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Review

Bioadhesive microspheres as a controlled drug delivery system

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Abstract

The concept of controlled drug delivery has been traditionally used to obtain specific release rates or spatial targeting of active ingredients. The phenomenon of bioadhesion, introduced by Park and Robinson [Park, K., Robinson, J.R., 1984. Bioadhesive polymers as platforms for oral controlled drug delivery: method to study bioadhesion. Int. J. Pharm. 198, 107–127], has been studied extensively in the last decade and applied to improve the performance of these drug delivery systems. Recent advances in polymer science and drug carrier technologies have promulgated the development of novel drug carriers such as bioadhesive microspheres that have boosted the use of "bioadhesion" in drug delivery. This article presents the spectrum of potential applications of bioadhesive microspheres in controlled drug delivery ranging from the small molecules, to peptides, and to the macromolecular drugs such as proteins, oligonucleotides and even DNA. The development of mucus or cell-specific bioadhesive polymers and the concepts of cytoadhesion and bioinvasion provide unprecedented opportunities for targeting drugs to specific cells or intracellular compartments. Developments in the techniques for in vitro and in vivo evaluation of bioadhesive microspheres have also been discussed.

Keywords: Bioadhesive; Microspheres; Controlled release; Drug delivery; Site specific

1. Introduction

Drug delivery systems (DDS) that can precisely control the release rates or target drugs to a specific body site have had an enormous impact on the healthcare system. The last two decades in the pharmaceutical industry have witnessed an avant-garde interaction among the fields of polymer and material science, resulting in the development of novel drug delivery systems (Mathiowitz et al., 1999).

Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etc. which modulates the release and absorption characteristics of the drug. Microspheres 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 the absorbing membranes. It can be achieved by coupling bioadhesion characteristics to microspheres and developing novel delivery systems referred to as "bioadhesive microspheres".

The present article is an attempt to review the potential of bioadhesive microspheres in controlled release systems, drug targeting and their administra-

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tion through various routes. Other applications such as mucosal immunization and delivery of macromolecules, e.g. protein drugs as well as DNA, have been highlighted. A brief review of various polymers, microencapsulation techniques and recent developments in methods to evaluate bioadhesive microspheres is also included.

2. Bioadhesion: basic concepts

"*Bioadhesion*" in simple terms can be described as the attachment of a synthetic or biological macromolecule to a biological tissue. An adhesive bond may form with either the epithelial cell layer, the continuous mucus layer or a combination of the two. The term "mucoadhesion" is used specifically when the bond involves mucous coating and an adhesive polymeric device, while "cytoadhesion" is the cell-specific bioadhesion. The mechanism of bioadhesion has been reviewed extensively (Ahuja et al., 1997; Lee et al., 2000). Adhesion between mucin and mucoadhesive polymers is usually analysed based on the molecular attractive and repulsive forces as listed in Table 1. In contrast, adhesion to cell surfaces involves highly specific receptor-mediated interactions.

The phenomenon of mucoadhesion, however, is unpredictable due to varying turnover time and composition of mucus, different behaviour of mucoadhesive devices over the pH range, and disease conditions (Table 2). The lack of specificity in adhering to specific mucous tissue seriously limits drug delivery/targeting through this technique. However, with the introduction of concept of cytoadhesion and the recent advances made in polymer science, bioadhesive microspheres have found new applications in drug targeting.

3. Bioadhesive microspheres

Bioadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1–1000 μm in diameter and consisting either entirely of a bioadhesive polymer or having an outer coating of it, respectively (Mathiowitz et al., 2001). Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of bioadhesive properties to microspheres has

Table 1
Different theories explaining the mechanism of bioadhesion

S. no.	Theory	Mechanism of bioadhesion	Comments
1	Electronic theory	Attractive electrostatic forces between glycoprotein mucin network and the bioadhesive material	Electron transfer occurs between the two forming a double layer of electric charge at the interface
2	Adsorption theory	Surface forces resulting in chemical bonding	*Strong primary forces*: covalent bonds *Weak secondary forces*: ionic bonds, hydrogen bonds and van der Waal's forces
3	Wetting theory	Ability of bioadhesive polymers to spread and develop intimate contact with the mucus membranes	Spreading coefficients of polymers must be positive Contact angle between polymer and cells must be near to zero
4	Diffusion theory	Physical entanglement of mucin strands and the flexible polymer chains Interpenetration of mucin strands into the porous structure of the polymer substrate	For maximum diffusion and best bioadhesive strength: solubility parameters (δ) of the bioadhesive polymer and the mucus glycoproteins must be similar
5	Fracture theory	Analyses the maximum tensile stress developed during detachment of the BDDS from the mucosal surfaces	Does not require physical entanglement of bioadhesive polymer chains and mucin strands, hence appropriate to study the bioadhesion of hard polymers, which lack flexible chains

Table 2
Factors affecting the performance of BDDS

S. no.	Factors	Comments
1	Polymer related factors	
	Molecular weight	*Low molecular weight polymer*: favours the interpenetration of polymer molecules *High molecular weight polymer*: favours physical entanglement *Optimum molecular weight*: at least 100,000 (threshold)
	Flexibility of polymer chains	Required for interpenetration and entanglement *Highly cross-linked polymers*: mobility of individual polymer chains decreases which leads to decreased bioadhesive strength
	Spatial conformation	
	Concentration of polymer	*Solid BDDS*: more is the polymer concentration higher is the bioadhesive strength *Liquid BDDS*: Optimum concentration is required for best bioadhesion High concentration may result in coiling of polymer molecules and hence reduced flexibility of the polymeric chains
2	Environment related factors	
	pH	*Surface charge on mucus*: varies with pH due to differences in dissociation of functional groups on the carbohydrate moiety and amino acids of the polypeptide backbone *Surface charge on polymer and degree of hydration*: e.g. polycarbophil—shows bioadhesive properties at pH below 5, protonated carboxyl groups form hydrogen bonds with mucin strands than the ionised carboxyl groups *Interpolymer complexation*: introduces a lag time in the drug dissolution and release, more at acidic pH
	Initial pressure applied at contact site	Affects the depth of interpenetration High pressure applied for a sufficiently long period promotes attractive interactions of bioadhesive polymer with mucin
	Initial contact time	Determines the extent of swelling and interpenetration of polymer chains Cannot be controlled for the BDDS in GIT
	Swelling	Depends on polymer concentration and presence of water Allows easy detachment of BDDS after the release of active ingredients
3	Physiological factors	
	Mucin turnover	Limits the residence time of BDDS on the mucous layer *In GI mucosa*: depends on presence of food *Intranasal mucociliary clearance*: inhibited by chitosans
	Disease states	May alter the physicochemical properties of mucus, e.g. common cold, gastric ulcers, ulcerative colitis, cystic fibrosis, bacterial and fungal infections and inflammation

additional advantages, e.g. 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, specific targeting of drugs to the absorption site achieved by anchoring plant lectins, bacterial adhesins and antibodies, etc. on the surface of the microspheres.

Bioadhesive 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 localised as well as systemic controlled release of drugs. Application of bioadhesive microspheres to the mucosal tissues of ocular cavity, gastric and colonic epithelium is used for administration of drugs for localised action. Prolonged release of drugs and a reduction in frequency of drug administration to the ocular cavity can highly improve the patient compliance. The latter advantage can also be obtained for the drugs administered intranasally due to the reduction in mucociliary clearance of drugs

Table 3
Applications of bioadhesive microspheres

Drug	Route of administration	Polymers used	Comments	Reference
Acyclovir	Ocular	Chitosan	Slow release rates Increased AUC	Genta et al. (1997)
Methyl prednisolone	Ocular	Hyaluronic acid	Slow release rates Sustained drug concentration in tear fluids	Kyyronen et al. (1992)
Gentamicin	Nasal	DSM + LPC	Increased nasal absorption	Farraj et al. (1990)
Insulin	Nasal	DSM + LPC	Efficient delivery of insulin into the systemic circulation via nasal route	Farraj et al. (1990)
Human growth hormone (hGH)	Nasal	DSM + LPC	Rapid and increased absorption	Illum et al. (1990)
Desmopressin	Nasal	Starch	Addition of LPC causes a five folds increase in C_{max} and two folds increase in bioavailability	Critchley et al. (1994)
Haemagglutinin (HA) obtained from influenza A virus	Nasal	HYAFF	With mucosal adjuvant: ↑ed serum IgG antibody response as compared to i.m. immunization	Singh et al. (2001)
Furosemide	GI	AD-MMS (PGEFs)	Increased bioavailability Higher AUC Effective absorption from the absorption window	Akiyama and Nagahara (1999)
Riboflavin	GI	AD-MMS (PGEFs)		
Amoxicillin	GI	AD-MMS (PGEFs)	Greater anti *H. pylori* activity	
Delapril hydrochloride (prodrug)	GI	PGEFs	MRT of drug is increased Plasma concentrations of the active metabolite are sustained	Akiyama et al. (1994)
Vancomycin	Colonic	PGEF coated with Eudragit S 100	Well absorbed even without absorption enhancers	Geary and Schlameus (1993)
Insulin	Colonic	PGEF coated with Eudragit S 100	Absorbed only in the presence of absorption enhancers, e.g. EDTA salts	Geary and Schlameus (1993)
Nerve growth factor (nGF)	Vaginal	HYAFF	Increased absorption from HYAFF microspheres as compared to aqueous solution of the drugs	Ghezzo et al. (1992)
Insulin	Vaginal	HYAFF	Increased absorption from HYAFF microspheres as compared to aqueous solution of the drugs	Illum et al. (1994)
Salmon calcitonin	Vaginal	HYAFF	Increased absorption from HYAFF microspheres as compared to aqueous solution of the drugs	Richardson and Armstrong (1999)
Pipemidic acid	Vesical	CMC as mucopolysaccharide + Eudragit RL as matrix polymer	–	Bogataj et al. (1999)

AD-MMS: adhesive micromatrix system; AUC: area under curve; CMC: carboxy methyl cellulose; DSM: degradable starch microspheres; EDTA: ethylenediaminetetraacetic acid; GI: gastrointestinal; HYAFF: hyaluronic acid esters; IgG: immunoglobulin G; i.m.: intramuscular; LPC: lysophosphatidylcholine; MRT: mean residence time; PGEFs: polyglycerol esters of fatty acids.

adhering to nasal mucosa. Microspheres prepared with bioadhesive and bioerodible polymers undergo selective uptake by the M cells of Peyer patches in gastrointestinal (GI) mucosa. This uptake mechanism has been used for the delivery of protein and peptide drugs, antigens for vaccination and plasmid DNA for gene therapy. Moreover, by keeping the drugs in close proximity to their absorption window in the GI mucosa, the bioadhesive microspheres improve the absorption and oral bioavailability of drugs like furosemide and riboflavin. The concept of a non-invasive single shot vaccine, by means of mucosal immunization, offers controlled release of antigens and thus forms another exquisite application of bioadhesive microspheres. All these applications have been reviewed in the subsequent sections and listed in Table 3.

4. Polymers used for bioadhesive microspheres

The properties of the bioadhesive microspheres, e.g. their surface characteristics, force of bioadhesion, release pattern of the drug, and clearance, are influenced by the type of polymers used to prepare them. Suitable polymers that can be used to form bioadhesive microspheres include soluble and insoluble, nonbiodegradable and biodegradable polymers. These can be hydrogels or thermoplastics, homopolymers, copolymers or blends, natural or synthetic polymers.

4.1. Classification of polymers

- *Hydrophilic polymers*: These are the water-soluble polymers that swell indefinitely in contact with water and eventually undergo complete dissolution.
- *Hydrogels*: These are water swellable materials, usually a cross-linked polymer with limited swelling capacity.
- *Thermoplastic polymers*: These polymers include the non-erodible neutral polystyrene and the semi crystalline bioerodible polymers, which generate the carboxylic acid groups as they degrade, e.g. polyanhydrides and polylactic acid. Various synthetic polymers used in bioadhesive formulations include polyvinyl alcohol, polyamides, polycarbonates, polyalkylene glycols, polyvinyl ethers, esters and halides, polymethacrylic acid, polymethyl methacrylic acid, methylcellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, and sodium carboxymethylcellulose.

Various biocompatible polymers used in bioadhesive formulations include cellulose-based polymers, ethylene glycol polymers and its copolymers, oxyethylene polymers, polyvinyl alcohol, polyvinyl acetate and HYAFF (esters of haluronic acid).

Various biodegradable polymers used in bioadhesive formulations are poly(lactides), poly(glycolides), poly(lactide-co-glycolides), polycaprolactones, and polyalkyl cyanoacrylates. Polyorthoesters, polyphosphoesters, polyanhydrides, polyphosphazenes are the recent additions to the polymers.

Many ligand molecules are often attached covalently to the surface of polymeric microspheres either to increase the strength of bioadhesion or to impart specificity to adhere to specific mucosal surfaces. Attachment of different anhydride oligomers (sebacic acid, bis(*p*-carboxy-phenoxy)propane, isophthalic acid, fumaric acid, maleic acid, adipic acid or dodecanedioic acid), positively charged ligands (polyethyleneimine, polylysine), polyamino acids (polyaspartic acid, polyglutamic acid), partially purified fractions of mucin (Santos et al., 2000), and metal ions (calcium, iron, copper, zinc) have been explored to modify the bioadhesive properties of the polymers (Jacob and Mathiowitz, 2000). Multivalent ions, such as divalent or trivalent cations in the metal compounds generally, have the strongest affinity for the negatively charged mucin chains. The ligand affinity need not be based solely on electrostatic charge, but other useful physical parameters such as solubility in mucin or specific affinity to carbohydrate groups. The in vivo chelation of calcium and other metal ions by the polyacrylic acid-based microparticles leads to higher rates of absorption and inhibition of enzymes. Depletion of extracellular calcium may affect the integrity of the epithelial cells, causing enhanced permeability and higher rate of absorption. Many enzymes require metal ions for their action and chelation of these ions by the polymer causes inhibition of the enzymes (Kriwet and Kissel, 1996). Polyethylene glycol has been reported to act as the adhesion promoter between polyacrylic acid and mucin by linear diffusion of the PEG chains into the polymeric networks of both mucin and the polymer (Lele and Hoffman, 2000). The release rate of indomethacin was studied from the bioadhesive

Table 4
Specific ligands corresponding to the glycosyl groups on cell membranes, which can be used for targeting the bioadhesive microspheres to a specific site

S. no.	Glycosyl groups on cell membranes	Specific ligands	Specific site
1	Mannose	*Galanthus nivalis* agglutinin (GNA)	Epithelial cells in stomach, caecum, and colon
2	*N*-Acetyl glucosamine	Wheat germ agglutinin (WGA)	Epithelial cells in stomach, caecum, colon and absorptive enterocytes in small intestine
		Lycopersicon esculentum or tomato lectin (LEA)	Strong binding to M cells
3	*N*-Acetyl galactosamine	Lectin ML-1 from *Viscum album*	Endocytosed by villus enterocytes and goblet cells Strong binding to epithelial cells in small intestine
4	Phytohaemagglutinin	*Phaseolus vulgaris* isoagglutinin	Surface cells of the stomach
5	Fucose	*Aleuria aurentia* agglutinin (AAA)	Specific binding and transcytosis by M cells

drug delivery system (BDDS) based on the above said complexes. The PEGylated drug was designed to be a prodrug, which was linked by an easily hydrolysable anhydride bond. The complex was found to dissociate and dissolve at pH 7.4 forming polyacrylate sodium and releasing free drug and PEG.

4.2. Specific site directed bioadhesives—the new generation

The specific mucosal surfaces can be targeted using site-specific chemical agents that are anchored onto the polymeric DDS. The first generation mucoadhesive polymers lack specificity and can bind to any mucosal surface. This limits their use for fabrication of BDDS for a particular tissue. However, the development of polymers and microspheres grafted with mucus or cell-specific ligands have increased therapeutic benefit and made site-specific drug delivery possible (Table 4). Any ligand with a high binding affinity for mucin can be covalently linked to the microspheres with the appropriate chemistry, such as CDI (carbonyl di-imidazole) and be expected to influence the binding of microspheres. Targeting of the drugs can be achieved by using the following ligands.

4.2.1. Lectins

Lectins can be defined as proteins of non-immune origin that bind to carbohydrates specifically and non covalently. According to the molecular structure, three groups of lectins can be distinguished (Haas and Lehr, 2002):

1. *Merolectins*: lectins having only one carbohydrate-recognising domain;
2. *Hololectins*: lectins with two or more carbohydrate-recognising domains;
3. *Chimerolectins*: lectins with additional unrelated domains.

Lectins can increase the adherence of microparticles to the intestinal epithelium and enhance penetration of drugs. They may be used to target therapeutic agents for different gut components or even for different cells (e.g. complex-specific lectins for parietal cells or fucose-specific lectins for M cells). A bioinvasive mechanism has been described for the activity of lectins as targeting moieties. After binding to specific cells, the lectins undergo cellular uptake and subsequently can also exhibit strong binding to nuclear pore membranes (Haas and Lehr, 2002). Polystyrene microparticles coated with tomato lectin were shown to be specifically adhesive to enterocytes (Gabor et al., 1997). Tomato lectin is a potential targeting moiety due to its low toxicity and high specificity, but its inactivation due to cross-reactivity with mucus limits its usefulness. The potential of tomato lectin can, however, be tapped by exploiting its cellular uptake for drug delivery (Lehr et al., 1992). The other useful lectin ligands include lectins isolated from: *Abrus precatroius*, *Agaricus bisporus*, *Anguilla anguilla*, *Arachis hypogaea*, *Pandeiraea simplicifolia*, and *Bauhinia purpurea*. Lectin-mediated drug delivery forms a promising approach for the peroral, specific bioadhesive formulations. The use of lectins

for targeting drugs to tumor tissue is currently under intensive investigation as the human carcinoma cell lines exhibit higher lectin binding capacity than the normal human colonocytes (Gabor et al., 1997).

4.2.2. Bacterial adhesins

Bacteria are able to adhere to epithelial surfaces of the enterocytes with the aid of fimbriae. Fimbriae are long, lectin like proteins found on the surface of many bacterial strains. Their presence has been correlated with pathogenicity, e.g. adherence of *Escherichia coli* to the brush border of epithelial cells mediated by K99 fimbriae is a prerequisite for subsequent production and cellular uptake of *E. coli* enterotoxin. Thus, the DDS based on bacterial adhesion factors could be an efficient mechanism to increase adhesion of bioadhesive microspheres to epithelial surfaces (Lee et al., 2000). Another study (Haltner et al., 1997) envisaging the importance of bacterial adhesins has been carried out using "invasin", which is a membrane protein from *Yersinia pseudotuberculosis*. Cellular uptake of polymeric nanospheres functionalised with invasin has been observed using confocal laser scanning microscopy.

4.2.3. Amino acid sequences

Certain amino acid sequences have complementary parts on the cell and mucosal surfaces and when attached to microparticles can promote binding to specific cell surface glycoproteins. The cell surface glycoproteins are altered in the presence of disease conditions and these altered protein sequences can be targeted by complementary amino acid sequences attached to the drug delivery device.

4.2.4. Antibodies

Antibodies can be produced against selected molecules present on mucosal surfaces. Due to their high specificity, antibody can be a rational choice as a polymeric ligand for designing site-specific mucoadhesives. This approach can be useful for targeting drugs to tumor tissues.

5. Preparation of bioadhesive microspheres

Bioadhesive microspheres can be prepared using any of the following techniques (Table 5).

5.1. Solvent evaporation

It is the most extensively used method of microencapsulation, first described by Ogawa et al. (1988). A buffered or plain aqueous solution of the drug (may contain a viscosity building or stabilising 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 emulsion

Table 5
Comparison of the various processes used for the preparation of bioadhesive microspheres

Process used	Particle size (μm)	Polymers	Comments
Solvent evaporation	1–100	Relatively stable polymers, e.g. polyesters, polystyrene	Labile polymers may degrade during the fabrication process due to the presence of water
Hot melt microencapsulation	1–1000	Water labile polymers, e.g. polyanhydrides, polyesters; with a molecular weight of 1000–50000	Smooth and dense external surfaces of the microspheres
Solvent removal	1–300	High melting point polymers especially polyanhydrides	Avoids use of water, only organic solvents are used
Spray drying	1–10	–	Primarily for microspheres used for intestinal imaging
Ionic gelation and size extrusion	1–300	Chitosan, CMC, alginate	Used for encapsulation of live cells
Phase inversion	0.5–5.0	Polyanhydrides	Involves low polymer loss and low drug loss during fabrication process

(w/o/w). The double emulsion, so formed, is then subjected to stirring until most of the organic solvent evaporates, leaving solid microspheres. The microspheres can then be washed, centrifuged and lyophilised to obtain the free flowing and dried microspheres.

5.2. Hot melt microencapsulation

This method was first used by Mathiowitz and Langer (1987) to prepare microspheres of polyanhydride copolymer of poly[bis(*p*-carboxy phenoxy) propane anhydride] with sebacic acid. In this method, 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 stabilised, 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 the moderate temperature to which the drug is exposed.

5.3. Solvent removal

It is a non-aqueous method of microencapsulation, particularly suitable for water labile polymers such as the polyanhydrides. In this method, drug is dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent like methylene chloride. This mixture is then suspended in silicone oil containing Span 85 and methylene chloride (Carino et al., 1999). After pouring the polymer solution into silicone oil, petroleum ether is added and stirred until solvent is extracted into the oil solution. The resulting microspheres can then be dried in vacuum.

5.4. 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 microdroplets 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 (1981) developed this method 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.

5.5. Spray drying

In this process, the drug may be dissolved or dispersed in the polymer solution and spray dried. The quality of spray-dried microspheres can be improved by the addition of plasticizers, e.g. citric acid, which promote polymer coalescence on the drug particles and hence promote the formation of spherical and smooth surfaced microspheres. The size of microspheres can be controlled by 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 (Bodmeier and Chen, 1988).

5.6. 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 a strong non-solvent (petroleum ether) in a solvent to non-solvent ratio of 1:100, resulting in the spontaneous production of microspheres through phase inversion. The microsphere in the size range of 0.5–5.0 μm can then be filtered, washed with petroleum ether and dried with air (Chickering et al., 1996). This simple and fast process of microencapsulation involves relatively little loss of polymer and drug.

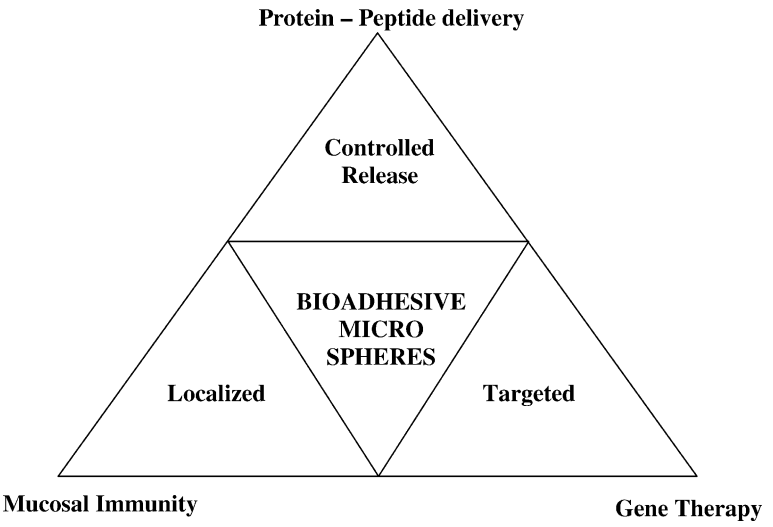

Fig. 1. Applications of bioadhesive microspheres in drug delivery.

6. Pharmaceutical applications

Bioadhesive microspheres have been extensively studied for a number of applications (Fig. 1). Majority of these can be understood by classifying these applications on the basis of route of administration (Table 6).

6.1. Topical

6.1.1. Ocular

Traditional ophthalmic formulations such as aqueous solutions and ointments have low (typically 2–10%) bioavailability of drugs due to the small surface area available for penetration, the presence

Table 6
Patents related to bioadhesive microspheres

Patent number	Assignee/inventor	Year of grant of patent	Title
US 6368586	Brown University Research Foundation	April 2002	Methods and compositions for enhancing bioadhesive properties of polymers
WO 0203955	Roversi Francesco, Cilurzo Francesco	January 2002	Fast release bioadhesive microspheres for the sublingual administration of Proximate principles
US 6274175	Immunex Corporation	August 2001	Prolonged release of GM-CSF
US 6197346	Brown University Research Foundation	March 2001	Bioadhesive microspheres and their use as drug delivery and imaging systems
US 6207197	West Pharmaceutical Services Drug Delivery and Clinical Research Centre	March 2001	Gastroretentive controlled release microspheres for improved drug delivery
CA 2060176	West Pharmaceutical Services Drug Delivery and Clinical Research Centre	January 2001	Small particle drug compositions
US 6123965	Brown University Research Foundation	September 2000	Methods and compositions for enhancing bioadhesive properties of polymers
US 6156348	Brown University Research Foundation	December 2000	Methods and compositions for enhancing bioadhesive properties of polymers using organic excipients
US 5935604	Danbiosyst Limited	August 1999	Nasal drug delivery compositions containing nicotine
US 5804212	Danbiosyst Limited	September 1998	Small particle compositions for intranasal drug delivery
WO 9640277	Brown University Research Foundation	December 1996	Spray dried polymeric microparticles containing imaging agents

of absorption barriers, and a number of pre-corneal elimination factors (Saettone et al., 1999). These elimination factors include drainage of instilled solutions, lacrimation and tear turn over, drug metabolism, tear evaporation and possible binding to lachrymal proteins. To prolong the residence time of drugs in the pre-ocular area, BDDS have been developed taking advantage of the presence of a mucin–glycocalyx domain in the external portion of the eye.

Various BDDS employed for ocular delivery of drugs include the semisolids, viscous liquids, solids/inserts and the particulate DDS including bioadhesive microspheres and liposomes. The advantages of microspheres, i.e. increased residence time and decreased frequency of administration were quite evident with chitosan microspheres of Acyclovir (Genta et al., 1997) and methyl prednisolone loaded hyaluronic acid microspheres (Kyyronen et al., 1992). Acyclovir loaded chitosan microparticles showed an increased drug bioavailability in the eye as compared to the drug administered alone. Genta et al. (1997) reported an approximately four times increase in the aqueous humour concentration of suspension (39.37 μg/ml min) after a single instillation into rabbit's eye. Increase in levels and the prolonged release of Acyclovir from bioadhesive microspheres can be used to overcome the inconvenience caused by frequently applied ointments. 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) (Kyyronen et al., 1992). Methyl prednisolone was either 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.

Clearance of microspheres which significantly limits their residence time in the ocular cavity is a direct function of the pH and hydration state of microspheres and follows a biphasic process with an initial rapid clearance followed by a much slower basal phase. Initial clearance phase is independent of pH and hydration state while the basal phase clearance values varies with these factors. Durrani et al. (1995) investigated the effect of these parameters on the pre-corneal clearance of In^{111}-labelled microspheres prepared using Carbopol 907. Clearance of microspheres administered in dry form was faster than in the hydrated form, probably due to incomplete hydration in the tear fluid. The in vivo slow basal phase clearance constants were found to be 0.007 and 0.034 min^{-1} for the suspension of microspheres at a pH of 5.0 and 7.4, respectively. At pH 5, presence of protonated carboxyl groups permits enhanced adhesion due to hydrogen bonding between the polymer and mucin strands resulting in reduced clearance values.

6.1.2. Nasal

The nasal cavity offers a large, highly vascularised subepithelial layer for efficient absorption. Also, blood is drained directly from nose into the systemic circulation, thereby avoiding first pass effect (Soane et al., 1999). However, nasal delivery of drugs has certain limitations due to the mucociliary clearance of therapeutic agents from the site of deposition resulting in a short residence time for absorption. Use of BDDS increases the residence time of formulations in nasal cavity thereby improving absorption of drugs. It has been shown (Illum et al., 1987) by gamma scintigraphy study that radiolabelled microspheres made from diethyl amino ethyl dextran (DEAE–dextran), starch and albumin are cleared significantly more slowly than solutions after nasal administration in human volunteers. Hence, it was suggested by Illum et al. that the intranasal application of bioadhesive microspheres (in powder form) causes them to swell on coming in contact with the nasal mucosa to form a gel and decrease their rate of clearance from the nasal cavity, thereby providing poorly absorbed drugs a longer time for absorption.

The excellent absorption enhancing properties of bioadhesive microspheres are now being used extensively for both low molecular weight as well as macromolecular drugs like proteins. Nasal cavity as a site for systemic drug delivery has been investigated extensively and many nasal formulations have already reached commercial status including leutinising hormone releasing hormone (LHRH) and calcitonin (Illum, 1999).

Chitosan and starch are the two most widely employed bioadhesive polymers for nasal drug delivery. It has been reported that the clearance half-life was 25% greater for chitosan microspheres than for starch microspheres. This may be due to the differences in the surface charge, molecular contact and flexibility of

two polymers. Chitosan exerts a transient inhibitory effect on mucociliary clearance of the bioadhesive formulations. The concept of using a bioadhesive delivery system in the form of degradable starch microspheres (DSM) for nasal delivery of drugs was introduced in 1988. DSM system when combined with absorption enhancers, such as lysophosphatidylcholine (LPC), successfully improved the nasal absorption of gentamicin (Illum et al., 1988). The bioavailability of gentamicin was increased to 10% with the use of bioadhesive microspheres and was further increased to 57% by the addition of LPC to microsphere formulation. The DSM/LPC system has also been proposed as an efficient method for delivery of insulin into the systemic circulation via nasal route (Farraj et al., 1990). A rapid and much higher absorption of the human growth hormone (hGH) has been observed when hGH was administered in the form of DSM/LPC system of microspheres (Illum et al., 1990).

Critchley et al. (1994) evaluated bioadhesive starch microspheres as a nasal delivery system for desmopressin, and observed significant improvement in the absorption of drug, both in terms of peak plasma levels and bioavailability. A five-fold increase in maximum plasma concentration (C_{max}) and a doubling of bioavailability was observed on addition of LPC in a concentration of 0.2% to the starch microspheres. Other bioadhesive microspheres used for nasal administration of peptides and proteins include the cross-linked dextran microspheres, which are water insoluble and water absorbable. Sephadex and DEAE–Sephadex were found to improve the nasal absorption of insulin, but to a lesser extent than the starch microspheres (Edman et al., 1992). Hyaluronic acid ester microspheres were used for the nasal delivery of insulin in sheep and the increase in nasal absorption was found to be independent of the dose of microspheres in the range of 0.5–2.0 mg/kg (Illum et al., 1994).

6.1.3. Vaginal

The vaginal route has been frequently used for delivery of therapeutic and contraceptive agents to exert a local effect (antifungal, spermicidal) and for the systemic delivery of drugs (Richardson et al., 1996). It has been used for the delivery of drugs, which are susceptible to gastrointestinal degradation or hepatic metabolism following peroral delivery. For example, oestrogens and progestogens for the treatment of postmenopausal symptoms and for contraception. This route has also been explored for the delivery of therapeutic peptides, e.g. calcitonin and for microbicidal agents to help prevent the transmission of human immuno-deficiency virus and other sexually transmitted diseases (STDs).

Absorption of peptides from the vagina can be increased by using absorption enhancers, e.g. surfactants and bile salts. The adverse effects of absorption enhancers on the mucosal integrity can however be bypassed by employing bioadhesive microspheres within the vaginal cavity. The advent of newer polymers known as HYAFF, produced by the chemical modification of hyaluronic acid, have opened new avenues for vaginal delivery of drugs from bioadhesive microspheres. HYAFF microspheres have been successfully used for the incorporation of peptides such as nerve growth factor (Ghezzo et al., 1992) and salmon calcitonin. HYAFF microspheres have demonstrated good bioadhesive properties both in vitro and in vivo. In an unconscious rat model, these microspheres maintained contact with the vaginal epithelium for at least 6 h after administration. Hypocalcemic effects in the rat and sheep confirmed that absorption of salmon calcitonin was increased after administration of bioadhesive (HYAFF) microspheres as compared with an aqueous solution of calcitonin (Richardson and Armstrong, 1999). HYAFF microspheres due to their high biocompatibility and controllable degradation rate have been used for the localised drug delivery of steroids, analgesics, anti-inflammatory and anti-infectives. This has led to a great deal of enthusiasm in the development of safe and effective bioadhesive vaginal contraceptive and anti-infective formulations to control pregnancy and help prevent the spread of sexually transmitted diseases (Richardson et al., 1996).

6.2. Oral

6.2.1. Buccal

The oral cavity, besides being highly accessible, has a highly permeable mucosa with rich blood supply which shows short recovery times after stress or damage. Furthermore, oral transmucosal drug delivery bypasses the first pass effect and avoids pre-systemic elimination in the GI tract. These factors make the oro-mucosal cavity a very attractive and feasible site

for systemic drug delivery (Harris and Robinson, 1992). Composition of the oral epithelium varies depending on the site in the oral cavity. The areas exposed to mechanical stress (the gingivae and hard palate) are keratinised similar to the epidermis. The mucosae of the soft palate, the sublingual, and the buccal regions, however, are not keratinised. The keratinised epithelia contain neutral lipids like ceramides and acylceramides, which have been associated with the barrier function. It is estimated that the permeability of the buccal mucosa is 4–4000 times greater than that of the skin. In general, the permeabilities of the oral mucosa decrease in the order of sublingual greater than buccal, and buccal greater than palatal. The daily salivary volume secreted in humans is between 0.5 and 2 l, which is sufficient to hydrate oral mucosal dosage forms. This water rich environment of the oral cavity is the main reason behind the selection of hydrophilic polymeric matrices as vehicles for oral transmucosal drug delivery systems.

Vyas and Jain (1992) prepared polymer grafted starch microspheres bearing Isosorbide dinitrate and evaluated their potential as sustained release buccal bioadhesive system both by in vitro release studies and in vivo absorption studies. Starch microspheres grafted with polymethyl methacrylate (PMMA) exhibited relatively slow drug release as compared to polyacrylate (PAA) grafted microspheres. Moreover, the C_{max} and AUC recorded for the acrylic acid grafted starch microspheres were found to be more than that for PMMA grafted starch microspheres. It has been revealed by the in vivo absorption studies that steady state plasma levels can be maintained above the minimum effective concentration (MEC) over a period of 12 h after buccal administration of the grafted microspheres.

6.2.2. Gastrointestinal (GI)

The development of peroral controlled release DDS has been hindered by the inability to restrain and localise the drug delivery system in selected regions of the gastrointestinal tract (GIT). BDDS form an important approach to decrease the GI transit of drugs. Drug properties especially amenable to bioadhesive formulations include a relatively short biological half-life of about 2–8 h, a specific window for the absorption of drug by an active, saturable absorption process and small absorption rate constants (Longer et al., 1985).

The GI epithelium consists of a single layer of simple, columnar epithelium lying above a collection of cells called the lamina propria and supported by a layer of smooth muscle known as the muscularis mucosae. The cells are held together by tight junctions or the zona occludens. A special type of GI epithelium the Peyer's patches (PP) of the gut-associated lymphoid tissue (GALT) is also present. The PP is lined by a specialised epithelium, the follicle-associated epithelium, containing microfold (M) cells, which have the ability to phagocytise antigens in the intestine. Polymeric microspheres can also be phagocytised by these microfold cells and hence can be used for vaccination purposes (Carino et al., 1999).

Specially engineered polymeric bioadhesive microspheres can traverse both the mucosal absorptive epithelium and follicle-associated epithelium covering the lymphoid tissues of Peyer's patches depending on the particle size, polymer composition and the surface charge of bioadhesive microspheres (Mathiowitz et al., 1997). Bioerodible bioadhesive microspheres have been reported to increase the peroral bioavailability of dicumarol, insulin and have been investigated for peroral gene delivery (Mathiowitz et al., 1999). The increased bioactivity of insulin and the plasmid DNA can be accounted to the uptake of microspheres by 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. Bioadhesive microspheres by keeping the drug in the region proximal to its absorption window allow targeting and localization of the drug at a specific site in the GIT. Plasma concentration of the active metabolite of a prodrug Delapril hydrochloride was reported to be sustained after oral administration of bioadhesive microspheres based on polyglycerol esters of fatty acids (PGEFs). AUC after administration of microspheres was found to be same as that of the solution, while the mean residence time (MRT) of drug in the form of microspheres was prolonged (Akiyama et al., 1994).

An adhesive micromatrix system (AD-MMS), a novel formulation approach, reported by Akiyama and Nagahara (1999) consists of the drug and an adhesive polymer dispersed in a spherical matrix of the PGEFs, 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 PGEFs. 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. The MRT values after PGEF microspheres and the AD-MMS administration were found to be 6.1 ± 0.6 and 6.7 ± 0.7 h, respectively. While the AUC (0–24 h) after AD-MMS administration (11.57 ± 1.84 μg h/ml) was 1.8 times that of the PGEF microsphere (6.56 ± 0.93 μg/ml). The results could be explained to be due to the adherence of the AD-MMS to a more proximal area of the GIT rather than the absorption window and furosemide was thereby effectively absorbed from the absorption window (Akiyama et al., 1998). AD-MMS containing amoxicillin have been evaluated against the amoxicillin suspension for *Helicobacter pylori* clearance in vivo using Mongolian gerbils as the animal model. A 10 times greater anti *H. pylori* activity after oral administration of AD-MMS as compared to the amoxicillin suspension has been reported which could be due to the difference in gastric residence provided by the two dosage forms (Nagahara et al., 1998). Amoxicillin AD-MMS adheres to the infected mucosa and thereby provides a higher *H. pylori* eradication rate.

6.2.3. Colon

Colon drug delivery has been used for molecules aimed at local treatment of colonic diseases and for delivery of molecules susceptible to enzymatic degradation such as peptides. The mucosal surface of colon resembles that of the small intestine at birth but changes with age causing the loss of villi leaving a flat mucosa with deep crypt cells. Therefore the absorptive capacity of the colon is much less as compared to small intestine. The mucus layer provides not only a stable pH environment but also acts as a diffusion barrier for the absorption of drugs. Mucus production is more in the elderly as the number of mucous secreting goblet cells increase with age. Colonic mucosal environment is also effected by the colonic microflora as they degrade the mucins.

Bioadhesive microspheres can be used during the early stages of colonic cancer (when systemic prevention of possible metastasis in the blood is still not necessary), for enhancing the absorption of peptide drugs and vaccines, for the localised action of steroids, and drugs with a high hepatic clearance, e.g. budesonide and for the immunosuppressive agents such as cyclosporine.

Colon-specific bioadhesive microspheres can be used for protection of peptide drugs from the enzyme rich part of the GIT and to release the biologically active drug at the desired site for its maximum absorption. The absorption efficiency of Vancomycin by colonic placement of the bioadhesive microspheres was found to be equivalent to absorption of the peptide without absorption enhancers (Geary and Schlameus, 1993). However, insulin was found to be absorbed well in the colon only in the presence of absorption enhancers, e.g. EDTA salts, which cause chelation of calcium ions present in the tight junctions and hence opening of water channels in the cell membranes.

Some of the applications are relatively difficult to classify on the basis of route of administration and are discussed in the following section.

6.3. Miscellaneous applications

6.3.1. Vesicular delivery

The mucosal layers in the urinary bladder are different from both small as well as large intestine with regards to their structure and thickness. The vesical mucus contains oligosaccharides–glycosaminoglycans (GAG) that carry a large number of sulfate groups and thus a high negative charge density. Despite these differences there are certain similarities between the mucus layers in urinary bladder and intestine as they both contain sugar chains completely or partly attached to proteins (Bogataj et al., 1999). Therefore it is expected that polymers, which show good mucoadhesive strength on the intestinal mucosa, will exhibit some mucoadhesiveness on the vesical mucosa as well. Bogataj et al. (1999) evaluated various polymers for the mucoadhesion strength, swelling and drug release from bioadhesive microspheres applied into the urinary bladder. It has been reported that the microspheres containing carboxy methyl cellulose (CMC) as mucoadhesive agent and Eudragit RL as matrix polymer provided the longest release time from microspheres and showed high strength of mucoadhesion.

6.3.2. Mucosal immunization

The majority of pathogens initially infect their hosts through mucosal surfaces, induction of mucosal immunity is therefore likely to make an important contribution to the protective immunity. Moreover, mucosal administration of vaccine avoids the use of needles and is thus an attractive approach for development of new generation vaccines. Current research in vaccine development has focused on treatment requiring a single administration, since the major disadvantage of many currently available vaccines is that repeated administrations are required. The ability to provide controlled release of antigens through bioadhesive microspheres has given an impetus to research in the area of mucosal immunization. Intravaginal immunization has been tried in sheep using DSM and LPC for the influenza virus haemagglutinin (TOPS) (O'Hagan et al., 1993). The highest levels of antibodies were detected after i.m. injection than after intravaginal immunization since the vagina unlike intestine, lungs and nasal cavity has no aggregates of lymphoid tissue within the epithelium. The HYAFF bioadhesive microspheres in the presence of a mucosal adjuvant-LTK 63 administered intranasally are reported to induce a significantly enhanced serum IgG antibody response in comparison to intramuscular immunization with haemagglutinin obtained from influenza A virus (Singh et al., 2001). Antigen-microsphere formulations prepared by adsorbing the antigen onto preformed polymeric hydrogel microspheres can be used to provide enhanced immune responses in animals. Polyphosphazene microspheres with adsorbed influenza antigen and tetanus toxoid can be administered intranasally to have increased immune responses (Payne et al., 2001).

6.3.3. Protein and peptide drug delivery

Protein and peptide drugs offer formidable challenges for peroral delivery due to their relatively large size, enzymatic degradation and very low permeability across the absorptive epithelial cells. Bioadhesive microspheres provide an interesting non-invasive patient compliant approach to improve the absorption of these drugs. The luminal enzymatic degradation of proteins and peptides can be effectively minimised by direct contact with the absorptive mucosa and avoiding exposition to body fluids and enzymes. Specific enzyme inhibitors can be attached to the surface of bioadhesive microspheres (Bernkop-Schnurch and Dundalek, 1996). Moreover, certain polymers, e.g. chitosan have been reported to possess permeability enhancing properties. Senel et al. (2000) observed a six- to seven-fold enhancement of permeability by chitosan for the bioactive peptide TGF-β to which the oral mucosa was reported to be relatively impermeable. This permeability enhancing effect can be attributed to the transient opening of the tight junctions in the cell membranes or due to an increase in the thermodynamic activity of penetrant or due to the ability of chitosan to disrupt the lipid organization of the cellular membranes. Microspheres prepared with polyacrylic acid derivatives can chelate the extracellular calcium ions in vivo and hence reduce the integrity of tight junctions, which results in a permeability enhancing effect (Borchard et al., 1996). Polyacrylates can also inhibit the proteolytic enzymes present in the GIT by binding to the essential enzyme cofactors, such as calcium and zinc ions, resulting in a conformational change of enzyme and loss of its activity (Luessen et al., 1995).

7. Evaluation of the bioadhesive microspheres

The best approach to evaluate bioadhesive microspheres is to evaluate the effectiveness of mucoadhesive polymer to prolong the residence time of drug at the site of absorption, thereby increasing absorption and bioavailability of the drug. The methods used to evaluate bioadhesive microspheres include the following.

7.1. Measurement of adhesive strength/in vitro tests

The quantification of the bioadhesive forces between polymeric microspheres and the mucosal tissue is a useful indicator for evaluating the bioadhesive strength of microspheres. In vitro techniques have been used to test the polymeric microspheres against a variety of synthetic and biological tissue samples, such as synthetic and natural mucus, frozen and freshly excised tissue etc. The different in vitro methods include the following.

7.1.1. Tensile stress measurement

7.1.1.1. Wilhelmy plate technique. The Wilhelmy plate technique is traditionally used for the measurement of dynamic contact angles and involves the

use of a microtensiometer or a microbalance. The CAHN dynamic contact angle analyser (model DCA 322, CAHN instruments, Cerritos) has been modified to perform adhesive microforce measurements. The DCA 322 system consists of an IBM compatible computer and a microbalance assembly (Chickering et al., 1999). The microbalance unit consists of stationary sample and tare loops and a motor powered translation stage. The instrument measures the bioadhesive force between mucosal tissue and a single microsphere mounted on a small diameter metal wire suspended from the sample loop in microtensiometer (Santos et al., 1999). 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 min, at which time the mobile stage is lowered and the resulting force of adhesion between the polymer and mucosal tissue is 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 software system, three essential bioadhesive parameters can be analysed. These include the fracture strength, deformation to failure and work of adhesion.

- *Fracture strength*: It is the maximum force per unit surface area required to break the adhesive bond.
- *Deformation 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.
- *Work of adhesion*: It is a function of both the fracture strength and the deformation to failure. It tends to be the strongest indicator of the bioadhesive potential.

This technique allows the measurement of bioadhesive properties of a candidate material in the exact geometry of the proposed microsphere delivery device and the use of a physiological tissue chamber mimics the in vivo conditions. From a single tensile experiment, 11 bioadhesive parameters can be analysed out of which 3 are direct predictors of the bioadhesive potential (Chickering and Mathiowitz, 1995).

The CAHN instrument, although a powerful tool has inherent limitations in its measurement technique, makes it better suited for large microspheres (with a diameter of more than 300 μm) adhered to tissue in vitro. Therefore, many new techniques have been developed to provide quantitative information of bioadhesive interactions of the smaller microspheres.

7.1.1.2. Novel electromagnetic force transducer (EMFT). The EMFT is a remote sensing instrument that uses a calibrated electromagnet to detach a magnetic loaded polymer microsphere from a tissue sample (Hertzog and Mathiowitz, 1999). It has the unique ability to record remotely and simultaneously the tensile force information as well as high magnification video images of bioadhesive interactions at near physiological conditions. The EMFT measures tissue adhesive forces by monitoring the magnetic force required to exactly oppose the bioadhesive force. To test a microsphere, it must first be attached to the sample of tissue; magnetic force is then generated by an electromagnet mounted on the microscope vertically above the tissue chamber. After the computer has calculated the position of microsphere, the tissue chamber is slowly moved down, away from the magnet tip. As the tissue slowly descends away from the magnet, the video analysis continuously calculates the position of microsphere until the latter is completely pulled free of the tissue. The computer can display the results either as raw data or convert it to a force versus displacement graph. The primary advantage of the EMFT is that no physical attachment is required between the force transducer and the microsphere. This makes it possible to perform accurate bioadhesive measurements on the small microspheres, which have been implanted in vivo and then excised (along with the host tissue) for measurement. This technique can also be used to evaluate the bioadhesion of polymers to specific cell types and hence can be used to develop BDDS to target-specific tissues.

7.1.2. Shear 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 (Kamath and Park, 1994). 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 (1990) 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 behaviour of the microparticle (Chickering and Mathiowitz, 1995).

7.1.3. Other tests to measure the adhesive strength

7.1.3.1. Adhesion number. Adhesion number for bioadhesive 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.

7.1.3.2. Falling 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 microspheres is allowed to flow down the intestinal strip. Particle concentrations entering 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 bioadhesion (Teng and Ho, 1987).

7.1.3.3. Everted sac technique. The everted intestinal sac technique is a passive test for bioadhesion 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 and saline, and agitated while incubating for 30 min. Sac is then removed, microspheres are washed and lyophilised, 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 is 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 bioadhesion in a very similar manner. Santos et al. (1999) established a correlation between the two in vitro bioadhesion assay methods which thereby allows one to confidentially utilise a single bioadhesion assay to scan a variety of bioadhesive polymers.

7.1.4. Novel rheological approach

The rheological properties of the mucoadhesive interface (i.e. of the hydrated gel) are influenced by the occurrence of interpenetration step in the process of bioadhesion. Chain interlocking, conformational changes and the chemical interaction, which occur between bioadhesive polymer and mucin chains, produce changes in the rheological behaviour of the two macromolecular species. The rheological studies provide an acceptable in vitro model representative of the in vivo behaviour of mucoadhesive polymers (Riley et al., 2001).

Due to intermolecular interactions between the two polymers (mucin and the bioadhesive polymer), experimentally measured viscosity of the mixture is generally higher than the viscosity calculated as a weighted average of the viscosities of the individual components. Thus, the magnitude of the intermolecular interactions can be quantitated by the relative change of the solution viscosity. A synergistic increase in the viscosity of the gastric mucus glycoprotein has been observed with polyacrylates, which thereby re-inforce the gastroduodenal mucus. It has been reported that an optimum polymer concentration is required for rheological synergy to be evident, above which any synergy is masked by the rheological properties of the polymer alone. The effect of pH on the mucus/polymer rheological synergism of polyacrylates has been examined using dynamic oscillatory rheology (Madsen et al., 1998). It has been shown that an optimum mucus polymer interaction occurs not only at the pK_a value but also at the pH regimes unique to each poly-

mer type, being influenced by the hydrogen-bonded interactions.

7.2. Measurement of the residence time/in vivo techniques

Measurements of the residence time of mucoadhesives at the application site provide quantitative information on their mucoadhesive properties. The GI transit times of many bioadhesive preparations have been examined using radioisotopes and the fluorescent labelling techniques.

7.2.1. GI transit using radio-opaque microspheres

It is a simple procedure involving the use of radio-opaque markers, e.g. barium sulfate, encapsulated in bioadhesive microspheres to determine the effectss of bioadhesive 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 labelled with Cr-51, Tc-99m, In-113m, or I-123 have been used to study the transit of the microspheres in the GI tract (Mathiowitz et al., 1999).

7.2.2. Gamma scintigraphy technique

Distribution and retention time of the bioadhesive intravaginal microspheres can be studied using the gamma scintigraphy technique. A study has reported the intensity and distribution of radioactivity in the genital tract after administration of technetium labelled HYAFF 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 bioadhesive-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 (Richardson et al., 1996).

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 BDDS, including microbicides.

7.3. Surface characterization of the bioadhesive microspheres

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 bioadhesive properties, the microsphere samples are lyophilised and analysed under SEM at 150× and 1000×. The smooth texture of the microsphere surface leads to weak bioadhesive properties, while the coarser surface texture improves the adhesion through stronger mechanical interactions. The morphological surface 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 (Mathiowitz et al., 1999).

8. Conclusion

Bioadhesive microspheres offer unique carrier system for many pharmaceuticals and can be tailored to adhere to any mucosal tissue, including those found in eyes, oral cavity and throughout the respiratory, urinary and gastrointestinal tract. The bioadhesive microspheres can be used not only for controlled release but also for targeted delivery of the drugs to specific sites in body. Recent advances in medicine have envisaged the development of polymeric drug delivery systems for protein/peptide drugs and gene therapy. These challenges put forward by the medicinal advances can be successfully met by using increasingly accepted polymers, e.g. HYAFF, polyacrylates, chitosan and its derivatives, polyphosphazenes, etc. Many studies have already been undertaken for exploring the prospects of bioadhesive microspheres in gene therapy, delivery of peptides (insulin, calcitonin, and desmopresin), localised and targeted release of antitumour agents and mucosal vaccination (influenza vaccine).

Although significant advances have been made in the field of bioadhesives, there are still many challenges ahead in this field. Of particular importance is the development of universally acceptable stan-

dard evaluation methods and development of newer site directed polymers. Efforts have been initiated on these lines in the form of novel EMFT techniques for evaluation of bioadhesion strength of microspheres to specific cell types. Polymeric science needs to be explored to find newer bioadhesive polymers with the added attributes of being biodegradable, biocompatible, bioadhesive for specific cells or mucosa and which could also function as enzyme inhibitors for the successful delivery of proteins and peptides. A multidisciplinary approach will therefore be required to overcome these challenges and to employ bioadhesive microspheres as a cutting edge technology for site targeted controlled release drug delivery of new as well as existing drugs.

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