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DIFFERENT TECHNIQUES OF FORMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERES

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ABSTRACT

Drug delivery systems (DDS) that can precisely control the release rates or target drugs to a specific body site have an enormous impact on the health care system. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles. Which modulates the release and absorption characteristics of the drug? Micro spheres constitute an important part of these particulate DDS by virtue of their small size and efficient carrier characteristics. However the success of these novel DDS is limited due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the DDS with absorbing membranes. It can be achieved by coupling mucoadhesion characteristics to microspheres and developing novel delivery systems referred to as "mucoadhesive" microspheres.

KEYWORDS

Carrier particle, microspheres, nanoparticles, microspheres

INTRODUCTION

Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1-1000µm in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it, respectively.[1] Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of mucoadhesive properties to microspheres has additional advantages. *eg* 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 achieved by anchoring plant lectins[3] Bacterial adhesion[2] and antibodies,[3] *etc* on the surface of microspheres. 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.

PREPARATION OF MUCOADHESIVE MICROSPHERES

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

Solvent Evaporation: It is the most extensively used method of microencapsulation first described by *Ogawa et al.*[4] Buffered or plain aqueous solution of the drug (may contain a viscosity building or stabilizing agent) is added to an organic phase consisting of the polymer solution in solvents like dichloromethane (or ethyl acetate or chloroform) with vigorous stirring to form the primary water in oil emulsion. This emulsion is then added to a large volume of water containing an emulsifier like PVA or PVP to form the multiple emulsions (w/o/w). The double emulsion, so formed, is then subjected to stirring until most of the organic solvent evaporates, leaving solid

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microspheres. The microspheres can then be washed, centrifuged and lyophilize to obtain the free flowing and dried microspheres.

Hot Melt Microencapsulation: In this method was first used by Mathiowitz and langer[5] to prepare microsphere of polyanhydride copolymer of poly[bis(*p*-carboxy phenoxy)propane anhydride]with sebacic acid , 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.

Solvent Removal: It is a non-aqueous method of microencapsulation, particularly suitable for water labile polymers such as the ploy anhydrides. In this method, drug is dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent like methylene chloride.[6] this mixture is then suspended in silicone oil containing span 80 and methylene chloride. After pouring the polymer solution into silicone oil, petroleum ether is added and stirred until solvent is extracted into the oil solution. The resulting microspheres can then be dried in vaccum.

Hydrogel Microspheres: Microspheres made of gel-type polymers, such as alginate, are produced by dissolving the polymer in an aqueous solution, suspending the active ingredient in the mixture and extruding through a precision device, producing micro droplets which fall into a hardening bath that is slowly stirred. The hardening bath usually contains calcium chloride solution, whereby the divalent calcium ions crosslink the polymer forming gelled microspheres. The method involves an "all-aqueous" system and avoids residual solvents in microspheres. Lim and Moss[100] develop this method. This method can be used for encapsulation of live cells, as it does not involve harsh conditions, which could kill the cells. The surface of these microspheres can be further modified by coating them with polycationic polymers, like polylysine after fabrication. The particle size of microspheres can be controlled by using various size extruders or by varying the polymer solution flow rates.

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, *eg* 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 the rate of spraying, the feed rate of polymer drug 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, and easy to scale up.[101]

Phase Inversion Microencapsulation: The process involves addition of drug to a dilute solution of the polymer (usually 1-5% w/v in methylene chloride). The mixture is poured into an unstirred bath of strong non-solvent (petroleum ether) in a solvent to non-solvent ratio of 1:100, resulting in the spontaneous production of microspheres in the size range of 0.5-5.0µm can then be filtered, washed with petroleum ether and dried with air.[102] This simple and fast process of microencapsulation involves relatively little loss of polymer and drug.

EVALUATION

The best approach to evaluate mucoadhesive microspheres is to evaluate the effectiveness of mucoadhesive polymer to prolong the residence

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time of drug at the site absorption, thereby increasing absorption and bioavailability of the drug. The methods used to evaluate mucoadhesive microspheres include the following.

Measurement of Adhesive Strength: The quantification of the mucoadhesive forces between polymeric microspheres and the mucosal tissue is a useful indicator for evaluating the mucoadhesive strength of micro-microspheres against a variety of synthetic and natural mucus, frozen and freshly excised tissue etc. The different in vitro methods include the following.

1.1Tensile Stress Measurement: The wilhelmy plate technique is traditionally used for the measurement of dynamic contact angles and involves the use of a microtensiometer or microbalance assembly.[7] The CAHN dynamic contact angle analyzer (model DCA 322, CAHN instruments, Cerritos) has been modified to perform adhesive micro force measurements. The DCA 322 system consists of an IBM compatible computer and microbalance assembly. The microbalance unit consists of stationary sample and tare loops and a motor powered translation stage. The instrument measures the mucoadhesive force between mucosal tissue and a single microsphere mounted on a small diameter metal wire suspended from the sample loop in microtesiometer.[8] The tissue, usually rat jejunum, is mounted within the tissue chamber containing Dulbecco's phosphate buffered saline containing 100 mg/dl glucose and maintained at the physiologic temperature. The chamber rests on a mobile platform, which is raised until the tissue comes in contact with the suspended microsphere. The contact is held for 7 minute, at which time the mobile stage is lowered and the resulting force of adhesion between the polymer and mucosal tissue recorded as a plot of the load on microsphere versus mobile stage distance or deformation. The plot of output of the instrument is unique in that it displays both the compressive and the tensile portions of the experiment. By using the CAHN soft ware system, three essential mucoadhesive parameters can be analyzed.

1.2Fracture strength: It is the maximum force per unit surface area required to break the adhesive bond.

1.3Deformation to failure: It is the distance required to move the stage before complete separation occurs. This parameter is dependent on the material stiffness and the intensity of strength of adhesion.

1.4Shear Stress Measurement: The shear stress measures the force that causes a mucoadhesive to slide with respect to the mucus layer in a direction parallel to their plane of contact. Adhesion tests based on the shear stress measurement involve two glass slides coated with polymer and a film of mucus. Mucus forms a thin film between the two polymer coated slides, and the test measures the force required to separate the two surfaces. Mikos and Peppas[9] designed the in vitro method of flow chamber. The flow chamber made of Plexiglass is surrounded by a water jacket to maintain a constant temperature. A polymeric microsphere placed on the surface of a layer of natural mucus is placed in a chamber. A simulated physiologic flow of fluid is introduced in the chamber and movement of microsphere is monitored using video equipment attached to a goniometer, which also monitors the static and dynamic behavior of the microparticles.

1.5Adhesion Number: Adhesion number for mucoadhesive microspheres is determined as the ratio of the number of particles attached to the substrate to the total number of applied particles, expressed as a percentage. The adhesion strength increases with an increase in the adhesion number.[10]

1.6Falling Liquid Film Method: It is a simple, quantitative in situ method, wherein an excised intestinal segment cut lengthwise, is spread on a plastic flute and positioned at an incline. The suspension of microsphere is allowed to flow down the intestinal strip. Particle concentrations entering

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the segment from the dilute suspension reservoir and leaving the intestinal segment can be determined with the help of coulter counter to quantify the steady state fraction of particles adhered to the intestinal mucosa. The percent of particles retained on the tissue is calculated as an index of mucoadhesion.[11, 12, and 13]

1.7 Everted Sac Technique: The everted intestinal sac technique is a passive test for mucoadhesion and involves polymeric microspheres and a section of the everted intestinal tissue. It is performed using a segment of intestinal tissue excised from the rat, everted, ligated at the ends and filled with saline. It is then introduced into a tube containing a known amount of the microspheres[14] and saline, and agitated while incubating for 30 minutes. Sac is then removed, microspheres are washed and lyophilized, and the percentage of binding to the sac is calculated from difference in the weight of the residual spheres from the original weight of the microspheres. The advantage of the technique is that no external force applied to the microspheres being tested; microspheres are freely suspended in buffer solution and made to come in contact with the everted intestinal tissue randomly. The CAHN technique and the everted intestinal sac technique, both predict the strength of mucoadhesion in a very similar manner. Established a correlation between the two in vitro mucoadhesion assay methods which thereby allows one to confidentially utilize a single mucoadhesion assay to scan a variety of mucoadhesive polymers.

1.8 *In Vivo* Techniques

1.2.1 Measurement of the Residence: Time measurements of the residence time of mucoadhesives at the application site provide quantitative information on their mucoadhesive properties. The GI transit times of many mucoadhesive preparations have been examined using radioisotopes and fluorescent labelling techniques.

1.20.2 GI Transit Using Radio-Opaque Micro spheres: It is a simple procedure involving the use of radio-opaque markers, e.g. barium sulfate, encapsulated in mucoadhesive polymers to determine the effects of mucoadhesive polymers on GI transit time. Faeces collection (using an automated faeces collection machine) and X-ray inspection provide a non-invasive method of monitoring total GI residence time without affecting normal GI motility. Mucoadhesives labeled with Cr-51, Tc-99m, In-113m, or I-123 has been used to study the transit of the microspheres in the GI tract.

1.20.3 Gamma scintigraphy technique: Distribution and retention time of the mucoadhesive intravaginal Microsphere can be studied using the gamma scintigraphy technique.A study has reported the intensity and distribution of radioactivity in the gential tract after administration of technetium labeled hyaluronic acid esters microspheres. Dimensions of the vaginal cavity of the sheep can be outlined and imaged using labelled gellan gum and the data collected is subsequently used to compare the distribution of radiolabelled HYAFF formulations. The retention of mucoadhesive-radiolabelled microspheres based on HYAFF polymer was found to be more for the dry powder formulation than for the pessary formulation after 12 h of administration to vaginal epithelium. The combination of sheep model and gamma scintigraphy method has been proved to be an extremely useful tool for evaluating the distribution, spreading and clearance of vaginally administered mucoadhesive drug delivery system, including microbicides.

1.20.4 Surface Characterization of the Mucoadhesive Micro-spheres: Surface morphology of microspheres and the morphological changes produced through polymer degradation can be investigated and documented using scanning electron microscopy (SEM), electron microscopy and scanning tunneling microscopy (STM). To assess the effect of surface morphology on the mucoadhesive properties, the

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microsphere samples are lyophilized and analyzed under SEM at 150µm and 1000 µm. The smooth texture of the microsphere surface leads to weak mucoadhesive properties, while the coarser surface texture improves the adhesion through stronger mechanical interactions. The morphological surfaces changes occurring due to the hydrolytic degradation of the polymers, E.g. polyanhydrides can be studied after incubating the microspheres in the PBS buffer for different intervals of time.

Summary of Research Work on Mucoadhesive Microsphere and Microcapsule

Drug	Polymer	Route	Purpose/Result
Amoxicillin	PEG	GI	Amoxicillin release from, PEG nanoparticles system was studied.
Amoxicillin	Ethyl cellulose	GI	Prolonged gastrointestinal residence time
Amoxicillin	Chitosan	GI	Grater anti *H.Pylori activity.*
Furosemide	AD-MMS(PGEFs)	GI	Increased bioavailability Higher AUC.
Glipizide	Sodium Alginate CMC/MC/Carbopol/HPMC	Oral	Slow release rate.
Ceftriaxone sodium	mucin-gelatin	Rectal	Successfully delivered rectally when embedded in microspheres.
prednisolone	Alginate/chitosan	GI	Colon-specific delivery.
Amoxicillin	Chitosan	GI	Grater anti *H.Pylori* activity.
Clarithromycin	Chitosan	GI	Provide prolonged contact time for drug delivery of antibiotics.
Metoclopramide	sodium alginate, chitosan hydrochloride	Nasal	Mucoadhesive microspheres for nasal administration of an antiemetic drug.

AD-MMS: Adhesive micro matrix system,
AUC: Area under curve,
CMC: Carboxy methyl cellulose,
EDTA: Ethylenediaminetetraacetic
HPMC: Hydroxy propyl methyl cellulose

Statistical Optimization Technique

2^3 Factorial Designs [16, 17, 18, 19]: The main objective of a factorial experiment is able to determine (or) at least estimate the factor effects, which indicates how each factor affects the process output. Factor effects need to be understood so that the factors can be adjusted to optimize the process output. The effect of each factor on the output can be due to it alone [main effect of the factor], [or] a result of the interaction between the factor and one [or] more of the other factor (interactive effects). When assessing factor effects (whether main (or) interactive effect), one needs to consider not only the magnitudes of the effects, but their directions as well. The directions of effects determined the direction in which factors need to be adjusted in a process in order to optimize the process out put. In factorial designs, the main effects are referred to

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using single uppercase letters,(e.g.) the main effects of a factor A,B&C is referred to simply as A,B&C respectively. An interactive effect, on the other hand, is referred denoting which factors are interacting to produce the effect, (e.g.,) the interactive effect produced by factors A, B&C is referred to as ABC. The magnitude and polarity (or direction) of the numerical values of main and interactive effects indicates how this effects influences the process output. A higher absolute value for an effect means that the factor responsible for it affects the out put significantly. A negative value means that increasing level(s) of the factor(s) responsible for that effect will decrease the output process.

Mechanism of drug release: In order to understand the complex mechanism of drug release from the mucoadhesive microspheres, the *in vitro* amoxicillin trihydrate release data were fitted to korsmeyer-peppa's release model and interpretation of release exponent values (n) enlightens in understanding the release mechanism from the dosage form. The release exponent values thus obtained were ranged from 0.7766 to 0.9352 are shown in Table No.35. All the formulations exhibited anomalous (non-fickian transport) diffusion mechanism. The drug release was diffusion controlled as the plot of Higuchi's model was found to be linear ($r > 0.9724$). These formulations are also showed as highest "r" values of zero order kinetics indicating the Amoxicillin trihydrate release from these mucoadhesive microspheres were by both diffusion and erosion.

CONCLUSION

Mucoadhesive microspheres include microparticles and microcapsules (having a core of the 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 mucoadhesive properties to microspheres has additional advantages. *eg* 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 achieved by anchoring plant lectins[63] Bacterial adhesion and antibodies, *etc* on the surface of microspheres. 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. Application of mucoadhesive microspheres to the mucosal tissues of ocular cavity, gastric and colonic epithelium is used for administration of drugs for localized action.

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