DIFFERENT METHODS OF FORMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERE

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ABSTRACT: Mucoadhesion is topic of current interest in the design of drug delivery system. Mucoadhesive microsphere exhibit a prolonged residence time at the site of application and facilitate an intimate contact with the underlying absorption surface and thus contribute to improved or better therapeutic performance of drug. Mucoadhesive drug delivery systems promises several advantages that arise from localization at a given target site, prolonged residence time at the site of drug absorption and an intensified contact with the mucosa increasing the drug concentration gradient. 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. The principles underlying the development of Mucoadhesive microsphere and research work carried out on these systems are reviewed here.

Key Words: Bioavailability, Mucoadhesive Microsphere, Polymers.

INTRODUCTION

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 polymers 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 (K. Ikeda, et.al., 1992). This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres. Bioadhesive 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 AND MUCOADHESIVE DRUG DELIVERY SYSTEM

Mucoadhesive drug delivery system are delivery system which utilizes the property of bioadhesion 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 (J. R. Robinson, et.al., 1990). Bioadhesion is a phenomenon in which two materials at least one of which is biological and are held together by means of interfacial forces. The attachment could be between an artificial material and biological substrate such as adhesion between polymer and a biological membrane in case of polymer attached to the mucin layer of mucosal tissue. The term mucoadhesion is used when the mucosal layer lines a number of regions of body including a gastrointestinal tract, urogenital tract, the airways, the ears, nose and eye. These represent potential sites for attachment of bioadhesive system and hence the mucoadhesive drug delivery system could be designed for buccal, oral, vaginal, rectal, nasal and ocular route of administration.

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 (N.K. Jain, et.al., 1997). A General Mechanism of Mucoadhesion Drug Delivery system is show in Figure 1.

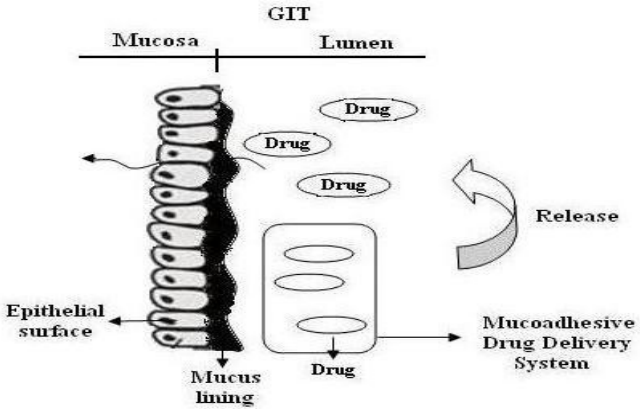

Figure 1. Mechanism of Mucoadhesion

Electronic theory

According to this theory, electron transfers occur upon contact of adhesive polymer with a mucus glycoprotein network because of difference in their electronic structures. This results in the formation of electrical double layer at the interface 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.

Absorption 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, including 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 crosslinking and decreases significantly as the cross linking density increases.

Wetting theory

The wetting theory postulates 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 substrate surfaces are brought in contact with each other in the presence of the liquid, the liquid may act as an adhesive among the substrate surface.

Cohesive theory

The cohesive theory proposes that the phenomena of bioadhesion are mainly due to intermolecular interaction amongst like molecule. Based upon the above theories, the process of bioadhesion can broadly be classified into two categories namely chemical (electron and absorption theory) and physical (wetting, diffusion and cohesive theory).

POLYMERS USED IN MUCOADHESIVE DRUG DELIVERY SYSTEM

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

Hydrophilic polymers

The polymers within this category are soluble in water. Matrices developed with these polymers swell when put into an aqueous media with subsequent dissolution of the matrix. The polyelectrolytes extend greater mucoadhesive property when compared with neutral polymers (A. Ludwig, et.al., 2005).

Anionic polyelectrolytes, e.g. poly (acrylic acid) and carboxymethyl cellulose 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 (G.P. Andrew, et.al., 1995 and S. Rossi, et.al., 2005). Chitosan provides an excellent example of cationic polyelectrolyte, which has been extensively used for developing mucoadhesive polymer due to its good biocompatibility and biodegradable properties (A. Portero, et.al., 2005). Chitosan undergoes electrostatic interactions with the negatively charged mucin chains thereby exhibiting mucoadhesive property. Structure of Chitosan is shown in Figure 2. The ionic polymers may be used to develop ionic complex with the counter-ionic drug molecules so as to have a drug delivery matrix exhibiting mucoadhesive property. Non-ionic polymers, e.g. poloxamer, hydroxypropyl methyl cellulose, methyl cellulose, poly (vinyl alcohol) and poly (vinyl pyrrolidone) have also been used for mucoadhesive properties (A. Ludwig, et.al., 2005).

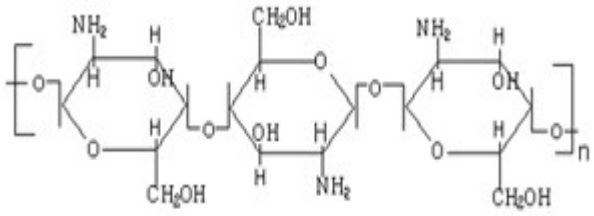

Figure. 2. Chemical structure of Chitosan

Hydrogels

Hydrogels can be defined as three-dimensionally crosslinked 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 indicated an increase in the mucoadhesive property with the increase in the crosslinking density and was attributed to increase in the poly (acrylic acid) chain density per unit area (S.J. Warren, et.al., 1998). Acrylates have been used to develop mucoadhesive delivery systems which have the ability to deliver peptide bioactive agents to the upper small intestine region without any change in the bioactivity of the peptides. Wheat germ agglutinin helped in improving the intestinal residence time of the delivery system by binding with the specific carbohydrate moieties present in the intestinal mucosa.

Thiolated polymers

The 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. poly (acrylic acid) and chitosan) in addition to the paracellular uptake of the bioactive agents (P.L. Soo, et.al., 2002, R. Saviae et.al., 2003, C. Allen, et.al., 1999, C.E. Kast, et.al., 2003 and V.M. Leitner, et.al., 2003). Various thiolated polymers include chitosan–iminothiolane, poly (acrylic acid)–cysteine, poly (acrylic acid)–homocysteine, chitosan–thioglycolic acid, chitosan–thioethylamidine, alginate–cysteine, poly (methacrylic acid)–cysteine and sodium carboxymethylcellulose–cysteine (G.P. Andrew, et.al., 1995)

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 (C.M. Lehr, et.al., 2000, E. Haltner, et.al., 1997 and J.D. Smart, 2004). 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 systems. Various lectins which have shown specific binding to the mucosa include lectins extracted from *Ulex europaeus* I and *Lens culinarius* (J. Hietanen, et.al., 2007).
A short list of Mucoadhesive polymers is given in Table no. 1

Table No 1. List of Natural and Synthetic polymers

Synthetic polymers	Natural polymers
Cellulose derivatives	Tragacanth
polycarbophil	Sodium alginate
Poly (ethylene oxide).	Karaya gum
Poly (vinyl pyrrolidone).	Guar gum
Poly (vinyl alcohol).	Gelatin
Poly (hydroxyethyl methylacrylate)	Chitosan
Hydroxyl propyl cellulose	Soluble starch

IDEAL CHARACTERISTICS OF AN MUCOADHESIVE POLYMER

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

METHOD OF PREPARATION

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% wt/vol) 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 (I. Orienti, et.al., 1996)

Preparation of Microspheres by Glutaraldehyde crosslinking

A 2.5% (wt/vol) 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% (wt/vol) Span 85 to form a water in oil (w/o) emulsion. Stirring was continued at 2000 rpm using a 3- blade propeller stirrer (Remi Equipments, Mumbai, India). A drop-by-drop solution of a measured quantity (2.5 mL each) of aqueous glutaraldehyde (25% vol/vol) 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 a vacuum desiccators (B.C. Thanoo, et.al., 1992).

Preparation of microspheres by Tripolyphosphate

Chitosan solution of 2.5% wt/vol 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% wt/vol TPP solution. Chitosan microspheres were separated after 2 hours by filtration and rinsed with distilled water, then they were air dried (R. Bodmeier, et.al., 1989 and S. Shiraishi, et.al., 1993).

Preparation of Microspheres by Emulsification and Ionotropic gelation by NaOH

Dispersed phase consisting of 40 mL of 2% vol/vol aqueous acetic acid containing 2.5% wt/vol chitosan was added to the continuous phase consisting of hexane (250 mL) and Span 85 (0.5% wt/vol) to form a w/o emulsion. After 20 minutes of mechanical stirring, 15 mL of 1N sodium hydroxide solution was added at the rate of 5 mL per min at 15-minute intervals. Stirring speed of 2200 rpm was continued for 2.5 hours. The microspheres were separated by filtration and subsequently washed with petroleum ether, followed by distilled water and then air dried (A.V. Singla, et.al., 2003 and K.V. Ranga Rao, et.al., 1988)

Preparation of Ethylcellulose Microspheres

A solution of Ethylcellulose in acetone was added to liquid paraffin containing emulgent (Span 85) while stirring at a speed of 1500 rpm. The emulsion was stirred for 5 to 6 hours at 25°C to 30°C. Subsequently, a suit able amount of petroleum ether was added to the dispersion, filtered, and dried at ambient temperature. The resultant microspheres were washed with water followed by petroleum ether to remove traces of liquid paraffin. The microspheres were desiccated under vacuum (K.V. Ranga Rao, et.al., 1988)

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 1-100µm . Micro particles 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 (U.S. Koff, et.al., 1963). This process is rapid and this leads to the formation of porous micro particles shown in Figure 3.

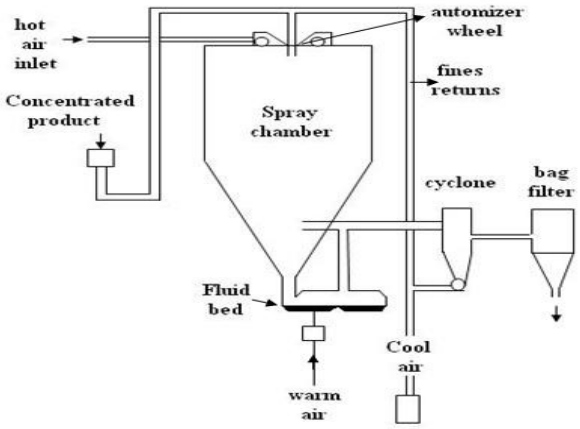

Figure 3. Spray drying method for preparation of microsphere

Solvent Evaporation

The processes are 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 4. 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. The comparison of mucoadhesive microspheres of hyaluronic acid, Chitosan glutamate and a combination of the two prepared by solvent evaporation with microcapsules of hyaluronic acid and gelating prepared by complex coacervation were made (S.T. Lim, et.al., 2000)

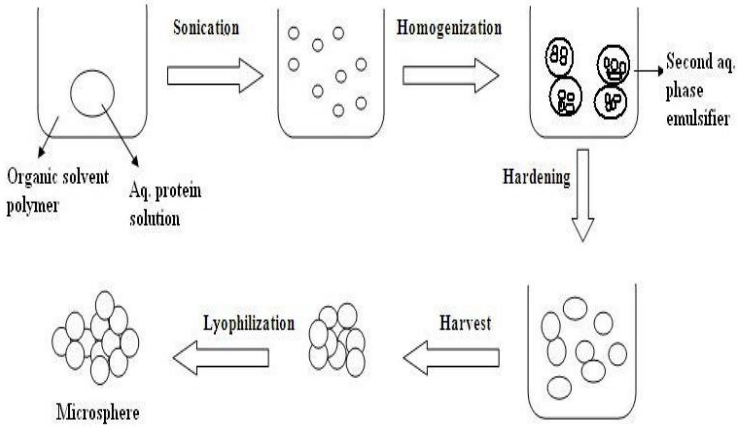

Figure 4. Solvent evaporation method for preparation of microsphere

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 (F.L. Mi, et.al., 2000)

Complex Coacervation

CS microparticles can also prepare by complex co acervation, Sodium alginate, sodium CMC and sodium polyacrylic acid can be used for complex coacervation with CS to form microspheres. These microparticles are formed by interionic interaction between oppositely charged polymers solutions and KCl & CaCl2 solutions. The obtained capsules were hardened in the counter ion solution before washing and drying (Y. Nishioka, et.al., 1990 and Y. Ohya, et.al., 1990)

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.* polyanhydrides. 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 (E. Mathiowitz, et.al., 1987)

CHARACTERIZATION OF MICROSPHERES

Particle size, Shape and Morphology

All the microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle diameters of more than 100 microspheres were measured randomly by optical microscope (M. Shirui, et.al., 2004, A. Martin, et.al., 1996 and Y.C. Huang, et.al., 2000). Scanning Electron photomicrographs of drug-loaded microspheres were taken. A small amount of microspheres was spread on gold stub. Afterwards, the stub containing the sample was placed in the Scanning electron microscopy (SEM). A Scanning electron photomicrograph was taken at an acceleration voltage of 20KV.

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.

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 hydrogel microspheres then dried in an oven at 60° for 5 h until there was no change in the dried mass of sample. The swelling index of the microsphere was calculated by using the formula (G. Fandueanu, et.al., 2004 and G.T. Kulkarni, et.al., 2004)

Swelling index= (mass of swollen microspheres - mass of dry microspheres/mass of dried microspheres) 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 using the formula.

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.

In Vitro diffusion studies

In Vitro diffusion studies were performed using in vitro nasal diffusion cell (J.K. Patel, et.al., 2005). 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 (S. Pisal, et.al., 2004)

Drug polymer interaction (FTIR) study

IR 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.

CONCLUSION

Mucoadhesive microsphere prepared by different method was evaluated for their mucoadhesive properties. The microsphere prepared by glutaraldehyde and thermal cross linking showed good stability in HCl as compared with microsphere prepared by tripolyphosphate and emulsification ionotropic gelation. Microspheres have the potential to be used for targeted and controlled release drug delivery but coupling of mucoadhesive properties to microspheres has additional advantages such as efficient absorption, enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of drugs to the absorption site. Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye, nasal cavity, urinary and gastrointestinal tract, thus offering the possibilities of localized as well as systemic controlled release of drugs.

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