was carried on pure drug, physical mixture, formulations and empty microspheres[64].

Percentage yield: Thoroughly dried microspheres were collected and weighed accurately[70]. The Percentage yield was then calculated using formula given below.

- % yield = Mass of microspheres obtained / Total weight of drug and polymer x 100.

Angle of repose: Angle of repose was calculated by static method using funnel. The angle of repose (θ) is calculated by the following formula, [71]

$$\theta = \tan^{-1} (h/r)$$

Where, h = pile height of microspheres,

r = radius of the circular are formed by the microspheres on the ground.

Bulk density:

The bulk density was determined by 3-tap method. Weighed quantities of prepared microspheres were filled in 10 mL of graduated cylinder the initial volume was noted. After tapping for three times the final volume was noted[72]. The bulk density was calculated as per following formula:

- Bulk density = Weight of sample (in grams) / final volume after tapping (in mL).

Drug entrapment and drug loading:

Microspheres equivalent to 50 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl (p^H-1.2) repeatedly. The extract was transferred to a 100mL volumetric flask and the volume was made up using 0.1N HCl. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically (UV 1700, Shimadzu, Japan) at wavelength of particular drug against appropriate blank. The amount of drug loaded and entrapped in the microspheres was calculated by the following formulae[73-74]**.**

- Percentage drug loading = Weight of the drug loaded in the microspheres / Total weight of the microspheres x 100.
- Percentage drug entrapment = Amount of actually drug present / Theoretical drug load expected x 100.

APPLICATION OF MUCOADHESIVE MICROSPHERES[75]

- Vaccine delivery for treatment of diseases like hepatitis, influenza, pertusis, ricin toxoid, diphtheria, birth control. Microsphere in vaccine delivery have a specific advantage like improved antigenicity by adjuvant action, modulation of antigen release, stabilization of antigen.
- Passive targeting of leaky tumour vessels, active targeting of tumour cells, antigens, by intra arterial / intravenous application. The concept of targeting i.e. site specific drug delivery is well established because placement of the micro particles in discrete anatomical compartment leads to their retention either because of physical properties of the environment or biophysical interaction of the particles with the cellular content of the target tissue.
- Monoclonal antibodies mediated microspheres targeting which is also called as immune microspheres used to achieve selective targeting to the specific sites. Mbs is directly attached to microspheres by means of covalent coupling. The free aldehyde, amino, hydroxyl groups on the surface of microspheres can be linked to antibodies. The antibodies attached to microspheres by one of the following methods,

1. Non specific adsorption
2. Specific adsorption
3. Direct coupling
4. Coupling via reagents

- Chemoembolisation is an endovascular therapy, which involves the selective arterial embolisation of a tumour together with simultaneous or subsequent local delivery the chemotherapeutic agent. The theoretical advantage is that such embolisations will not only provide vascular occlusion but will bring about sustained therapeutic levels of chemotherapeutics in the areas of the tumour. Chemoembolisation is an extension of traditional percutaneous embolisation techniques
- **Imaging:** The microspheres have been extensively studied and used for the targeting purposes. Various cells, cell lines, tissues and organs can be imaged using radio labeled microspheres. The particle size range of microspheres is an important factor in determining the imaging of particular sites. The particles injected intravenously apart from the portal vein will become entrapped in the capillary bed of the lungs. This phenomenon is exploited for the scintiographic imaging of the tumour masses in lungs using labeled human serum albumin microspheres.1.Release of proteins, hormones and peptides over extended period of time.2.Gene therapy with DNA plasmids and also delivery of insulin.
- **Topical porous microspheres:** Microsponges are porous microspheres having myriad of interconnected voids of particle size range 5-300 µm. These microsponges having capacity to entrap wide range of active ingredients such as emollients, fragrances, essential oils etc., are used as the topical carries system
- **Surface modified microspheres -** Different approaches have been utilized to change the surface properties of carriers to protect them against phagocytic clearance and to alter their body distribution patterns. The most studied surface modifiers are; Antibodies and their fragments, Proteins, Mono-oligo- and polysaccharide, Chelating compounds (EDTA, DTPA or desferroxamine), synthetic soluble polymers.

  Such modifications are provided surface of microspheres in order to achieve the targeting to the discrete organs and to avoid rapid clearance from the body. Past Research work on mucoadhesive microspheres of various drugs is carried out which is summarized in table 2.

Table: 2 past research works on mucoadhesive microspheres

Drug	Ref.	Polymer	Route	Purpose/Result
Acyclovir	76	Chitosan	Ocular	Slow release rates increased *AUC*
Methyl prednisolone	77	Hyaluronic acid	Ocular	Slow release rates,Sustained drug concentration in tear fluids
Gentamicin	78	DSM+LPC	Nasal	Increase nasal absorption
Insulin	79	DSM+LPC	Nasal	Effective delivery of insulin into the systemic circulation *via* nasal route
Human growth hormone (hGH)	79	DSM+LPC	Nasal	Rapid and increased absorption
Desmopressin	80	Starch	Nasal	Addition of LPC causes a five folds increase in *C*max and two folds increase in bioavailability
Beclomethasone	81	HPC	Nasal	Increasing the bioavailability
Furosemide	82	AD-MMS (PGEFs)	GI	Increased bioavailability. Higher *AUC* Effective absorption from the absorption window
Riboflavin	82	AD-MMS (PGEFs)	GI	—
Amoxicillin	82	AD-MMS (PGEFs)	GI	Greater anti *H. pylori* activity
Cephradine	83	Chitosan/ethylcellulose	GI	Prolonged the intestinal absorption
Vancomycin	84	PGEF coated with Eudragit S 100	Colonic	Well absorbed even without absorption enhancers
Acriflavine	85	MC/SodiumCMC/Alginate/Carbopol 974	Vaginal	Controlled release
Pipedimic acid	86	CMC as mucopolysaccharide, Eudragit RL as matrix polymer	Vesical	Controlled release
Indomethacin	87	Alginate Sodium CMC/MC/Carbopol/HPMC	Oral	Slow release rates
Glipizide	87	Alginate Sodium CMC/MC/Carbopol/HPMC	Oral	Slow release rates

AD-MMS: adhesive micro matrix system, *AUC*: area under curve, CMC: Carboxy Methyl Cellulose, DSM: Degradable Starch Microspheres, GI: gastrointestinal, LPC: Lysophosphatidylcholine, MC: Methyl Cellulose, HPC: Hydroxy Propyl Cellulose, HPMC: Hydroxy Propyl Methyl Cellulose, PEGs: Polyglycerol Esters of Fatty Acids

CONCLUSION:

Mucoadhesive microspheres offer unique carrier system for many pharmaceuticals and can be tailored to adhere to any mucosal tissue, including those found in eyes, oral cavity and throughout the respiratory, urinary and gastrointestinal tract. The mucoadhesive microspheres can be used not only for controlled release but also for enhancing bioavailability, for targeted delivery of the drugs to specific sites in the body. Drug delivery through mucoadhesive microspheres is a promising area for continued research with the aim of achieving controlled

release with enhanced bioavailability over longer periods of time, and for drug targeting to various sites in the body.

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