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AN EXTENSIVE REVIEW ON MUCOADHESIVE MICROSPHERES AS CARRIERS IN DRUG DELIVERY

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ABSTRACT

Among modified-release oral dosage form increasing interest has currently turned to systems designed to achieve prolonged retention at the site of drug delivery. The mucoadhesive microspheres offer better retention and controlled release. To overcome inherent drawbacks associated with conventional dosage forms, an attempt was made to develop an alternative drug delivery system in the form of mucoadhesive microspheres. The concept of mucosal adhesives, or mucoadhesives, was introduced into the controlled drug delivery arena in the early 1980s. Mucoadhesives are synthetic or natural polymers which interact with the mucus layer covering the mucosal epithelial surface and mucin molecules constituting a major part of the mucus. The concept of mucoadhesion has alerted many investigators to the possibility that mucoadhesive polymers can be used to overcome physiological barriers in long term drug delivery. The use of mucoadhesive polymers can solve a number of problems encountered in controlled drug delivery. They localize the formulation at a particular region of the body thereby improving bioavailability of drugs with low bioavailability. The increased contact time and localization of the drug due to the strong interaction between the polymer and mucus is essential for the modification of tissue permeability.

KEYWORDS: mucoadhesive microspheres, polymers, bioavailability, controlled release.

INTRODUCTION

Drug action can be improved by developing new drug delivery system, such as the mucoadhesive microsphere drug delivery system. These systems remain in close contact with the absorption tissue, the mucous membrane, releasing the drug at the action site leading to a bioavailability increase and both local and systemic effect.[1] The oral route of drug administration constitutes the most convenient and preferred means of drug delivery to systemic circulation of body. However oral administration of most of the drugs in conventional dosage forms has short-term limitations due to their inability to restrain and localize the system at gastro-intestinal tract. Microspheres constitute an important part of these particulate drug delivery systems by virtue of their small size and efficient carrier capacity. Microspheres are the carrier linked drug delivery system in which particle size is ranges from 1-1000 µm range in diameter having a core of drug and entirely outer layers of polymer as coating material. However, the success of these microspheres is limited due to their short residence time at 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 "mucoadhesive microspheres". Mucoadhesive microspheres have advantages like efficient absorption and enhanced bioavailability of the drugs due 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.

MUCOADHESION

Mucoadhesion or bioadhesion can be defined as the state in which two materials, at least one of which is biological in nature, are held together for a prolonged time period by means of interfacial forces. In biological systems, bioadhesion can be classified into 3 types.

• **Type 1**: adhesion between two biological phases, for example: platelet aggregation and wound healing.

• **Type 2**: adhesion of a biological phase to an artificial substrate, for example tissue: cell adhesion to culture dishes and biofilm formation on prosthetic devices and inserts.

• **Type 3:** adhesion of an artificial substance to a biological substrate, for example: adhesion of synthetic hydrogels to soft tissues. For drug delivery purpose, the term "bioadhesion" implies attachment of a drug carrier system to a specific 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 mucous coat, the phenomenon is referred to as "Mucoadhesion".

Mucoadhesion is defined as the interaction between a mucin surface and a synthetic or natural polymer.[3] Mucoadhesion has been widely promoted as a way of achieving site-specific drug delivery through the incorporation of mucoadhesive hydrophilic polymers within pharmaceutical formulations such as "microspheres" along with the active pharmaceutical ingredient (API).

MICROSPHERES

Microspheres are defined as spherical particles having size less than 200µm and made up of polymer matrix in which therapeutic substance is dispersed throughout the matrix at the molecular or macroscopic level. The rationale of developing mucoadhesive microsphere drug delivery system lies behind the fact that the formulation will be 'held' on a biological surface for localized drug delivery. The API will be released close to the site of action with a consequent enhancement of bioavailability.

Mucoadhesive microspheres include microparticles and microcapsules (having a core of drug) of 1- 1000µm in diameter and consisting either entirely of a Mucoadhesive polymer or having an outer coating of it, respectively. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of bioadhesive properties to microspheres has additional advantages e.g. efficient absorption and bioavailability of the drugs due to high surface to volume ratio, a much more intimate contact with the mucous layer, specific targeting of drugs to the absorption site.[4]

MUCOADHESIVE DRUG DELIVERY SYSTEMS

Since the early 1980s, there has been increasing interest in the use of mucoadhesive polymers to prolong the contact time in the various mucosal sites of drug administration. The ability to maintain a delivery system at a particular location for an extended period of time has great appeal for both local disease treatments as well as for systemic drug bioavailability.[5] This utilize the property of certain polymers to adhere to mucus membranes upon hydration and hence can be used for targeting a drug to a particular region of the body for extended period of times.[6] Mucoadhesion is an interfacial phenomenon in which two materials, at least one of which is biological, are held together by means of interfacial forces.

The mucosal layer lines a number of regions of the body including the gastrointestinal tract, the urogenital tract, the airways, the ear, the nose and the eyes. These represent potential sites

for attachment of any mucoadhesive system and hence, the mucoadhesive drug delivery systems include buccal, vaginal, oral, rectal, nasal and ocular systems.[7]

The various shapes and forms of mucoadhesive drug delivery systems used include tablets, powders, gels, patches, liposomes and microspheres.[8] General aspects of mucoadhesion, mucoadhesive polymers and mucoadhesive dosage forms have been reviewed[9], however the present review mainly focuses on the formulation and applications of mucoadhesive microspheres in drug delivery.

ADVANTAGE OF MUCOADHESIVE MICROSPHERES

1. Provide constant and longer therapeutic effect.
2. Reduces the frequency of daily administration and thereby improve the patient compliance.
3. Improve the absorption of drug hence improve the bioavailability of drug and reduce the chances of adverse effects.
4. The morphology of microspheres permits a controllable variability in degradation and drug release.

LIMITATION OF MUCOADHESIVE MICROSPHERE

1. The release from the formulations may get modified.
2. The release rate may vary from a variety of factors like food and the rate of transit though gut, mucin turnover rate etc.
3. Differences in the release rate can be found from one dose to another.
4. Any loss of integrity in release pattern of the dosage form may lead to potential toxicity.
5. These kinds of dosage forms cannot be crushed or chewed.

Mucoadhesion

Bioadhesion is a phenomenon in which two materials at least one of which is biological in nature are held together by means of interfacial forces. The term “mucoadhesion” define the adhesion of the polymers with the surface of the mucosal layer.[10]

Mucus Membranes

Mucus membranes are the moist surfaces lining walls of various body cavities such as the gastrointestinal and respiratory tracts. Mucus is secreted by the goblet cells. Mucus is present either as a gel layer adherent to the mucosal surface or in suspended form or as a luminal

soluble. The major components of all mucus gels are mucin glycoprotein, water, lipids, and inorganic salts. The mucus serves as a protective barrier and for lubrication also.[11]

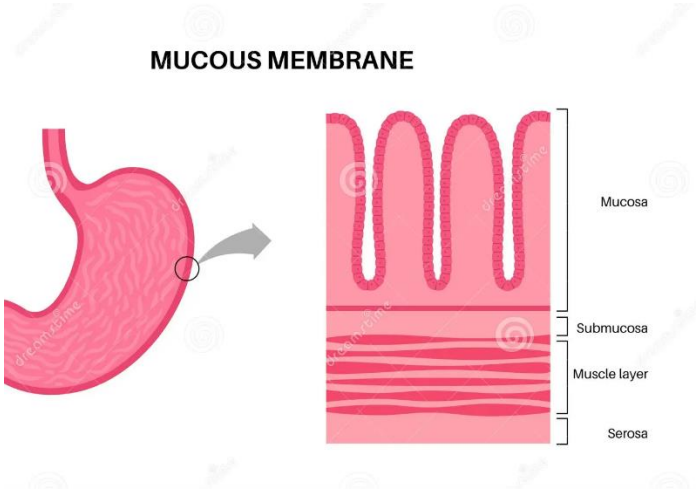
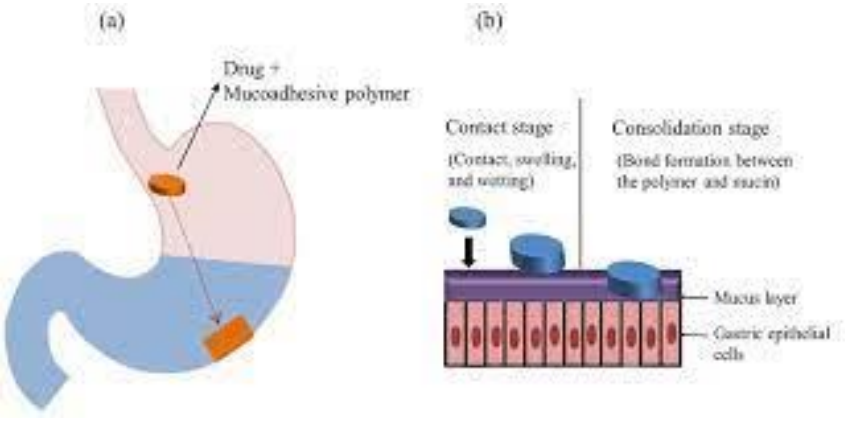


Figure 1: Structure of Mucus Membrane.

MECHANISM OF MUCOADHESION

Mucoadhesion is the attachment of the drug along with a suitable carrier to the mucosal layer. Mucoadhesion is a complex phenomenon which involves wetting, adsorption and interpenetration of polymer chains. Mucoadhesion has the following mechanism.[12]

- Intimate contact between a mucoadhesive delivery system and mucosal membrane (wetting or swelling phenomenon).
- Penetration of the mucoadhesive delivery system into the tissue or into the surface of the mucous membrane.[13]



MUCOADHESIVE POLYMERS

Mucoadhesive polymers are water soluble or water insoluble polymers with swellable networks. The polymer should possess optimal polarity to make sure it is sufficiently wetted

by the mucus and should have optimal fluidity that permits the mutual adsorption and interpenetration of polymer and mucus to take place. Mucoadhesive polymers fulfill the following desirable features of controlled release systems.[14]

a. Localization in specified regions to improve and enhance bioavailability of drugs.

b. Optimum contact with the absorbing surface to permit modification of tissue permeability, which is especially important in the case of peptides and proteins and ionized species, and.

c. Prolonged residence time to permit once-daily dosing, thus improving patient compliance.

An polymer for a mucoadhesive drug delivery system should have the following ideal characteristics.[15]

1. It should be a nonirritant to the mucus membranes.
2. The polymer and its degradation products should be non toxic.
3. It should adhere quickly to moist tissue and should possess some site specificity.
4. It should preferably form a strong non covalent bond with the mucin/epithelial cell surfaces.
5. The polymer must not decompose on storage or during shelf life of the dosage form.
6. It should allow easy incorporation of the drug and offer no hindrance to its release.
7. It should be economical.

TYPES OF POLYMER

(1) Natural Polymers: Tragacanth, sodium alginate, karaya gum, guar gum, xanthan gum, lectin, soluble starch, gelatin, pectin, chitosan etc.

(2) Synthetic Polymers: Cellulose derivatives (hydroxyethyl cellulose, hydroxypropyl methyl cellulose, hydroxypropyl cellulose, sodium carboxymethyl cellulose), poly (acrylic acid) polymers (carbomers, polycarbophil), poly (hydroxylethyl methylacrylate), poly (ethylene oxide), poly (vinyl pyrrolidone), poly (vinyl alcohol), etc.

METHODS OF MICROSPHERE PREPARATION

The microspheres can be prepared by using any of the several methods described in the following section, but the choice mainly depends on the nature of the mucoadhesive polymer, the drug, the intended use and the therapy. Moreover, the method of preparation and its choice are equivocally determined by some formulation and technology related factors as follows: It should satisfy the particle size requirement.

- The drug should not be adversely affected by the method of preparation.
- There should be no stability problem.

- There should be no toxic products associated with the final product.
- There should be reproducibility of the release profile and the method.
- Various methods used for the preparation of mucoadhesive microspheres are as follows:

1. Single Emulsion Method

The microspheres of natural polymers, i.e. those of proteins and carbohydrates are usually prepared by this method. The polymers are dissolved or dispersed in an aqueous medium followed by dispersion in the non-aqueous medium e.g., oil. In the second step, cross linking can be achieved either by means of heat or by using chemical cross linkers e.g. formaldehyde, gluteraldehyde, terephthaloyl chloride, diacid chloride, etc. Cross linking by heat is affected by adding the dispersion to previously heated oil. Heat denaturation is however, not suitable for thermolabile drugs while the chemical cross linking suffers from the disadvantage of excessive exposure of active ingredients to the cross linking chemicals.[16]

2. Solvent Evaporation Method

This is the most widely used method for preparation of microspheres. Microspheres can be formed by the evaporation of an organic solvent from dispersed oil droplets containing both the polymer and the drug.[17] Often, a double emulsion is employed; first the drug for encapsulation is dissolved in water; this aqueous phase is dispersed in an organic solvent, usually dichloromethane (or chloroform or ethyl acetate), which contains the degradable polymer and the primary water-in-oil (w/o) emulsion is formed. Dispersion of the primary emulsion in a stabilized aqueous medium (usually using poly (vinyl alcohol) as a stabilizer) forms the final water-in-oil-in-water (w/o/w) emulsion; microspheres are formed as the organic solvent evaporates and the polymer hardens, trapping the encapsulated drug.[18]

3. Solvent Removal Method

This method is suitable for water labile polymers such as the polyanhydrides. In this non-aqueous method of microencapsulation, the 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 oil containing emulsifier like Span 85 Mucoadhesive Microspheres Current Drug Delivery, 2008, Vol. 5, No. 4 3 and organic solvent. Then petroleum ether is added and stirred until solvent is extracted into the oil solution. The resulting microspheres can then be dried in vacuum.[19,20]

4. Hot Melt Microencapsulation

This method is also suitable for water labile polymers. In this method of microsphere preparation, the polymer is first melted and then mixed with solid particles of the drug that have been sieved to less than 50µm. The mixture is suspended in a non-miscible solvent (like silicone oil) and stirred continuously at 5 0 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 microspheres obtained by this method are in the range of 1–1000 µm. The disadvantage of this method is the exposure of the drug to the melting temperature of the polymer.[21]

5. Extrusion-Gelling Method (Hydrogel Microspheres)

Hydrogel microspheres are prepared by dissolving the polymer (like sodium alginate) in an aqueous solution, suspending the drug 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 particle size of the microspheres can be controlled by varying the size of extru, polymer solution flow rates or the stirring rate.[22]

6. Phase-inversion Microencapsulation

This simple and fast process of microencapsulation involves relatively little loss of polymer and drug. The drug is added to a dilute solution of the polymer (usually 1–5% w/v in an organic solvent like methylene chloride). The mixture is poured into an unstirred bath of a strong non-solvent (petroleum ether) at a solvent to non-solvent ratio of 1:100, resulting in the spontaneous production of microspheres through phase inversion. The microspheres obtained in the size range of 0.5–5.0 µm can then be filtered, washed with petroleum ether and air dried.[23]

7. Spray Drying Method

Spray drying is a one step process transforming liquid into a dried particulate form. In this process, the drug may be dissolved or dispersed in the polymer solution and spray dried. Qualitative and quantitative composition of the liquid feed and drying conditions strongly affect the properties of the spray dried particles such as size, morphology, density, shape, porosity and flowability. 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 the microspheres can be controlled by the rate of spraying, the feed rate of the solution, nozzle size and the drying temperature. This method of microencapsulation is particularly less dependent on the solubility characteristics of the drug and polymer and is simple, reproducible.[24]

THEORIES OF MUCOADHESION

Different theories are involved in the mucoadhesion which are as follows:

1. The electronic theory
2. The wetting theory
3. The adsorption theory
4. The diffusion theory
5. The mechanical theory, and
6. The cohesive theory

1. The Electronic Theory: According to this theory an electrical double layer is formed on the transfer of the electrons among the mucoadhesive and mucosal membrane.
2. The Wetting Theory: This theory is applicable for liquids, postulates that the lower the contact angle of liquid on substrate surface there will be greater affinity for adhesion.
3. The Adsorption Theory: According to this theory the mucoadhesive get adsorbed on the mucosal surface by intermolecular forces, viz. Vander Waal's forces, hydrogen bonding etc.
4. The Diffusion Theory: This theory illustrates the forming of a network structure among the mucoadhesive and the mucosal surface by diffusion of the polymers chains present on the mucoadhesive surface.
5. The Mechanical Theory: Explains the formation of an interlocked structure by the diffusion of the liquid adhesives into the micro-cracks and irregularities present on the mucoadhesive substrate resulting in mucoadhesion.
6. The Cohesive Theory: According to this theory the phenomena of mucoadhesion is mainly due to the intermolecular interactions amongst like-molecules.[25]

FACTORS AFFECTING MUCOADHESION

The mucoadhesion of a drug carrier system to the mucous membrane depends on the below mentioned factors.

Polymer Based Factors

- Molecular weight of the polymer,

- concentration of polymer,
- stereo chemistry of polymer,
- chain length of polymer,

Physical Factors

- pH at polymer substrate interface,
- swelling of polymer,
- applied strength,
- contact time.

Physiological Factors

- Mucin turnover rate and diseased state.
- Materials Used In the Formulation of Mucoadhesive Microspheres.[26]

Mucoadhesive microspheres are made up by using mucoadhesive polymers. Mucoadhesive polymers can be of either natural or synthetic in origin. Mucoadhesive polymers that adhere to the mucin-epithelial surface can be conveniently divided into three broad classes.

- Polymers that become sticky on placing them in water and achieve their mucoadhesion due to stickiness.
- Polymers that adhere through nonspecific, noncovalent interactions that is primarily electrostatic in nature.
- Polymers that bind to specific receptor site on tile self surface.

CLASSIFICATION OF MUCOADHESIVE POLYMERS

There are various mucoadhesive polymers of synthetic and natural origin, which are classified in Table 1. Table 1: A short list of mucoadhesive polymers16. Synthetic polymers Natural polymers Hydroxy propyl methyl cellulose (HPMC) Chitosan Poly(acrylic acid) polymers (carbomers, polycarbophil) Sodium alginate Poly vinyl pyrrolidone (PVP) Pectin Poly vinyl alcohol (PVA) Locust bean gum Poly hydroxyethyl methylacrylate Guar gum Poly ethylene oxide Xanthan gum Sodium carboxy methyl cellulose (Na CMC) Karaya gum Hydroxyl ethyl cellulose (HEC) Gelatin Hydroxy propyl cellulose (HPC) Tragacanth Ethyl cellulose (EC) Soluble starch Methyl cellulose (MC) Lecithin Methods Of Preparation Of Mucoadhesive Microspheres Mucoadhesive microspheres can be prepared by using different techniques like.

1. Complex coacervation

2. Hot melt microencapsulation
3. Single emulsion technique
4. Double emulsion method
5. Solvent removal
6. Ionotropic gelation
7. Phase inversion method
8. Spray drying

1. Complex Coacervation

Principle of this method is under suitable conditions when solutions of two hydrophilic colloids were mixed, result into a separation of liquid precipitate. In this method the coating material phase, prepared by dissolving immiscible polymer in a suitable vehicle and the core material is dispersed in a solution of the coating polymer under constant stirring. Microencapsulation was achieved 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 nonsolvent 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 microsphere.[28,29]

1. Hot Melt Microencapsulation

Microspheres of polyanhydride copolymer of poly bis(p-carboxy phenoxy) propane anhydride with sebacic acid were firstly prepared by this method.[30] In this method the polymer is firstly melted and then the solid drug particles are added to it with continuous mixing. The prepared mixture is then suspended in a non-miscible solvent like silicone oil with stirring and heated at the temperature above the melting point of the polymer with continuous stirring so as to get stabilized emulsion. The formed emulsion is cooled to solidify polymer particles followed by filtration and washing of the microspheres with petroleum ether.

2. Single Emulsion Technique

The microspheres of natural polymers are prepared by single emulsion technique. The polymers and drug are dissolved or dispersed in aqueous medium followed by dispersion in organic medium e.g. oil, results in formation of globules, and then the dispersed globule are cross linked by either of heat or by using the chemical cross-linkers. The chemical cross-linkers used are formaldehyde, glutaraldehyde, diacid chloride etc.

3. Double Emulsion Method

This method is firstly described by Ogawa Y et al. in year 1988, and is the most widely used method of microencapsulation 20. In this method an aqueous solution of drug and polymer is added to the organic phase with vigorous stirring to get primary water-in-oil emulsion. This emulsion was then poured to a large volume of water containing an emulsifier like polyvinyl alcohol or Garg et al. Asian J Pharm Clin Res, Vol 5, Suppl 3, 2012, 24-27 26 polyvinylpyrrolidone, under stirring, to get the multiple emulsions (w/o/w); and stirring was continued until most of the organic solvent evaporates, leaving solid microspheres. The microspheres are then washed and dried.[31]

5. Solvent Removal

This is a non-aqueous method of microencapsulation and is most suitable for water labile polymers such as the polyanhydrides. The method involves dissolving the polymer into volatile organic solvent and the drug is dispersed or dissolved in it, this solution is then suspended in the silicone oil containing span 85 and methylene chloride under stirring, then petroleum ether is added and stirred until solvent is extracted into the oil solution. The obtained microspheres were then subjected for vacuum drying.[32]

4. Ionotropic Gelation

This method was developed by Lim F and Moss RD.[33] Using this method Microspheres are formed by dissolving the gel-type polymers, such as alginate, in an aqueous solution followed by suspending the active ingredient in the mixture and extruding the solution through needle to produce micro droplets which fall into a hardening solution containing calcium chloride under stirring at low speed. Divalent calcium ions present in the hardening solution crosslink the polymer, forming gelled microspheres.

5. Phase Inversion Method

The method involves addition of drug into dilute 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. Microspheres produced are then clarified, washed with petroleum ether and air dried.[34,35]

6. Spray Drying

This method involves dissolving/dispersing of the drug into the polymer solution which is then spray dried. By this method 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.[36-38]

DRUG LOADING IN MICROSPHERE

The drugs are loaded in the microspheres principally using two methods i.e. during the preparation of the microsphere or after the preparation of the microsphere by incubating them with the drug solution. The active components may be loaded by means of the physical entrapment, chemical linkage and surface absorption. It was found that maximum of drug loading in microspheres may be achieved by incorporating the drug during the time of preparation but it may get affected by many other process variables like presence of additives, method of preparation, heat of polymerization, agitation intensity etc. The loading of drug after the preparation of microspheres may be achieved by incubating them with high concentration of the drug in a suitable solvent. Here drug may be loaded in the microspheres via penetration or diffusion of the drug through the pores present in the microsphere as well as by absorption of drug on the surface of microspheres. The solvent is then removed, leaving drug-loaded microsphere.

DRUG RELEASE KINETICS

Release of drug is an important consideration in case of microspheres. Many theoretically possible mechanisms for the release of drug from the microsphere may be as follows:

- Liberation of the drug due to polymer erosion or degradation.
- Self diffusion of drug through the pore of the microspheres.
- Release of the drug from the surface of the polymer.
- Pulsed delivery initiated by the application of an oscillating or sonic field.[39]

EVALUATION OF MUCOADHESIVE MICROSPHERES

1. Interaction study by TLC/ FTIR

IR spectroscopic studies.

The IR spectra of the free drug and the microspheres are recorded. The identical peaks corresponding to the functional groups features confirm that neither the polymer nor the method of preparation has affected the drug stability.

Thin layer chromatographic studies

The drug stability in the prepared microspheres can also be tested by the TLC method. The Rf values of the prepared microspheres can be compared with the Rf value of the pure drug. The values indicate the drug stability.

UV-FTTR (Fourier transform infra red)

The drug polymer interaction and also degradation of drug while processing for microencapsulation can be determined by FTIR. In this method the pellets of drug and potassium bromide are prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra are scanned in the wave number range of 4000- 600 cm-1. FTIR study is carried on pure drug, physical mixture, formulations and empty microspheres.[40]

2. Particle size distribution of prepared microspheres. The size of the prepared microspheres can be measured by the optical microscopy method. Optical microscopy:- This method is used to determine particle size of microspheres by using optical microscope (Meizer OPTIK) The measurement is done under 45x (10x eye piece and 45x objective) and100 particles are calculated.

3. Surface topography by Scanning Electron Microscopy (SEM). SEM of the microspheres shows the surface morphology of the microspheres like their shape and size.

Scanning electron microscopy (SEM):-Surface morphology of microspheres is determined by the method SEM. In this method microspheres are mounted directly on the SEM sample slub with the help of double sided sticking tape and coated with gold film under reduced pressure. Scanning Electron photomicrographs of drug-loaded microspheres are taken. A small amount of microspheres s spread on gold stub. Afterwards, the stub containing the sample is placed in the Scanning electron microscopy (SEM). A Scanning electron photomicrograph is taken at an acceleration voltage of 20KV and chamber pressure of 0.6 mm Hg.

4. Particle size analysis. The particle sizes and particles size distributions are further analyzed by using dynamic light scattering technique, Microspheres are dispersed into 100 ml of water and sonicated for 1 min to remove agglomerations. The mean volume diameter (Vd) is recorded and polydispersity is determined by the SPAN factor. A high value of SPAN indicates a wide distribution in size and a high polydispersity.

5. Swelling index. This technique is used for Characterization of sodium alginate microspheres. Different solution (100mL) are taken such as (distilled water, buffer solution of pH (1.2, 4.5, 7.4) are taken and alginate microspheres (100mg) are placed in a wire basket and kept on the above solution and swelling is allowed at 37 0C and changes in weight variation between initial weight of microspheres and weight due to swelling is measured by taking weight periodically and soaking with filter paper. The swelling index of the microsphere is calculated by using the formula:- Swelling index= (mass of swollen microspheres - mass of dry microspheres/mass of dried microspheres) 100.

6. Entrapment Efficiency. The capture efficiency of the microspheres or the percent entrapment can be determined by allowing washed microspheres to lyse. The lysate is then subjected to the determination of active constituents as per monograph requirement. The percent encapsulation efficiency is calculated using following equation:- % Entrapment = Actual content/Theoretical content x 100.

7. Stability studies. By placing the microspheres in screw capped glass container and stored them at following conditions:- 1. Ambient humid condition 2. Room temperature (27+/-2 0C) 3. Oven temperature (40+/-2 0C) 4. Refrigerator (5 0C -80C). It is carried out of a 60 days and the drug content of the microsphere is analyzed.

8. Density determination. The density of the microspheres can be measured by using a multi volume pychnometer. Accurately weighed sample in a cup is placed into the multi volume pychnometer. Helium is introduced at a constant pressure in the chamber and allowed to expand. This expansion results in a decrease in pressure within the chamber. Two consecutive readings of reduction in pressure at different initial pressure are noted. From two pressure readings the volume and density of the microsphere carrier is determined.

9. Bulk density. The microspheres fabricated are weighed and transferred to a 10-ml glass graduated cylinder. The cylinder is tapped using an autotrap until the microsphere bed volume is stabilized. The bulk density is estimated by the ratio of microsphere weight to the final volume of the tapped microsphere bed.

10. Angle of contact. The angle of contact is measured to determine the wetting property of a micro particulate carrier. It determines the nature of microspheres in terms of hydrophilicity or hydrophobicity. This thermodynamic property is specific to solid and affected by the

presence of the adsorbed component. The angle of contact is measured at the solid/air/water interface. The advancing and receding angle of contact are measured by placing a droplet in a circular cell mounted above objective of inverted microscope. Contact angle is measured at 200c within a minute of deposition of microspheres.

11. In vitro drug release studies. In-vitro release studies can be performed according to USP XXII type 2 dissolution apparatus at suitable pH conditions. The temperature should be maintained at 37±0.5°C and the rotation speed of 100 rpm. Then 5 ml of sample should be withdrawn at various time intervals and replenished with an equal volume of fresh dissolution media. The drug content in the sample can be analyzed spectrophotometrically at specific wavelength (nm).

12. In vitro mucoadhesion test. The mucoadhesive property of the optimized microspheres prepared by different methods is evaluated by an in vitro mucoadhesion testing method known as the wash-off method. A rat stomach mucosa is tied onto the glass slide using a thread. In this method microspheres are spread onto wet rinsed tissue specimen and the prepared slide is hung onto one of the grooves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus is switched on and the tissue specimen is given up and down movements for 2 h in the beaker of the disintegration test apparatus, which contained the stimulated gastric fluid (pH 1.2). The microspheres remaining at the surface of gastric mucosa are then collected, and the percentage of the remaining microspheres is calculated. The experiment is performed in triplicate. The percentage mucoadhesion is calculated by the following formula: Percent mucoadhesion = (Weight of adhered microsphere /Weight of applied microspheres) ×100.[41]

13. In situ Bioadhesivity Studies. Bioadhesivity testing is done by a novel in situ method. A freshly cut 5-6cm long piece of small intestine of rat is obtained and cleaned by washing with isotonic saline. The piece is cut open and the mucosal surface is exposed. Known weights of microspheres are added evenly on the mucosal surface. The intestinal piece is maintained at 80% (RH) relative humidity for 30mts in a desiccator. The piece is taken out and phosphate buffer pH 6 is allowed to flow over the intestinal piece for about 2 mts at a rate of 20ml/min. The perfusate is collected and dried to get the particles not adhered. The percent of bioadhesion is estimated by the ratio of amount applied to adhere micro matrices.

Future Challenges

Future challenges of microspheres look bright particularly in the area of medicinal field because of its wide spectrum of application in molecular biology, e.g. microsphere based genotyping platform is used to detect six single nucleotide polymorphism, yittrium-90 microspheres is used to prevent tumour after liver transplantation and it's advanced way in delivery of vaccines and proteins.

APPLICATIONS OF MUCOADHESIVE MICROSPHERES

The applications of mucoadhesive microspheres can be classified depending upon the routes of administration as follows.

1. Buccal

Buccal drug delivery has lately become an important route of drug administration. This route of drug administration has recently been extensively reviewed by Shojaei. The buccal cavity has a relatively small surface area (approximately 50 cm2), but there are some advantages to it as a site for systemic drug absorption, as well as the need to treat oral lesions. First pass metabolism is avoided and the non – keratinized epithelium is relatively permeable to drugs. Materials in the buccal cavity have a short residence time largely due to the flow of saliva and swallowing, and so is a prime candidate for the development of mucoadhesive devices, which adhere to the buccal mucosa and remain in place for a considerable period of time.

Vyas and Jain prepared polymer grafted starch microspheres bearing isosorbide dinitrate for buccal administration. It was observed that compression of grafted starch microspheres modified drug release and extended drug action via slow release following buccal application. Kockisch et al. developed mucoadhesive microspheres for the controlled release of triclosan in oralcare formulations. Mucoadhesive microspheres were prepared from Gantrez™ MS-955 {mixed sodium and calcium salt of poly(methylvinyl ether/maleic anhydride)}, Carbopol™ 974P (polyacrylic acid), polycarbophil and chitosan. Giunchedi et al. developed buccal tablets based on chitosan microspheres containing chlorhexidine diacetate. The microparticles were prepared by a spray-drying technique, their morphological characteristics were studied by scanning electron microscopy and in vitro release behavior was investigated in pH 7.0 buffer. Chlorhexidine in the microspheres dissolved quickly in vitro than did chlorhexidine powder. The loading of chlorhexidine into chitosan was able to maintain or improve the antimicrobial activity of the drug.

2. Oral

The gastro intestinal (GI) tract is the most important route for drug administration, and has been the subject of extensive study as a site for the use of mucoadhesive formulations to improve drug bioavailability. The idea of mucoadhesives began with the clear need to localize a drug at a certain site in the GI tract. Therefore, a primary objective of using mucoadhesive systems orally would be achieved by obtaining a substantial increase in the residence time of the drug for local effect and to permit once-daily dosing. Bioerodible mucoadhesive microspheres have been reported to increase the peroral bioavailability of dicumarol, insulin and have been investigated for peroral gene delivery. The increased bioactivity of insulin and plasmid DNA can be accounted by the uptake of the microspheres by the cells lining the GI epithelium. Thus, these uptake pathways can be used as a platform for the systemic delivery of a variety of therapeutic agents showing poor absorption through GI epithelium. An adhesive micromatrix system (AD-MMS), a novel formulation approach, consists of the drug and an adhesive polymer dispersed in a spherical matrix of the polyglycerol esters of fatty acids, with a diameter of 177–500 μm. This formulation showed strong adherence to the stomach mucosa. Drug release from this system could be regulated by appropriate selection of HLB value of the polyglycerol esters of fatty acids. Various channeling agents were reported to regulate drug release through the micromatrix systems, e.g. mannitol, acrylic acid and lactose. In experiments using rats, prolongation of GI transit time and improvement in the bioavailability of furosemide (with a narrow absorption window) have been shown. Specially engineered polymeric mucoadhesive microspheres can traverse both the mucosal absorptive epithelium and follicleassociated epithelium covering the lymphoid tissues of Payer's patches depending on the particle size, polymer composition and the surface charge of mucoadhesive microspheres. The release of delapril hydrochloride was reported to be sustained after oral administration of mucoadhesive microspheres based on polyglycerol esters of fatty acids (PGEFs). AUC after administration of the microspheres was found to be same as that of solution, while the mean residence time (MRT) of drug in the form of microspheres was prolonged. Cuna et al. prepared microparticles consisting of amoxycillin-loaded ion-exchange resin encapsulated in mucoadhesive polymers (polycarbophil and Carbopol 934) with the aim of increasing the efficacy of amoxycillin in the treatment of peptic ulcers by achieving targeted delivery to the gastric mucosa and prolonged drug release. Van der Lubben et al. prepared chitosan microparticles by precipitation/coacervation method which were positively charged and had an average

diameter of 4.3 μm. These microparticles were proposed as good candidates for vaccination since the M-cells of the Peyer's patches can retain only microparticles less than 10 μm.

3. Nasal

Ease of access, avoidance of first–pass metabolism and a relatively permeable, well – vascularised membrane, contribute to the nasal cavity being an attractive site for drug delivery. Although, the surface area is not large, being between 150 – 200 cm2, a more important disadvantage is the rapid removal of substances by mucociliary action (residence time half life 15 – 30 minutes). This makes it a prime target for mucoadhesive formulations which could prolong the residence time to allow drug release and absorption. The nasal cavity is also perceived as a good site for the administration of therapeutic peptides, particularly insulin, and for vaccine administration to induce mucosal immunity. Nasal administration has been the subject of intense research with the emphasis moving towards the use of mucoadhesive microparticles as a preferred medium, and with chitosan leading the field as the mucoadhesive. The microparticles can be administered into the nasal cavity as a dry powder. Illum et al. demonstrated the bioadhesive properties of several microspheres (albumin, starch and DEAE – dextran microspheres) for nasal use. The half life of clearance for starch microspheres was found to be in order of 240 min, compared to 15 min for the liquid and powder control formulations. Patil et al. investigated chitosan microspheres for nasal delivery of amlodipine besylate and studied the influence of the process variables in the preparation of the microspheres. The nasal retention of chitosan microparticles has been quantified in human volunteers using gamma scintigraphy. The retention time half life at 84 minutes was 4 times that of a control, clearly demonstrating the mucoadhesion. Recently, it was demonstrated that insulin loaded microspheres administered intranasally to rabbits could lower the blood glucose by 60 % compared with 15.5 % for the control containing the same amount of insulin in solution. Farraj et al. showed that the nasal bioavailability of insulin in sheep was improved from 1-11% to 32% when administered as a freeze dried powder of starch microspheres without and with the addition of LPC (lysophosphatylcholine), respectively. Kellaway et al. reported the preparation of microspheres of hydrophilic polymers like carbopol 934P, chitosan and HPMC, by the w/o emulsification solvent evaporation technique with as drug carriers for nasal administration by insufflation. Lim et al. investigated the effect of the novel hyaluronic acid/chitosan glutamate microparticles compared with hyaluronic acid and chitosan glutamate microparticles on the nasal absorption of a model drug, gentamicin, in vivo. Krauland et al. developed a microparticulate delivery

system based on a thiolated chitosan conjugate for the nasal application of peptides by using insulin as a model peptide It should be noted that a major area of investigation is the use of intranasal microparticulate systems for vaccination. Apart from the attraction of the ease of administration, a major advantage is that both systemic and mucosal immunity can be generated, and the latter is of considerable value in combating air – born infectious agents. Protection has been achieved against B. pertussis and Y. pestis in mice using microspheres consisting of polylactic acid or poly (lactide-co-glycolide). Nasally instilled mucoadhesive chitosan particles containing tetanus toxin have been reported to elicit an important systemic immune response in mice.

4. Ocular

As the major reason for the failure of conventional ocular drug delivery systems is the drainage of the drug before adequate absorption can occur, it is a target for prolonging residence time by mucoadhesives. Various mucoadhesive drug delivery systems employed for ocular delivery of drugs include semisolids, viscous liquids, solids/inserts and particulate drug delivery systems including mucoadhesive microspheres and liposomes. Mucoadhesive microparticles are being investigated for ocular delivery and show promise for local drug administration. The advantages of microspheres, i.e. increased residence time and decreased frequency of administration were quite evident when acyclovir loaded chitosan microparticles showed an increased drug bioavailability in the eye as compared to the drug administered alone. The release of methyl prednisolone from hyaluronic acid ester films and microspheres has been investigated in vitro and in vivo (in tear fluid of rabbits). Methyl prednisolone waseither physically dispersed in the polymeric matrix or covalently linked to hyaluronic acid. Microspheres containing methyl prednisolone chemically bonded to the polymeric backbone of hyaluronic acid showed slower release of drug in vitro and produced sustained drug concentrations in the tear fluids of rabbits. De Campos et al. evaluated the potential of cyclosporin loaded chitosan microspheres in rabbits and concluded that the advantage of the system includes the ability to contact intimately the corneal/conjunctival epithelium. This increases delivery to external ocular tissues without compromising inner ocular structures and systemic drug exposure, and provides these target tissues with longterm drug levels.

5. Vaginal

Vaginal delivery may offer a number of advantages over the other routes of administration for drugs which are susceptible to gut or hepatic metabolism or which cause GI side effects.

However, in common with other mucosal sites, the bioavailability and local action of drugs administered vaginally is generally very low and may be increased by use of the principle of mucoadhesion. In recent years, vaginal mucoadhesive preparations have been developed as a new type of controlled-release form for the treatment of both topical and systemic diseases. The most important advantage of these dosage forms is the possibility of maintaining them in the vagina for extended periods of time. The mucoadhesive polymers used for vaginal preparations include hydroxypropyl methyl cellulose (HPMC) and polyacrylic acid.

HYAFF (esters of hyaluronic acid) microspheres have been successfully used for the incorporation of peptides such as nerve growth factor and salmon calcitonin. HYAFF microspheres have demonstrated good mucoadhesive properties both in vitro and in vivo. In an unconscious rat model, these microspheres maintained contact with the vaginal epithelium for at least 6 hour after administration. Hypocalcemic effects in the rat and sheep confirmed that absorption of salmon calcitonin was increased after administration of mucoadhesive HYAFF microspheres as compared with an aqueous solution of calcitonin. Due to the high biocompatibility and controllable degradation rate, HYAFF microspheres have been used for the localized drug delivery of steroids, analgesics, anti-inflammatory and anti-infectives. This led in the development of safe and effective mucoadhesive vaginal contraceptives and anti-infective preparations for control of pregnancy and to prevent the spread of sexually transmitted diseases. Rochira et al. prepared HYAFF microspheres by a solvent extraction method for the vaginal administration of salmon calcitonin.

6. Rectal

The feasibility and effectiveness of the rectal route has been examined for the delivery of peptides and proteins. The main advantages of the rectal mucosal membrane are its thickness and vascularity in comparison with the colon. Another reason for preferring this route is that when drugs are delivered rectally, the liver can perhaps be avoided to some extent. The rectal dosage forms are prepared by considering all the major physical and chemical factors, including pKa, drug solubility, distribution coefficient and particle size. However, one of the main problems with this route is patient acceptability. Ofokansi et al. prepared mucin gelatin mucoadhesive microspheres containing ceftriaxone sodium for rectal delivery. The microspheres were prepared by the emulsification cross-linking method using arachis oil as the continuous phase. However, the rectal delivery route still lacks any significant progress or major breakthrough with regard to its application in humans.[42]

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