Mucoadhesive Microspheres-A Promising Carrier in Drug Delivery: A Review

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Abstract:
Carrier technology offers a promising approach for drug delivery system by coupling the drug to a carrier particle such as microspheres,nanoparticles, niosomes, liposomes etc. which modulates the release characteristics of the drug. Mucoadhesion had been a topic of interest in the design of novel drug delivery system to extend the residence time of the dosage form at the site of application or absorption and improve an intimate contact with the underlying absorption surface and enhance the bioavail ability or therapeutic performance of drugs. Mucoadhesive microspheres delivery system is an attractive concept, in which the drug can be entrapped inside the carrier to be released at the mucosal surface where the carriers are adhered due to their mucoadhesiveness. Nowadays mucoadhesive microspheres have been also developed for oral, buccal, ocular, nasal, vaginal and rectal routes for either systemic or local effects. The aim of this article is review the principles underlying the formulation and evaluation of mucoadhesive microspheres.

Keywords: Microsphere, mucoadhesion, controlled drug delivery, bioavailability.

Introduction

The oral route is the most suitable and most widely accepted one by the patients and preferred means of delivery of drugs to systemic circulation [1,2]. However oral administration of most of the drugs in conventional dosage forms has drawbacks such as inability to restrain and localize the system at gastro-intestinal tract [3]. In order overcome this limitation, it has been proposed, to coupling the drugs to polymeric carrier systems because of their propensity to interact with biological surface for local or systemic drug delivery [4,5]. Microspheres constitute an important part of these carrier drug delivery systems due to their small size and efficient carrier capacity. Microspheres are defined as spherical particles having size ranges from 1-1000 µm range in diameter and made up of polymer matrix in which core of drug is dispersed throughout the outer layers of polymer at the molecular or macroscopic level. However, the success of microspheres is limited due to their short residence time at absorption site [6]. It would, therefore, be advantageous to have means for providing an intimate contact of the microspheres with absorbing membranes. It can be achieved by associate mucoadhesion characteristics to microspheres and developing mucoadhesive microspheres [7]. Mucoadhesive microspheres have advantages like efficient absorption and improved bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus membrane and

drug targeting to absorption site [8,9]. Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue present in eye, nasal cavity, urinary, and GI tract [10].

The mucoadhesive drug delivery system may include the following [11]

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

Advantages [12,13]

➢ Prolongs the residence time of the dosage form at the site of absorption or action.

➢ A localization of drug action of the delivery system at a given target site.

➢ A direct contact with intestinal cells that is the first step before particle absorption.

➢ Better patient compliance- ease of drug administration.

➢ Drugs which are unstable in the acidic environment or destroyed by enzymatic or alkaline environment of intestine can be administered by this route. Eg. Buccal, sublingual, vaginal.

➢ Increased safety margin of high potency API due to better control of plasma levels.

➢ Maximum utilization of drug enabling reduction in total amount of drug administered.

➢ The use of specific bioadhesive molecules allows for possible targeting of particular sites or tissues, for example the gastrointestinal (GI) tract.

➢ Increased residence time combined with controlled API release may lead to lower ad ministration frequency, cost reductions may

be achieved and dose-related side effects may be reduced.

Limitations

➢ The release rate may vary from a variety of factors like food and the rate of transit though gut, mucin turnover rate etc.

➢ Any loss of integrity in release pattern of the dosage form may lead to toxicity.

➢ Occurrence of local ulcerous effect due to prolonged contact of the API possessing ulcerogenic property.

➢ Lack of a good model for *in vitro* screening to identify drugs suitability.

Mechanism of mucoadhesion [14-17]

As stated, mucoadhesion is the attachment of the drug along with a carrier to the mucous membrane. The mechanisms responsible in the formation of mucoadhesive bonds are not fully known, however most research has described mucoadhesive bond formation as a three step process:-

➢ Spreading, wetting and swelling of the bioadhesive dosage form at the mucus surface, initiates intimate contact between the bioadhesive polymer and biological tissue.

➢ Inter diffusion and interpenetration of the mucoadhesive polymer chains into the tissue or surface of the mucous membrane creating a greater area of contact (Fig. 1).

➢ Entanglements and the formation of second dary chemical bonds between the muco adhesive polymer chain and mucus gel network.

Step 1:-The wetting and swelling of bioadhesive dosage form (tablet or paste) occurs when the polymer chain spreads over the surface of the biological tissue or mucosal membrane in order to develop an intimate contact with the substrate.

Bioadhesive dosage form is able to adhere with biological subtract by the help of the surface tension and forces that exist at the site of adsorption or contact.

Step 2:-

Inter diffusion and interpenetration take place between mucoadhesive polymers chains and the mucous gel network creating a great area of contact. The strength of this bioadhesive bond depends on the degree of penetration between the polymer chain and glycoprotein. In order to form strong adhesive bonds, one polymer group should soluble in the other one and both polymer types must be of similar chemical structure.
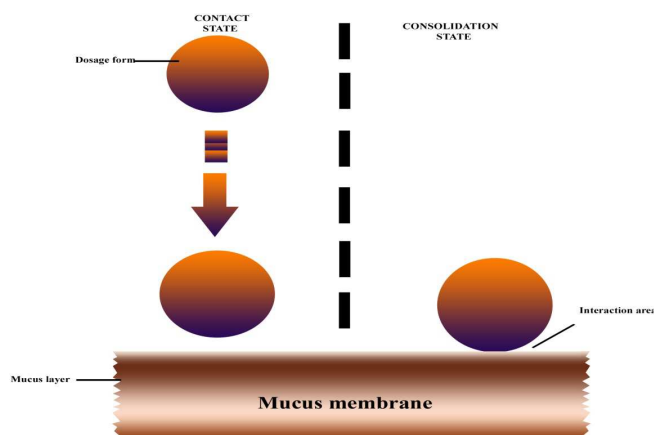

Figure 1: The two stages in mucoadhesion

Step 3:- In this step entanglement and formation of covalent bonds as well as van der Waals Interactions and hydrogen bonds between the polymers chains mucin molecule. The mucoadhesive polymer should require at least one of the following characteristics to obtain adhesion (i) sufficient number of hydrogen bonding chemical groups (- OH and –COOH) (ii) high molecular weight (iii) high chain flexibility (iv) anionic surface chain (v) surface tension that will induce spreading of polymer chain into the mucus layer.

Theories of adhesion

Wetting Theory

The wetting theory emphasizes the intimate contact between the adhesive and mucus, and, primarily in liquid systems, it uses interfacial tension to predict spreading and subsequent adhesion. The wetting surface is controlled by structural similarity, degree of cross linking of the adhesive polymer, or use of a surfactant .This affinity can be found by using measuring techniques such as the contact angle. The general rule states that the lower the contact angle then the greater the affinity. The contact angle which should ideally be zero for adequate spreading is related to interfacial tensions as per the Young's equation [18, 19].

Electronic theory

The electronic theory assumes that the adhesive polymer and mucus glycoprotein network typically have different electronic characteristics. When two surfaces come in contact with each other, electron transfer occurs resulting in the formation of a double layer of electrical charge at the interface of the bioadhesive and the biologic surface. E.g. 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. The bioadhesive force is believed to be present due to the attractive forces across this double layer [20,21].

Adsorption theory

Adsorption theory states that a mucoadhesive polymer adheres to the mucus by secondary chemical interactions, such as in Van der Waals and hydrogen bonds, electrostatic attraction, hydrophobic interactions, or other

related forces. It is one of the most widely accepted theories of bioadhesion [22,23].

Diffusion theory

The diffusion theory states that interpenetration of the chains of polymer and mucin 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 both interacting polymers, and the diffusion co-efficient is known to depend on molecular weight and cross-linking density. In addition, this penetration rate depends on the segment mobility, flexibility of the bioadhesive polymer, mucus glycoprotein, and the expanded nature of both network are important parameters that need to be considered [24,25].

Fracture theory

This is by-far the most accepted theory on bioadhesion. It explains the forces required to separate the two surfaces after adhesion has taken place. It is considered to be appropriate for the calculation of fracture strengths of the adhesive bonds involving rigid mucoadhesive polymers, and has frequently been applied to the analysis of tensile strength .The maximum tensile strength produced during detachment can be determined by dividing the maximum force of detachment (F_m) by the total surface area (A_m) involved in the adhesion interactions.

The equation can be written as: $S_m = F_m / A_m$

Since the fracture theory is concerned only with the force required to separate the parts, it does not take into account the interpenetration or diffusion of polymer chains [26,27].

The Mechanical Theory

Mechanical theory considers adhesion to be due to the filling of the irregularities on a rough surface by an mucoadhesive liquid .Explains the formation of an interlocked structure by 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 [28,29].

The Cohesive Theory

The cohesive theory proposes that the phenomenon of mucoadhesion is mainly due to the inter molecular 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 [30].

Polymers used for mucoadhesive microspheres

Mucoadhesive delivery systems are being explored for the localization of the drugs to a particular location /application site. Mucoadhesive polymers have played a prominent role in designing such systems so as to increase the residence time of the drugs at the particular location. Mucoadhesive polymers are water-soluble and water insoluble polymers, having swellable networks, jointed by crosslinking agents. These polymers possess optimal polarity to ensure that they allow sufficient wetting by the mucus and satisfactory fluidity that permits the mutual adsorption and inter penetration of polymer and mucus to take place [31, 32].

Characteristics of an ideal mucoadhesive Polymer [33-35]

1. The mucoadhesive polymer and its degradation products should be nontoxic,

nonirritant and should be non absorbable from the gastro intestinal tract.

2. It should be nonirritant to the mucus membrane.

3. It should preferably form a strong non covalent bond with the mucin or epithelial cell surfaces.

4. It should adhere quickly to most tissue and possess some site specificity.

5. It should allow easy incorporation of the drug and should offer no barrier to its release.

6. The polymers must not decompose on storage or during the shelf life of the dosage form.

7. The cost of the polymer should not be high so that the prepared dosage form remains competitive.

8. It should be inert and compatible with the environment.

9. It should be easily available in the market and economical.

10. It should have good spreadability, wetting, swelling,solubility and biodegradability properties.

11. pH should be biocompatible and should possess good viscoelastic properties.

12. It should possess peel, tensile and shear strengths at the bioadhesive range.

13. It should show mucoadhesive properties in both dry and liquid state.

14. It should demonstrate local enzyme inhibition and penetration enhancement properties.

Traditional non - specific first - generation muco adhesive polymers may be divided into three types

1. Anionic polymers
2. Cationic polymers
3. Non-ionic polymers

Anionic polymers

Have been most widely employed mucoadhesive polymers within mucoadhesive delivery systems due to their high mucoadhesive functionality and low toxicity. Anionic polymers are characterize by the presence of carboxyl and sulphate functional groups that give negative charge at pH values exceeding pKa of the mucoadhesive polymer. Typical examples include poly (acrylic acid), Na CMC, Polycarbophil and carbomers (Carbopol). Anionic polymers possess good muco adhesive characteristics due to their ability to exhibit strong hydrogen bonding interactions with mucin [36,37] .

Cationic polymers

Chitosan used for developing mucoadhesive drug delivery system due to its good biocompatibility and biodegradable properties. Chitosan will unde rgo electrostatic interactions with the negatively charged mucin present in the mucosal layer thereby exhibiting mucoadhesive property. Chitosan is a cationic polysaccharide, produced by the deacetylation of chitin. Chitosan binds to the mucus membrane via ionic bonds between the amino group and sialic acid residues [38].

Non-Ionic Polymers:

This Non-Ionic Polymers hydrophilic polymers form viscous solutions when dissolved in aqueous media and hence may also is used as viscosity enhancing agents in the development of various mucoadhesive delivery systems to increase the bioavailability of the boiactives. E. g. poloxamer, HPMC, Methyl cellulose, poly (vinyl alcohol) and poly (vinyl pyrrolidone) [39].

Table 1: A list of mucoadhesive polymers

Anionic	Cationic	Non-ionic
Sodium Carboxymethylcellulose	Aminodextran	Hydroxyethyl starch
Chitosan-EDTA	Polylysine	Hydroxy propyl cellulose
Sodium alginate	Chitosan	Polyvinyl pyrrolidone
Alginic acid	Dimethylaminoethyl (DEAE)-dextran	Poly (ethylene oxide)
Polyacrylic acid	Polybrene,	Poly vinyl alcohol
Dextran	Trimethylated chitosan	Polyethylene glycol
Pectin		Scleroglucan
Xanthan gum		Hydroxyethyl Cellulose
Carageenan		Hydroxypropylmethyl cellulose
Polycarbophil		
Carbomers (Carbopol),		

New generation of mucoadhesive polymers

The first generation of mucoadhesive polymers, lack specificity and targeting capability. They adhere to any mucosal surface, and suffer short retention times due to the turnover rate of the mucus. This limits their use for development of mucoadhesive drug delivery system for a particular mucosal tissue. Second generation polymers are less susceptible to mucus turnover rates, with more site specific binding directly to mucosal surfaces; more accurately called cytoadhesives [40].

Lectins

Lectins are naturally occurring proteins that play a fundamental role in biological recognition involving cells and proteins. Lectins are a group of structurally diverse proteins and glycoprotein that bind reversibly to specific carbohydrate residues. After mucosal cell-binding the lectins may either remain on the cell surface or may be taken inside the cell via a process of endocytosis , they hence allow a method for targeted site specific and controlled drug delivery. The use of lectins for tumor targeting is currently under intensive research as the human carcinoma cell lines exhibit higher lectin. The lectins have numerous advantages but they also have the disadvantage of being immunogenic or toxic [41].

Thiolated polymers

Thiolated polymers (thiomers) are derived from hydrophilic polymers such as polyacrylates, chitosan or deacetylated gellan gum. The presence of thiol groups promoting covalent bonds with cysteine-rich sub domains of the mucus, leading to increased residence time and improved bioavailability. The presence of disulphide bonds may significantly alter the mechanism of drug release from the delivery system due to increased rigidity and cross-linking. E.g. Chitosan–iminothiolane, chitosan thioglycolic acid,chitosan thioethyl amidine,alginate cysteine poly (acrylic acid) cysteine, Polyacrylic acid homocysteine,and sodium CMC–cysteine [42].

Hydrogels:

Hydrogels were the class of polymeric biomaterial, usually a three-dimensionally cross linked polymer chains which have the ability to hold water within its porous structure and interacts by means of adhesion with the mucus that covers epithelia. The water holding capacity of the hydrogels is mainly due to the presence of hydrophilic functional groups like hydroxyl, amino,

carboxyl groups and possesses excellent mucoadhesive properties.

E.g. poly (acrylic acid co acrylamide) copolymer, guar gum, sodium alginate, carrageenan and modified guar gum etc [43].

Hydrophilic polymers

Hydrophilic polymers were the water soluble poly mers . Dosage form developed with hydrophilic polymers swell when put into an aqueous media with subsequent dissolution of the matrix. The polyelectrolyte's polymers have greater muco adhesive property than neutral polymers [44].

Polyox WSR

Polyox WSR is high molecular weight polyethylene oxide homopolymers having the good water solubility, hydrophilic in nature, biocompatible and non toxic can be formulated into tablets, films, gels, microcapsules [45].

Novel polymers

Tomato lectin showed selective binding ability to the small intestine epithelium. For optimal buccal adhesion Shajaei and Li have designed and characterized a co polymer of PAA and PEG mono ethyl ether mono methacrylate (PAA-co-PEG) [46].

Factors Affecting Mucoadhesion

Polymer related factors: The mucoadhesive bond between a drug carrier system and mucin gel network can be investigated in term of contribution of the following factors:

a. **Molecular Weight:** For successful mucoadhe sion depends upon the type and molecular weight of mucoadhesive polymer and bioadhesive tissue. Numerous studies have identified that there is a certain molecular weight at which bioadhesive is at a maximum. The interpenetration of polymer chain is favorable for low molecular weight polymers (PEG MW 20,000), whereas entanglements of polymer chain are favors for high molecular weight polymers (PEG MW 400,000). The mucoadhesive forces increases with the molecular weight of mucoadhesive polymer up to 100,000 and that beyond this level there is not much different [47].

b. **Flexibility of polymer chains:** Chain flexibility is important for interpenetration and entangl ement for mucoadhesive polymer. As water-soluble mucoadhesive polymer becomes cross-linked, the mobility of the individual polymer chain decreases. As the cross linking density increases the effective length of the polymer chain, which can penetrate into mucus layer decreases even further and mucoadhesive strength is also decreased [48].

c. **Spatial conformation:** Along with molecular of mucoadhesive polymer , spatial or helical conformation the polymer chain, that may shield many adhesively active groups primarily responsible for adhesion in comparison to that with linear conformation; plays important role in the mucoadhesion property of polymer [49].

d. **Concentration**: The effect of polymer concentration is dependable on the physical state (solid / liquid) of the muco adhesive drug delivery systems more is the polymer concentration results the higher muco adhesive strength in Solid mucoadhesive drug delivery system while an optimum concentration is required for best mucoadhesion in liquids.

Environment related Factors [50-53]

a. **pH:** The hydrogen ion concentration can influence the formal charge on the surface of mucus layer as well as certain ionizable mucoadhesive polymers. Mucus layer will have a different charge density depending on pH because of differences in dissociation of functional groups on the carbohydrate moiety and amino acids of polypeptide backbone. For example polyacrylic acid does not show any mucoadhesive property above pH 5 but shows maximum adhesive strength at pH 3 that gradually decreases with an increase in pH up to 5.

b. **Applied strength:** The pressure initially applied on the solid mucoadhesive system to apply on mucosal tissue can affect the depth of interpenetration and If high pressure is applied for a satisfactory period of time the adhesive strength of polymer will be satisfactory even though they do not have attractive interaction with mucins.

c. **Initial contact time:** The initial contact time between mucoadhesive dosage form and the mucus layer determines the extent of swelling and the interpenetration of mucoadhesive polymer chains. In addition increase in initial contact time increases mucoadhesive strength.

d. **Secretion of the model substrate surface:** Studies on biological substrate variability should be confirmed by examining properties like permeability, electro physiology, or histology etc., before and after performing the *in vitro* tests. Such studies may be using tissues for the better *in vitro / in vivo* correlation using.

e. **Swelling:** Swelling depends both on mucoadhesive polymer concentration and amount of water present. In order to achieve sufficient mucoadhesion of the system, too early swelling must not occur. When swelling of mucoadhesive polymer is too great, decrease in mucoadhesion occurs.

Physiological Variables:

a. **Mucins Turnover:** The natural turnover of mucins molecules from the mucus layer is important factor two reasons. First, the mucins turnover limits the residence time of the mucoadhesive on the mucous layer even mucoadhesive strength high. Second, mucin turnover released out of soluble mucin molecules in substantial amount. These soluble mucin molecules interact with mucoadhesives before they have a chance to interact with mucus layer. High mucin turnover decrease mucoadhesion [54].

b. **Disease state:** Pathological changes during the course of a disease like common colds, cystic fibrosis ,gastric ulcers, ulcerative colitis, inflammatory conditions of the eye ,bacterial and fungal infections of the female reproductive tract and; the physicochemical properties of the mucous changes. There is no clear understanding of structural changes of mucus under these conditions. The mucoadhesive property needs to be evaluated, if mucoadhesive are intended to be used in the diseased state, the mucoadhesive property needs to be evaluated under it.

Methods of preparation of mucoadhesive microspheres

Polymerization

The process involves the reaction of monomeric sub units located at the interface 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.

Pan coating:

In this method, 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.

Phase Inversion Method

The method involves addition of drug into dilute (1-5% w/v) polymeric solution, in methylene chloride; and resultant mixture is poured into an unstirred bath of strong non-solvent, petroleum ether, in a ratio of 1: 100., resulting in the spontaneous production of microcarriers in the size range of 0.5—5.0mm. Microcarriers produced are then clarified, washed with petroleum ether [56].

Coacervation

The 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. Microencapsule can be prepared by utilizing one of the methods of phase separation, that is, by changing the temperature of the polymer solution; by changing the pH of the medium, by adding a salt or an incompatible polymer or a non-solvent to the polymer solution; by inducing a polymer polymer interaction. Generally coating is hardened by thermal cross linking or desolvation techniques, to form a self sustaining hard microsphere [57].

Hot Melt Microencapsulation

This method was first used to prepare microspheres of polyanhydride copolymer of poly[bis(*p*-carboxy phenoxy) propane anhydride] with sebacic acid. In this method the polymer is firstly melted and then the solid drug particles are added to it with continuous mixing. The mixture is suspended in a non-miscible solvent (like silicone oil), continuously stirred, and heated slightly above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify followed by filtration and washing of the microspheres with petroleum ether. The only disadvantage of this method is moderate temperature to which the drug is exposed [58].

Spray Drying

In this process, the drug may be dissolved or dispersed in the polymer solution and spray dried. The quality of spray-dried microspheres can be improved by the addition of plasticizers, *e.g.* citric acid, which promote polymer coalescence on the drug particles and hence promote the formation of spherical and smooth surfaced microspheres. The size of microspheres can be controlled by manipulating the rate of spraying, feeding rate of polymer drug solution, nozzle size, and the drying temperature 25-27. This technology of microencapsulation is particularly less dependent on the solubility characteristics of

the drug and polymer and is simple, reproducible, and easy to scale up [59].

Solvent Removal

This is a non aqueous method of microencapsulation and is most suitable for water labile polymers such as the polyanhydrides. This method involves dissolving the polymer into volatile organic solvent like methylene chloride and the drug is dispersed or dissolved in it, followed by the mixture is then suspended in the 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 formed microspheres were then subjected for vacuum drying [60].

Solvent evaporation

It is the most extensively used method of microencapsulation. A buffered or plain aqueous solution of the drug along with a viscosity building or stabilising agent was poured to an organic phase consisting of the polymer solution in dichloromethane or ethyl acetate or chloroform, with vigorous stirring to form primary water-in-oil emulsion. This obtained emulsion was then poured to a large volume of water containing an emulsifier like PVA or polyvinyl pyrrolidone, under stirring, to form the multiple emulsions (w/o/w); and stirring was continued until most of the organic solvent evaporates, leaving solid microspheres. The Microspheres could then be washed, centrifuged, and lyophilised to get the free flowing and dried Microspheres [61].

Ionotropic Gelation

In this method Microspheres are formed by dissolving the gel-type polymers, such as alginate or the mucoadhesive polymer are dispersed in pure water followed by suspending the drug in the mixture and mixed thoroughly to form a smooth viscous dispersion .Resulting dispersion is then extrude through needle or sprayed to a hardening solution containing calcium chloride under stirring at low speed for 30 min to complete the curing reaction and to produce rigid microspheres. The formed microspheres are collected by decantation, and the product thus separated is washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of microspheres and then air [62].

Evaluation of mucoadhesive microspheres

Particle size, shape & surface morphology analysis

All the microspheres were evaluated with respect to their size and shape using an optical microscope method or sieving method or dynamic light scattering Technique method. In optical microscope method, a compound microscope fitted with calibrated ocular micrometer and a stage micrometer. The particle diameters of more than 100 mucoadhesive microspheres were measured randomly. The average particle size was determined using the Edmondsons equation.

Average particle size = Σ nd / Σ n

Where n = No. of microspheres observed, d = mean size range

In sieve analysis method all the microspheres were separated into different size fractions by sieving using standard sieves of 12, 14, 16, 18 and 22 (mesh apertures *i.e.* 1.4 mm, 1.18 mm, 1.0 mm, 0.85 mm and 0.71 mm respectively) for 5 min. After 5 min microspheres retained on each sieve were collected separately and weighed. The study was conducted in triplicate and mean particle size of microspheres was calculated using the following formula.

Mean particle size = Σ (mean particle size of the fraction × weight fraction) / Σ weight fraction

In dynamic light scattering technique , Micro spheres are dispersed into 100 ml of water and sonicated for 1 min to remove agglomerations. The mean volume diameter (Vd) is recorded and poly dispersity is determined by the SPAN factor. A high SPAN values indicates a wide distribution in size and a high polydispersity.

The shape and surface morphology of the microspheres was studied by using scanning electron microscopy. In this method a thin film of aqueous dispersion of microspheres was applied uniformly in to circular aluminum stubs using double adhesive tape, and coated with gold using sputter gold coater. Afterwards, the stub containing the sample is placed in the Scanning electron microscopy (SEM).The parameters of SEM were an acceleration voltage of 10KV and chamber pressure of 0.6 mm Hg [63,64].

Surface Characterization of Mucoadhesive Microspheres

Data from the scanning electron microscopy, the electron microscopy and scanning tunneling Micro scopy (STM) provides information related to the surface morphology of microspheres and the morphological changes produced through degradation of polymers. It was observed that muco adhesive microspheres with the coarser surface texture improve the mucoadhesive properties, where as smooth surface microspheres lead to weak mucoadhesive properties [65].

Surface Charge Study

Measurement of zeta potential of microspheres and mucus helps to predict electrostatic interactions during mucoadhesion .From zetasizer (Malvern Instruments) data the surface charge (zeta potential) of the mucoadhesive microspheres can be determined. The surface charge can be determined by measuring the electrophoretic mobility in micro electrophoresis flow cell. Zeta potential is an indicator of particle surface charge, which can be used to predict and control the adhesive strength, stability, and the mechanisms of mucoadhesion. Process of mucoadhesion involves interactions between the mucus and mucoadhesive polymers, and is influenced by their structure including their charge [66].

Percentage yield

The percentage yield of each batch was calculated on weight basis with respect to the weight of starting material .Thoroughly dried mucoadhesive microspheres of each batch were collected and weighed accurately. The Percentage yield was then calculated using formula given below [67].

Percentage yield = Mass of microspheres obtained / Total weight of drug and polymer x 100

Angle of repose: Angle of repose of all formulation was calculated by static method using funnel. The angle of repose (θ) is calculated by the following formula [68],

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

Where, h = pile height of microspheres,

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

Micromeritic properties of microspheres

The flow properties of mucoadhesive micro spheres were studied by determining various parameters like the bulk density, tapped density and hausner ratio. The Bulk and tapped density

is determined by 3-tap method cylinder method and were obtained.

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 [69]

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

The entrapment efficiency of prepared microsphere was determined by method of extraction of drug present in microsphere. The dried microspheres (100mg) were crushed and extracted in 100 mL of suitable buffer for 24 hours. Then the dispersion of microspheres was sonicated for 30 min and filtered through a 0.45 µm filter. The absorbance was measured spectrophotometrically at wave length of particular active constituents against appropriate blank after suitable dilution. Each determination was made in triplicate. The amount of drug loaded and entrapped in the microspheres was calculated by the following formula [70].

$$\text{Percentage drug loading} = \frac{\text{Weight of the drug loaded in the microspheres} \times 100}{\text{Total weight of the microspheres}}$$

$$\text{Percentage drug entrapment} = \frac{\text{Amount of actually drug present} \times 100}{\text{Theoretical drug load expected}}$$

Swelling Index

Swelling index demonstrate the ability of the mucoadhesive microspheres to get swelled at the absorbing surface by absorbing fluids available at the site of absorption, which is a primary requirement for initiation of muco adhesion.

Swelling index was determined by measuring the extent of swelling of microspheres in the given buffer. To ensure the complete equilibrium, a weighed amount of mucoadhesive microsphere was placed in 100 ml of buffer and allowed to swell. At predetermined time intervals the swollen microspheres were removed from the media and the excess surface adhered liquid drops were removed by blotting and 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 [71-72]

$$\text{Percentage swelling index} = \frac{(\text{Mass of swollen microspheres} - \text{Mass of dried microspheres}) \times 100}{\text{Mass of dried microspheres}}$$

In vitro wash-off test

The mucoadhesive property of the microspheres is evaluated on goat's or hen or rat intestinal mucosa by using buffer, as per monograph .The freshly excised pieces of intestinal mucosa (1x1 cm) from hen or rat or goat were mounted onto glass slides (3 inch x 1 inch) with cyanoacrylate glue. About 100 number of mucoadhesive microspheres were spread onto the wet, rinsed, tissue specimen and the prepared slide was hung onto the arm of a one of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated, the tissue specimen was given a slow, regular up and down movement in the test buffer at 37^0C contained in a one liter beaker. At different time intervals the number of microsphere still adhering on to the

tissue was counted and percentage mucoadhesion was calculated [73].

Percentage mucoadhesion = $\frac{(Wi - Wt)}{Wi}$ X 100

Where Wi = Initial number of adhere microsphere , Wt = Number of microsphere adhered after particular time period.

In Vitro drug release

In-vitro release profile of drug from the mucoadhesive microspheres was examined in dissolution media using standard IP/BP/USP dissolution test apparatus (rotating basket or paddle type) .To carry out the test, microspheres equivalent to 100 mg of drug were dispersed in dissolution media that is similar to the fluid present at the absorption site as per monograph and maintained at 37±2 °C under continuous stirring at 50 rpm. An aliquot of 5 ml was 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 after suitable dilution. The released drug content was determined from the standard calibration curve of given drug [74].

Drug Permeation through Mucosal Membrane

Drug permeation through mucosal membrane to be assess using selected test animal mucosa for ensuring *in vivo* drug absorption. The mucosal membrane is placed between the donor & receptor compartment of the Franz diffusion cell. The receptor chamber was filled with suitable buffer maintained at 37 ± 2 °C. Accurately weighed microspheres equivalent to 10 mg were spread on mucosal membrane. At predeter mined time intervals 0.5 mL of samples were withdrawn through a hypodermic syringe and replaced with the same volume of pre warmed fresh buffer solution to maintain a constant volume. The drug content of samples was analyzed spectrophotometrically [75].

Stability studies of microsphere

The success of an effective mucoadhesive microspheres were evaluated only through stability studies that were aimed to obtain a stable product which assures its safety and efficacy, and peak profile up to the end of shelf life, at prescribed storage conditions. The preparation was divided into 3 groups and placing the microspheres in screw capped glass container. The containers were stored at ambient humidity conditions, 4°C (refrigerator), room temperature and 40°C±2°C (thermostatic oven). After 15, 30 and 60 days drug content of all the formulation were analyzed using spectrophoto metrically [76].

Drug polymer interaction (FTIR) study

FTIR study was performed by using 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 cm^{-1} - 600 cm^{-1}. FTIR study was carried on pure drug, pure polymers, formulations containing both drug and polymers and empty mucoadhesive microspheres were performed to study the drug polymer interaction.

Kinetics of Drug Release

In order to understand the mechanism and kinetic of drug release, the drug release data of the *in vitro* dissolution study were analyzed with various kinetic model like zero order, first order, Higuchis, Peppas and Coefficient of correlation (r) values were calculated for the liner curves by regression analysis of the above plots [77].

Table 2: List of drugs which are given as mucoadhesive microspheres [78-81]

Drug	Polymer	Route	Purpose/Result
Riboflavin	AD-MMS (PGEFs)	GI	Effective absorption from the absorption window
Amoxicillin	AD-MMS (PGEFs)	GI	Greater anti H.Pylori activity
Furosemide	AD-MMS (PGEFs)	GI	Increased bioavailability
Delapril Hcl (prodrug)	AD-MMS (PGEFs)	GI	MRT of drug is increased
Indomethacin	Alginate sodium/CMC/MC/HPMC/carbopol	oral	Slow release rate
Glipizide	Alginate sodium/CMC/MC/HPMC/carbopol	oral	Slow release rate
Amoxicillin	Polycabopol/Carbopol934/Ion exchange resin	oral	Greater H.Pylori activity
Cephradine	Chitosan/EC	GI	Prolonged the intestinal absorption
Amoxicillin	Chitosan/EC	GI	Greater H.Pylori activity
Amoxicillin	EC	GI	Prolonged GIT residence time
Clarithromycin	Chitosan	GI	Provide prolonged contact time for the drug delivery of antibiotics
Predinisolone	Alginate/chitosan	GI	Colon specific delivery
Amoxicillin	PEG	GI	Amoxicillin release from, PEG nanoparticles system was studied
Pioglitazone Hcl	Carbopol 934	GI	Slow release rate
Trimetazidine Hcl	Chitosan	GI	Prolonged the intestinal absorption
Furazolidine	Eudragit RS100, Carbopol 974P, HPMC	GI	Prolonged GIT residence time
Aceclofenac	Sodium alginate,HPMC, Chitosan,Carbopol	GI	Prolong the gastric residence time
Acyclovir	Sodium alginate	GI	Prolong the gastric residence time
Atenolol	Polyacrylic acid,PVP	GI	Slow release rate
Captopril	Sodium alginate,HPMC, Chitosan,Carbopol 934P,CAP	GI	sustained delivery of Captopril in the stomach
Salbutamol sulphate	Carbopol, HPMC	GI	Slow release rate
Acetazolamide	Eudragit RS & RL	GI	Sustained release
Famotidine	Sodium CMC,Sodium alginate	GI	Prolongation of gastric residence time
Metronidazole	Guargum, Sodium alginate	GI	Controlled release of Metronidazole
Torsemide	Sodium alginate ,HPMC	GI	Effective absorption from the absorption window

CONCLUSIONS

Mucoadhesive microspheres offer a promising carrier system for many pharmaceutical components and can be modified to adhere to any mucosal tissue including those found in eye, nasal cavity, rectal, urinary and oral mucosal delivery, thus providing the potential for localized as well as systemic controlled release of drugs. The various advantages of mucoadhesive micro spheres can be used not only for controlled release but also for enhancing bioavailability of many drugs by prolongation of the residence time of the drug which in turn increases the absorption of the drug, for targeted delivery of drugs to various sites in the body. The most commonly studied polymers for mucoadhesion have been the high molecular weight, hydrophilic, anionic molecules like carbomers. Recently several novel second generation polymers like the thiolated polymers, lectins and lecithins considered to have complying properties of mucoadhesion. Mucoadhesive delivery system is a promising area for continued research with the aim of achieving controlled release with enhanced bioavailability and for drug targeting to specific sites in the body.

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