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ORAL MUCOADHESIVE MICROCARRIERS FOR CONTROLLED AND EXTENDED RELEASE FORMULATIONS

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ABSTRACT

Nowadays oral mucoadhesive microcarriers were exploited extensively to improve the performance of delivery system and the patient compliance through controlling and extending release profile of drug. Present review work is aimed to explore the aspects of mucoadhesives, development and evaluation techniques of mucoadhesive microcarriers that will aid in designing an efficient extended release oral mucoadhesive microcarrier system. Data revealing several aspects like advantages, development and evaluation techniques of microcarrier systems; and properties of mucoadhesives influencing development and performance of microcarriers; were collected from databases, compiled and analysed. Presented data will help pharmaceutical scientists engaged in designing dosage forms for enhancing the bioavailability of drug(s) and performance of delivery system. Oral mucoadhesive microcarriers were having potentiality for controlling and extending release profile so as to improve performance and patient compliance.

Keywords: Controlled, extended, microcarrier, mucoadhesion, mucoadhesive, oral.

1. INTRODUCTION

Historically oral route of administration had been used for both conventional as well as novel drug delivery systems, was still preferred due to declared advantages but fails to restrain and localize the delivery system in gastrointestinal (GI) tract (Khan GM. 2001). However, performance of oral conventional dosage form can be improved with controlled/extended-release or targeted-delivery products (Chang RK and Robinson JR, 1982). Microcarrier system based controlled/extended-release (C/E-R) formulation were generally intended for oral and topical use (Benita S. 1996); modulates the release and the absorption characteristics of the drug(s), to achieve CR and drug targeting; while their short GI retention/transit time decreases their performance and success (Helliwell M. 1993) that can be improved by coupling mucoadhesion characteristics to the microcarriers (Costa MS and Margarida Cardoso MM, 2006). This review is aimed to provide information on mucoadhesion, mucoadhesive materials, and properties of mucoadhesives influencing performance and development of mucoadhesive microcarriers; and preparation and evaluation method of mucoadhesive microcarrier system.

2. OVERVIEW OF ORAL DRUG DELIVERY SYSTEMS

Historically oral route is preferred due to the ease of administration; the self- medication; the improved

patient compliance; their compact nature, stability and low cost; and the ease of packaging, transports, and manufactures (Banker GS and Anderson NR, 1987). Administration of oral conventional dosage forms in multiple daily dosing produces wide ranging fluctuations in drug concentration in blood stream and tissues with consequent undesirable toxicity and poor performance associated with non-adherence to dosage regimen (Vyas SP and Khar RK, 2002), and also had short-term limitations due to their inability to restrain and localise the delivery system in GI tract (Khan GM. 2001); however their performance can be improved with C/E-R or targeted-delivery products (Chang RK and Robinson JR, 1982) that excellently control drug levels in plasma; minimises dosing frequency; improves patient convenience/compliance, safety margin and efficacy; reduces intensity of local or systemic side effects, health care costs, and expenses and complications involved in marketing new drug entities; and many more to list (Belgamwar V et al. 2009). The real hurdle in the development of oral C/E-R drug delivery systems was to control release profile over extended period of time by special technological construction (Lee TW and Robinson JR, 2000); and to improve short GI retention/residence time or GI transit time, associated with the rapid GI transit phenomenon of the GI tract, that diminishes the extent of absorption of drug associated with diminished exposure time of the delivery system at the absorption site (Helliwell M. 1993) which in turn limits the duration of action to approximately 8-12 hours. The GI retention time of solid dosage forms may be improved by the mechanisms of mucoadhesion (Arya RKK et al. 2010), flotation (Hoffman A and Stepensky D, 1999), sedimentation (Singh BN and Kim KH, 2000), or by expansion (Vasir JK et al. 2003). Due to convenience and safety oral C/E-R mucoadhesive system was widely exploited (Costa MS and Margarida Cardoso MM, 2006).

From early 1980s mucoadhesive polymers was introduced to be used for developing controlled drug delivery system so as to improve their performance and since then this had became a thrust area of research for pharmaceutical scientists (Kamath KR and Park K, 1992; Mathiowitz E et al. 1999b; Vasir JK et al. 2003; Arya RKK et al. 2010) exploring the fundamental aspects of mucoadhesion and the potential application of mucoadhesive dosage forms for improving and enhancing the bioavailability of drug(s).

Microcarrier system based delivery systems offers an well-informed approach for drug delivery where the drug particles are impinged entrenched either in a polymeric or proteinic matrix network in a solid aggregated state or in a molecular dispersion (Nur AO and Zhang JS, 2000) resulting carrier particle such as pellets, beads, microcapsules, microspheres, lipospheres, etc. which modulates drug(s) release and absorption characteristics; had been accepted as a process to achieve CR and targeting of drug (Benita S. 1996). Short GI retention/transit time of microcarriers (Helliwell M. 1993), limiting their performance and success, can be improved by conjugating mucoadhesion characteristics to it (Costa MS and Margarida Cardoso MM, 2006; Asane GS et al. 2008) and developing microcarriers with mucoadhesive property was referred as “mucoadhesive microcarrier system” (Chowdary KPR and Rao YS, 2004) which may simultaneously localise delivery system in selected regions of the GI tract.

3. MUCOADHESION AND BIOADHESION

Mucoadhesion/bioadhesion was defined as the state in which two materials, at least one is biological in nature, held together for an extended period of time by interfacial forces (Good RJ. 1976), alternately it was defined as the ability of a material (synthetic or biological) to adhere to a biological tissue for an extended period of time (Peppas NA and Buri PA, 1985; Jiménez-castellanos MR et al. 1993). Bioadhesion involves adhesion of the polymer with the biological membrane while mucoadhesion involves adhesion of the polymer with the mucus membrane.

4. MECHANISM OF MUCOADHESION

In solid systems, mucoadhesion was believed to occur in following few steps. First stage involves an intimate contact between a mucoadhesive polymer and a membrane, either from good wetting of the surface of mucoadhesive or from the swelling

of the mucoadhesive. In the second stage, after contact was established, penetration of the mucoadhesive into the crevice of the tissue surface or interpenetration of the chains of the mucoadhesive with those of the mucous takes place. Third stage involves entanglements and the formation of secondary chemical bonds between the polymer chain and the mucin molecules (Ducheňe D et al. 1988; Leung SHS and Robinson JR, 1990).

5. THEORIES OF MUCOADHESION

Theories that had been adapted to study the mucoadhesion were as follows (Ducheňe D et al. 1988; Chickering DE et al. 1999; Lee JW et al. 2000; Shaikh R et al. 2011).

5.1. Wetting theory

The wetting theory was based upon prediction of the intimate contact between the mucoadhesive polymer and the mucous leading to dispelling of barrier substances, spreading, and subsequent adhesion, in liquid state, utilising interfacial tension. This theory involves calculation of the contact angle and the thermodynamic work of adhesion; and the work done related to the surface tension of both the adhesive and the substrate, calculated with the Dupre's equation (Pritchard WH. 1970), the horizontal resolution of the forces with the Young equation, and the spreading coefficient (S_b).

Dupre's equation: $\omega_A = \gamma_b + \gamma_\tau - \gamma_{bt}$
Young equation: $\gamma_{ta} = \gamma_{bt} + \gamma_{ba}\mathrm{COS}\theta$
Spreading coefficient: $S_b = \gamma_{ta} - \gamma_{bt} - \gamma_{ba}$

Where ω_A was the specific thermodynamic work of adhesion and γ_b represents the surface tensions of the bioadhesive polymer, γ_τ represents the surface tension of the substrate, γ_{bt} represent the interfacial tension between the tissue and polymer, θ represent the angle of contact, γ_{ba} represent the interfacial tension between polymer and air, and γ_{ta} represent the interfacial tension between tissue and air.

Young equation state that wetting will be complete if θ will approach zero, that was the vector γ_{ta} greatly exceeds $\gamma_{bt} + \gamma_{ba}$; while a θ vale greater than zero will result in incomplete wetting. In order to achieve adhesion of mucoadhesives to a biological membrane spreading coefficient should be positive (Jasti B et al. 2003) that is bioadhesion is favoured by large values of γ_{ta} or by small values of γ_{bt} and γ_{ba} (Wake WC. 1982).

5.2. Adsorption theory

According to adsorption theory mucoadhesion results from secondary molecular interactions like electrostatic attraction, hydrophobic interactions, hydrogen bonds, van der Waals forces, or other related forces (Jasti B et al. 2003); associated with re-orientation of polar molecules or groups at the interface or with chemisorptions (Wake WC. 1982).

5.3. Electronic theory

This theory assumes that, mucoadhesion occurs from the formation of an electric double layer at the mucoadhesive interface by the transfer of electrons between the mucin glycoprotein network and the mucoadhesive polymer (Derjaguin BV and Smilga VP, 1967).

5.4. Diffusion theory

The diffusion theory states that interpenetration of the chains of polymer and mucin to a sufficient depth results from the existing concentration gradients and consequential interpenetration; until an equilibrium penetration depth was achieved, in the range of 0.2 to 0.5 μm (Ducheňe D et al. 1988); creates a semi-permanent bond through entanglement and mechanical interlocking between mucin and mucoadhesives (Jasti B et al. 2003). The mean diffusional depth (S) of the bioadhesive polymer segments can be calculated from contact time (t) and diffusion coefficient value (D) with the following relation (Peppas NA and Buri PA, 1985).

$$S = (2tD)\ ½$$

But the time to bioadhesion of a particular polymer (t) can be calculated from the diffusion coefficient of a bioadhesive through the substrate value (D_b) and the interpenetrating depth (l), with the following relation (Peppas NA and Buri PA, 1985).

$$t = l^2/D_b$$

5.5. Fracture theory

The fracture theory was based on analysis of the force required for the separation of two surfaces after adhesion (Chickering DE and Mathiowitz E, 1999) using tensile apparatus employing following relation that relates fracture strength (σ), fracture energy (ϵ), young modulus of elasticity (E) and critical crack length (L).

$$\sigma = (E \times \epsilon/L)\ ½$$

6. FACTORS AFFECTING MUCOADHESION

The adhesive bond between a bioadhesive system and mucin gel can be investigated in term of contribution of the following factors (Chickering D et al. 1996).

6.1. Polymer related factors

6.1.1. Concentration of active polymer

The polymer concentration was dependable on the physical state (solid/liquid) of the mucoadhesive drug delivery systems and an increase in the polymer concentration increases the mucoadhesive strength in solid dosage form while an optimum concentration in liquid system was required for best mucoadhesion (Ducheňe D et al. 1988; Vasir JK et al. 2003). In liquid systems, beyond the threshold concentration the coiled molecules become separated from the medium limiting availability of chain for interpenetration thereby dropping adhesive strength significantly.

6.1.2. Hydrophilicity

Numerous hydrophilic functional groups, like hydroxyl and carboxyl, of the bioadhesive polymers; aids swelling in aqueous media leading to maximal exposure of potential anchor sites and subsequent hydrogen bonding with the substrate (Shaikh R et al. 2011).

6.1.3. Spatial conformation

Along with molecular weight or chain length; spatial or helical conformation the polymer chain, that may shield many adhesively active groups responsible for adhesion in comparison to that with linear conformation; plays important role in the mucoadhesion (Jiménez-castellanos MR et al. 1993).

6.1.4. Molecular weight

Low-molecular-weight of polymer favours interpenetration of molecules while higher molecular weight favours entanglements. Type of the mucoadhesive polymer and the tissue determines the optimum molecular weight for maximum mucoadhesion (Kamath KR and Park K, 1992). The bioadhesive/mucoadhesive force increases with an increase in the molecular weight of polymer up to 100,000 and beyond this level there was not much effect (Roy S et al. 2009).

6.1.5. Flexibility of polymer chains associated with cross-linking and swelling

Flexibility was important for interpenetration and entanglement. As the cross linking density of water-soluble polymer increases; the mobility of the individual polymer chain decreases; and the effective length of the chain that can penetrate into mucous layer decreases even further consequently mucoadhesive strength decreases (Kamath KR and Park K, 1992; Chowdary KPR and Srinivas L, 2000). Too great degree of swelling results in slippy mucilage and can be easily removed from the substrate (McCarron PA et al. 2004). Polymers grafting onto the preformed network; and the inclusion of adhesion promoters in the formulation (free polymer); enhances mucoadhesion of cross-linked polymers (Peppas NA et al. 2000a).

6.2. Environment related factors

6.2.1. pH of polymer-substrate interface

The hydrogen ion concentration can influence charge on the surface of mucous, associated with dissociation of functional groups on the carbohydrate moiety and amino acids of polypeptide backbone; as well as certain ionisable mucoadhesive polymers. Studies depicted that the pH of the medium was important for the degree of hydration of cross linked polyacrylic acid that consistently increases from pH 4 through pH 7 and then decrease as alkalinity and ionic strength increases. Polycarbophil shows maximum adhesive strength at pH 3 that gradually decreases with an

increase in pH up to 5 and above pH 5 it does not show any mucoadhesive property (Kamath KR and Park K, 1992; Chowdary KPR and Srinivas L, 2000). Protonated carboxyl groups, rather than the ionised carboxyl groups, react with mucin molecules, apparently by the concurrent formation of numerous hydrogen bonds (Park H and Robinson JR, 1985).

6.2.2. Initial contact time

Initial contact time between the mucoadhesive and the mucus layer determines the extent of swelling and the interpenetration of polymer chains. An increase in initial contact time increases mucoadhesive strength (Chowdary KPR and Srinivas L, 2000).

6.2.3. Applied strength

The pressure initially applied on the solid bioadhesive system to apply on mucosal tissue can affect the depth of interpenetration, and the adhesive strength increases with an increase in the applied strength or with the density up to an optimum value (Kamath KR and Park K, 1992; Chowdary KPR and Srinivas L, 2000).

6.2.4. Secretion of the model substrate surface

Studies on the variability of biological substrate should be confirmed by examining properties like permeability, electro physiology, or histology etc., before and after performing the *in vitro* tests using tissues for the better *in vitro*/*in vivo* correlation (Vasir JK et al. 2003).

6.2.5. Swelling

Bioadhesion decreases with too great swelling that depends on the presence of water and on the polymer concentration. In order to achieve sufficient bioadhesion of the system, too early swelling must not occur (Kamath KR and Park K, 1992; Chowdary KPR and Srinivas L, 2000).

6.3. Physiological variables

6.3.1. Mucin turnover

The natural turnover of mucin molecules from the mucous layer not only limits the residence time of the mucoadhesive on the mucous layer but also released out soluble mucin molecules, in substantial amount, interacts with mucoadhesives before they have a chance to interact with mucous layer (Lehr CM. 1996). An increase in mucin turnover decrease mucoadhesion.

6.3.2. Disease state

In diseased conditions; like common colds, gastric ulcers, ulcerative colitis, cystic fibrosis, bacterial and fungal infections of the female reproductive tract, and inflammatory conditions of the eye; the physicochemical properties of the mucous changes. The mucoadhesive property needs to be evaluated, if mucoadhesives are intended to be used in the diseased state (Kamath KR and Park K, 1992; Chowdary KPR and Srinivas L, 2000).

7. CHARACTERISTICS OF AN IDEAL MUCOADHESIVE POLYMER

An ideal mucoadhesive polymer should stick quickly to most tissue, preferably form a strong non-covalent bond with the mucin-epithelial cell surfaces, possess some site-specificity, allow daily amalgamation of the drug and must not hinder its release, and should be inexpensive and non-irritant to the mucous membrane; should provide adequate stability and shelf-life to dosage form; and the polymer and its degradation products should be non-absorbable into the system and non-toxic (Jiménez-castellanos MR et al. 1993). The properties of the mucoadhesive drug delivery system like surface characteristics, force of mucoadhesion, release pattern of the drug, and clearance; are predisposed by the type of polymers used to organise them. Apposite polymer should be selected from soluble and insoluble; non-biodegradable and biodegradable; hydrogels or thermoplastic homopolymers, copolymers, or blends; and natural or synthetic polymers.

8. MUCOADHESIVE POLYMER

Mucoadhesives were the swellable or non-swellable, synthetic or natural polymers that upon hydration becomes adhesive (Nagai T and Machida Y, 1985) thereby network with the mucosal layer casing the mucosal epithelial surface and the mucin, in order to prolong the residence time of dosage form at the site of absorption/application so as to facilitate their intimate contact with the absorption surface

(Ikeda K et al. 1992; Chowdary KPR and Rao YS, 2004). Diverse classes of polymers including synthetic polymers like poly(acrylic acid), polyvinyl alcohol, polyamides, hydroxypropyl methylcellulose, poly(methylacrylate) derivatives, polycarbonates, polyalkylene glycols, polyvinyl ethers/esters/halides, methylcellulose, sodium carboxymethylcellulose, polymethylmethacrylic acid, and hydroxypropyl cellulose; biocompatible polymers like cellulose-based polymers, ethylene glycol polymers and its copolymers, oxyethylene polymers, polyvinyl alcohol, polyvinyl acetate, and esters of hyaluronic acid; biodegradable polymers like chitosan, polyorthoesters, polycaprolactones, poly(lactides), poly(glycolides), poly(lactide-co-glycolides), polyalkyl cyanoacrylates, polyphosphoesters, polyanhydrides, polyphosphazenes, polyethylene oxide; as well as naturally occurring polymers such as sodium alginate, pectin, tragacanth, gelatin, carrageenan have been investigated for their potentiality to be used as mucoadhesives.

8.1. Classification of mucoadhesive polymers

Basically there were three broad classes of mucoadhesive polymers (Roy S et al. 2009).

8.1.1. Thermoplastic polymers

Thermoplastic polymers include the non-erodable neutral polystyrene and semi crystalline bioerodable polymers that generate the carboxylic acid groups upon degradation, e.g. polyanhydrides and polylactic acid.

8.1.2. Hydrogels

Hydrogels were the class of polymeric biomaterial, usually a cross-linked water swellable polymer with limited swelling capacity, that swells by absorbing water and interacts by means of adhesion with the mucus that covers epithelia, e.g. poly(acrylic acid-co-acrylamide) copolymers, carrageenan, sodium alginate, guar gum, modified guar gum, etc. Amongst all bioadhesive polymeric hydrogels, poly(acrylic acid-co-acrylamide) had been considered to be superior mucoadhesive. But its higher transition temperature and higher interfacial free energy, does not let it to wet the mucosal surface to the optimal level, causing loose interpenetration and inter-diffusion of the polymer; thus was copolymerised with polyethylene glycol or polyvinylpyrrolidone to improve wetting properties (Peppas NA et al. 2000b).

8.1.3. Hydrophilic polymers

Hydrophilic polymers were the water-soluble polymers that swell indefinitely in contact with water and ultimately undergo complete dissolution; e.g. methylcellulose, carbomers, hydroxyethyl cellulose, hydroxypropyl methylcellulose, sodium carboxy methylcellulose, chitosan, plant gums, etc. Hydrophilic polymers containing carboxylic group (Hui HW and Robinson JR, 1985) that includes polyvinylpyrrolidone, methylcellulose, sodium carboxy methylcellulose, hydroxypropyl cellulose, and other cellulose derivative exhibits best mucoadhesive properties.

8.2. Novel mucoadhesive polymers under development

Copolymer of poly(acrylic acid-co-acrylamide) and PEG monoethylether monomethacrylate (PAA-co-PEG) (Shojaei AH and Li X, 1997); AB block copolymer of oligo(methyl methacrylate) and poly(acrylic acid-co-acrylamide) (Inoue T et al. 1998); PEGylated poly(acrylic acid-co-acrylamide) (Lele BS and Hoffman AS, 2000) and PEGylated polyvinylpyrrolidone (Cleary GW et al. 2003); and cysteine grafted (Bernkop-Schnürch A et al. 1999) were under extensive study.

9. SITE SPECIFIC BIOADHESIVES/ MUCOADHESIVES

Development of polymers and microcarriers grafted with mucus or cell-specific ligands increases therapeutic benefit, and provides site-specific drug delivery using any of the following ligands.

9.1. Lectins

Lectins were the proteins of non-immune origin that binds to carbohydrates specifically and non-covalently. Lectins could enhance penetration of drugs by improving adherence of microcarriers to the intestinal epithelium thereby (Lee JW et al. 2000), may be used for targeting drug to different gut components or even different cells like: complex-specific lectins for parietal cells (Lavelle EC. 2001) and GI tumour cells (Park K. 2009), morniga G

lectins for tumour-associated T/Tn antigen (Poiroux G et al. 2011), fucose-specific lectins for M cells (Jepson MA et al. 2004), *Aleuria aurantia* lectins for M cells (Roth-Walter F et al. 2004), polystyrene microcarriers coated with tomato lectin for enterocytes (Lehr CM et al. 1992b) and Peyer's patches (Woodley JF. 2000), wheat germ agglutinin conjugated poly(ethylene glycol)-poly(lactic acid) nanoparticles for the brain (Liu Q et al. 2010), and many more. The other useful lectin ligands comprises lectins isolated from: *Abrus precatroius*, (Olsnes S et al. 1974), *Agaricus bisporus* (Yu L et al. 1993), *Anguilla anguilla* (Gercken J and Renwrantz L, 1994), *Arachis hypogaea* (Avichezer D and Arnon R, 1996), *Bauhinia purpurea* (Allen HJ and Johnson EA, 1976), *Pandeiraea simplicifolia* (Goldstein IJ et al. 1981), *Phaseolus vulgaris* (Kruszewska D, 2003), and *Polygonatum cyrtonema* (Wang SY et al. 2011). Lectins from *Arachis hypogea*, *Lens culinaris*, *Dolichus biflorus*, *Solanum tuberosum* and *Triticum vulgare*, were having affinity for human colonocytes and monolayer-forming Caco-2 and HT-29 cells, were found to be stable on *in vitro* exposure to GI-located enzymes, and can be exploited in the development of lectin-mediated particulate pharmaceutical devices (Gabor F et al. 1997). Lectins for targeting the human carcinoma cell was intensively investigated as this cell lines exhibit higher lectin binding capacity than the normal colonocytes (Haas J and Lehr CM, 2002). Algal lectins from *Bryothamnion triquetrum* and *Bryothamnion seaforthii* for targeting human colon carcinoma cell (Pinto VP et al. 2009) are under extensive study.

9.2. Antibodies

Antibodies produced against selected molecules present on mucosal surfaces were of high specificity, could be well thought-out as a rational polymeric ligand for designing site-specific mucoadhesives (Singh M et al. 2001; Zhao X et al. 2009) and can be useful for targeting drugs to tumour tissues (Takeuchi H et al. 2001). Example: the hyaluronic acid esters bioadhesive microcarrier in the presence of a mucosal adjuvant-LTK 63 upon intranasal administration induces serum IgG antibody response. Polyphosphazene microcarrier with adsorbed influenza antigen and tetanus toxoid upon intranasal administration increases immune responses.

9.3. Bacterial adhesions

Mucoadhesive microcarriers based on fimbriae, were the long lectin like proteins found on the surface of many bacterial strains have correlation with pathogenicity (Brandsch M et al. 1995), could be employed for adhering it to epithelial surfaces (Lee JW et al. 2000).

9.4. Amino acid sequences

Amino acid sequences like Arg-Gly-Asp and others, that were the complementary part of the cell and mucosal surfaces, were attached to microcarriers for targeting specific cell surface glycoprotein (Huang Y et al. 2000).

10. Bioadhesive/mucoadhesive devices

Using mucoadhesives mucoadhesive devices were designed as an effort for extending systemic and local delivery of drugs through different mucosa, for targeting drug to a particular region of the body or GI tract (Kamath KR and Park K, 1992; Vasir JK et al. 2003), and to improve and enhance the bioavailability of drugs (Nagai T et al. 1984; Ilium L et al. 1988; Ikeda K et al. 1992; Chowdary KPR and Srinivas L, 2000). Studies reported; mucoadhesive drug delivery system in the form of tablets, films, patches, and gels for oral, buccal, nasal, ocular, vaginal, rectal, and topical routes (Chowdary KPR and Rao YS, 2004); adhere to the mucosal layer lining the GI tract, the urogential tract, the airways, the ear, the nose and the eye.

10.1. Advantages of mucoadhesive systems

Immobilization of drug delivery system at mucosal surface would result in its prolonged residence time at the site of drug action or absorption thereby enhances the bioavailability (Lueßen HL et al. 1994); and minimises the dosing frequency thereby improves the patient compliance (Robinson JR and Lee VH, 1987). Mucoadhesive delivery systems results in the greater bioavailability of furosemide (Ozdemir N et al. 2000), riboflavin (Kunisawa J et al. 2000), vasopressin (Morimoto K et al. 1991), dopamine (Ikeda K et al. 1992), insulin (Nagai T et al. 1984), peptides (Lehr CM et al.

1992a), gentamycin (Ilium L et al. 1988), and many more to list; localises drug action at the target site (Lueßen HL et al. 1994); and increases the drug concentration gradient associated with the passionate contact of particles with the mucosa and insertion of penetration enhancers like sodium glycocholate results in adaptation of tissue permeability for absorption of macromolecules like peptides and proteins (Lueßen HL et al. 1994).

10.2. Classification of mucoadhesive devices

10.2.1. Based upon the mechanism

Based on the mechanism by which a drug was released mucoadhesive devices can be classified into the monolithic (or matrix) systems where the drug was dissolved or dispersed in the polymer system in which diffusion of drug from the drug/polymer matrix controls the overall rate of its release from the device; and the reservoir (or membrane) systems, where diffusional resistance across a polymeric membrane controls the overall drug release rate.

Basing upon the key limiting factor that wheel the rate of drug transport and its delivery to the systemic circulation, either a monolithic or reservoir system was selected. When the desired rate of drug transfer was significantly less than that through the mucosal membrane, an appliance that control the drug delivery was needed to attain therapeutic steady state concentrations of drug in the plasma and to avoid overdosing; and in such cases, an appliance with a rate controlling membrane was requisite. A monolithic or matrix type of delivery system was used, if drug access through the mucosal membrane was the rate-controlling step.

10.2.2. Based upon the potential sites for attachment

Based upon the potential sites for attachment the mucoadhesive drug delivery system include: GI or gastro retentive delivery system, nasal delivery system, ocular delivery system, buccal delivery system, sublingual delivery system, vaginal delivery system, cervical and vulval drug delivery systems, and rectal delivery system (Woolfson AD et al. 1998; Donnelly RF et al. 2009).

11. Oral mucoadhesive microcarriers

Oral mucoadhesive microcarriers includes microspheres, microbeads, and microcapsules of 1-100 μm in diameter, that encloses drug in core, and consisting either exclusively of a mucoadhesive polymer or having an outer coating of it (Mathiowitz E et al. 2001). Generally microcarriers had potentiality to be employed for targeted and C/E-R of drug(s); but blending mucoadhesive properties to microcarriers will furthermore improve absorption and bioavailability of the drug(s) (Ozdemir N et al. 2000; Kunisawa J et al. 2000; Chowdary KPR and Rao YS, 2004; Belgamwar V et al. 2009) linked with high surface to volume ratio, enhanced intimate contact with the mucus layer, and drug targeting to the absorption site by anchoring plant lectins (Lehr CM et al. 1992b), bacterial adhesions (Yuehuei H and An Friedman JR, 2000), antibodies (Wright S and Huang L, 1989), etc., on the surface of the microcarriers. Tailored mucoadhesive microcarriers offers the possibilities of localised as well as CR of drug(s); achieved by adherence to any mucosal tissue those present in eye, nasal cavity, urinary, and GI tract; prolonged release of drug(s); and reduced dosing frequency for improving patient compliance (Robinson JR and Lee VH, 1987). Mucoadhesive microcarriers of bioerodable polymers undertake selective uptake by the M cells of Peyer's patches in GI mucosa (Heel KA et al. 1997); and can be employed for the delivery of protein and peptide drug(s), antigens for vaccination, and plasmid DNA for gene therapy. Non-invasive single shot vaccine, by way of mucosal immunisation, offers C/E-R of antigens and thus forms another fantastic application of mucoadhesive microcarriers (Kunisawa J et al. 2000).

11.1. Polymer selection in the preparation of mucoadhesive microcarriers

The type of polymer used to practise mucoadhesive microcarriers influences their surface characteristics, force of mucoadhesion, release pattern and clearance of drug. Polymers that can be used to form mucoadhesive microcarriers include soluble or insoluble; non-biodegradable and biodegradable polymers; that can be hydrogels or thermoplastics, homopolymers, copolymers or blend,

natural or synthetic polymers (Vasir JK et al. 2003; Chowdary KPR and Rao YS, 2004).

To serve as mucoadhesive polymers, polymer should have at least one characteristic; that includes sufficient number of hydrogen bonding chemical groups (-OH and –COOH), anionic surface chain, high molecular weight, high chain flexibility, surface tension that will provoke spreading into the mucus layer favouring the creation of bonds that are either of chemical or mechanical origin are vital to obtain adhesion (Leung SHS and Robinson JR, 1990; Chickering DE and Mathiowitz E, 1999).

11.2. Preparation of mucoadhesive microcarriers

Mucoadhesive microcarriers can be prepared using different techniques like solvent evaporation method, hot melt microencapsulation, solvent removal technique, hydrogel microcarrier technique, spray drying technique (de Oliveira BF et al. 2004), phase inversion technique, etc. (Ozdemir N et al. 2000; Vasir JK et al. 2003; Chowdary KPR and Rao YS, 2004; Shivanand P. 2010).

11.2.1. Complex coacervation

This refers to the phase separation of a liquid precipitate, or phase, when solutions of two hydrophilic colloids were mixed under suitable conditions. The core material was dispersed in a solution of the coating polymer in the liquid manufacturing vehicle phase; the coating material phase, prepared by dissolving immiscible polymer in a suitable vehicle. Physical mixing of the coating material phase and the core material phase in the manufacturing vehicle was done under stirring. Microencapsulation was achieved by utilising one of the methods of phase separation, that is, by changing the temperature of the polymer solution; by adding a salt or a non-solvent or an incompatible polymer to the polymer solution; by inducing a polymer-polymer interaction. Usually coating was hardened by thermal, cross linking or desolvation techniques, to form a self sustaining microcarrier (Zhang L et al. 2009; Mathiowitz E et al. 1999a).

11.2.2. Hot melt microencapsulation

Microcarriers of polyanhydride copolymer of poly [bis(p-carboxy phenoxy) propane anhydride] with sebacic acid were firstly primed by this method (Mathiowitz E and Langer R, 1987). The method involves melting of the polymer followed by pouring solid particles of the drug, less than 50 μm, and continued mixing. The mixture was suspended in a non-miscible solvent like silicone oil with stirring and heated to a temperature that was 5°C above the melting point of the polymer with continuous stirring, so as to get stabilized emulsion that is cooled to solidify polymer particles followed by decantation to separate microcarriers and washing of the microcarriers with petroleum ether. Moderate temperature to which the drug was exposed, which may be deteriorating, was the only disadvantage of this method.

11.2.3. Solvent evaporation

It was firstly described by Ogawa Y et al. in year 1988, was the most widely used method of microencapsulation (Bogataj M et al. 1999). A buffered or plain aqueous solution of the drug along with a viscosity building or stabilising agent was poured to an organic phase consisting of the polymer solution in dichloromethane or ethyl acetate or chloroform, with vigorous stirring to get primary water-in-oil emulsion. This emulsion was then poured to a large volume of water containing an emulsifier like polyvinyl alcohol or polyvinylpyrrolidone, under stirring, to get the multiple emulsions (w/o/w); and stirring was continued until most of the organic solvent evaporates, leaving solid microcarriers. The microcarriers could then be washed, centrifuged, and lyophilised to get the free flowing and dried microcarriers.

11.2.4. Solvent removal

This was a non-aqueous method of microencapsulation and was suitable for water labile polymers such as the polyanhydrides. The method involves dispersing or dissolving the drug in a polymeric solution, in a volatile organic solvent like methylene chloride; followed by suspending the polymer solution in the silicone oil containing span 85 and methylene chloride under stirring, then petroleum ether was added and stirred until solvent

was extracted into the oil solution (Carino PG et al. 1999). The resulting microcarriers were then subjected for vacuum drying.

11.2.5. Hydrogel methods

This method was developed by Lim F and Moss RD, was an 'all-aqueous' system, avoids residual solvents in microcarriers and was suitable for encapsulating live cells (Lim F and Moss RD, 1981; Sparnacci K et al. 2005). Microcarriers were formed by dissolving the gel-type polymers, such as alginate, in an aqueous solution followed by suspending the active ingredient in the mixture and extruding through a precision device, to produce micro droplets which fall into a hardening bath kept under stirring at low speed. Divalent calcium ions present in the hardening bath, calcium chloride solution, crosslink the polymer forming gelled microcarriers.

11.2.6. Phase inversion method

The process involves addition of drug into dilute (1-5% w/v) polymeric solution, in methylene chloride; and pouring resultant mixture into an unstirred bath of strong non-solvent, petroleum ether, at a solvent to non-solvent ratio of 1: 100, consequentially microcarriers in the size range of 0.5-5.0 μm was produced suddenly which was then clarified, washed with petroleum ether and air dried (Chickering D et al. 1996; Costa MS and Margarida Cardoso MM, 2006). It was an elementary process of microencapsulation with relatively little loss of drug and polymer.

11.2.7. Spray drying

This process involves dissolving/dispersing of drug in the polymer solution which was spray dried; and the quality of microcarriers can be improved by the addition of plasticizers (citric acid) that promote polymer coalescence on the drug particles, and hence promotes formation of spherical and smooth surfaced microcarriers. Microcarrier size can be controlled by manipulating the rate of spraying, the feed rate of polymer drug solution, nozzle size, and the drying temperature (Bodmeier R and Chen HG, 1988; de Oliveira BF et al. 2004; Yassin AE et al. 2009). This technique was independent on the solubility characteristics of the drug and polymer; was elementary and reproducible, and can be easily scaled up (Bodmeier R and Chen HG, 1988; Nagda C et al. 2009).

11.3. Evaluation of mucoadhesive microcarriers

Various *in vivo*, *ex vivo* and *in vitro* methods were used for characterising, and evaluating the effectiveness and the efficacy of the mucoadhesive microcarriers.

11.3.1. Measurement of adhesive strength (*in vitro* techniques)

Various methods used for studying mucoadhesive properties were illustrated here. Mucoadhesive strength of microcarriers can be evaluated and indicated by quantifying mucoadhesive forces between the polymeric microcarriers and the mucosal tissue. Several *in vitro* techniques had been used to test the effectiveness of polymeric microcarriers against a variety of synthetic and natural mucus, frozen and freshly excised tissue, etc. Commonly used *in vitro* and *ex vivo* methods include tensile strength measurement, shear strength measurement, and chip based systems. Important *in vitro* adhesive strength determination methods were as follows.

a. Falling liquid film method:

It was a simple, quantitative *in situ* method, involves flowing down the suspension of microcarriers on the intestinal strip (obtained by cutting the excised intestinal segment, lengthwise) that was spread on a plastic flute, and kept at an inclined position. Particle concentrations entering the intestinal segment and leaving the segment, determined suitably (mostly with coulter counter), to quantify the steady state fraction of microcarriers adhered to the intestinal mucosa; and reported in percent, as an index of mucoadhesion (Teng CLC and Ho NFH, 1987).

b. Novel electromagnetic force transducer:

The electromagnetic force transducer measures tissue adhesive forces by monitoring the magnetic force required to detach a magnetic loaded polymer microcarrier from a tissue sample (Hertzog BA and Mathiowitz E, 1999). The microcarrier was

firstly attached to the sample of tissue; magnetic force was then generated by an electromagnet mounted on the microscope vertically above the tissue chamber. The position of microcarrier was determined by computer, and then the tissue chamber was slowly moved down, away from the magnet tip. The slow descending movement of the tissue away from the magnet was continuously video analysed to calculate the position of microcarrier until the latter was completely pulled free of the tissue. The results were displayed either as raw data or as a plot of force versus displacement. This method eliminates the physical attachment between the force transducer and the microcarrier, making it suitable to perform accurate mucoadhesive measurements on the small microcarrier that had been implanted *in vivo* and then excised along with the host tissue for measurement. Evaluation of the mucoadhesion of polymers to specific cell types can be done with this technique and can aid to develop tissue specific targeted mucoadhesive drug delivery system (Singh M et al. 2001).

c. Tensile stress measurement by Wilhelmy plate technique:

Modified CAHN dynamic contact angle analyser was used to perform adhesive micro force measurements (Chickering DE et al. 1999). The mucoadhesive force between the mucosal tissue and a single microcarrier mounted on a small diameter metal wire suspended from the sample loop in microtensiometer was measured by the instrument (Santos CA et al. 1999). The tissue was mounted within the tissue chamber containing Dulbecco's phosphate buffered saline containing 100 mg/dl glucose, maintained at the physiologic temperature. Contact of the tissue with the suspended microcarriers was achieved by raising the chamber that was rested on a mobile platform. The contact was held for 7 min, subsequently the mobile stage was lowered, and the resulting force of adhesion was recorded as a plot of the load on microcarrier versus mobile stage distance or deformation. The plot displays both the compressive and the tensile portions of the experiment. Using CAHN software system, three essential mucoadhesive parameters; the fracture strength, the deformation to failure, and the work of adhesion; can be analysed.

d. Shear stress measurement:

This method involves measurement of the force that causes a mucoadhesive to slide with respect to the mucus layer in a direction parallel to their plane of contact (Kamath KR and Park K, 1992). The test measures the force required to separate the surface of two glass slides coated with polymer and a film of mucus, where the mucus forms a thin film between the polymers coated slides. An *in vitro* method using flow chamber made of Plexi-glass surrounded by a water jacket, maintaining a constant temperature had been used to measure shear stress (Mikos AG and Peppas NA, 1990). This method involves placing the polymeric microcarriers on the surface of a layer of natural mucus which was placed in the chamber, simulated physiologic fluid was introduced in the chamber, and movement of microcarrier was monitored using video equipment attached to a goniometer, which also monitors the static and dynamic behaviour of the microcarrier (Hertzog BA and Mathiowitz E, 1999).

e. Everted sac technique:

It was a passive test of mucoadhesion; and was carried out using a section of intestinal tissue excised from the rat that was everted and ligated at the ends followed by filling with saline. The sac was then introduced into a tube containing known amount of microcarriers in saline, and incubated for 30 min with occasional agitation. Microcarriers contained in the tube were washed and lyophilised, after removing the sac, and the percent binding to the sac was calculated from difference in the weight of the residual microcarriers from that of initial (Jacob J et al. 1995). This technique does not utilise any external force and was reliable.

f. Adhesion number:

Adhesion number was determined as the ratio of the number of microcarriers attached to the substrate with respect to the total number of applied microcarriers, and was expressed in percent. An increase in the adhesion number indicates increase in adhesion strength (Kamath KR and Park K, 1992).

11.3.2. Measurement of adhesive strength (*in vivo* techniques)

Various imaging techniques were used for the evaluation of mucoadhesive properties of the delivery systems under *in vivo* conditions (Shivanand P. 2010).

a. Measurement of the residence time:

Quantification of mucoadhesive properties of microcarriers can be achieved by measuring their residence time at the application site and can be examined using radioisotopes and fluorescent labelling techniques.

b. Gamma scintigraphy technique:

This technique can enumerate the distribution and retention time of the intra-vaginal mucoadhesive microcarriers. Combination of sheep model and gamma scintigraphy method had been proved to be an extremely useful tool for evaluating the distribution, the spreading, and the clearance of vaginally administered mucoadhesive drug delivery system (Richardson JL et al. 1996).

c. Gastrointestinal transit time measurement (using radio-opaque microcarriers):

This technique involves use of radio-opaque marker; like barium sulphate, Cr-51, Tc-99m, In-113m, or I-123 (Mathiowitz E et al. 1999b); labelled or encapsulated mucoadhesive microcarriers, to access the effect of mucoadhesive polymers on GI transit time. Using an automated faeces collection machine and X-ray inspection, this technique provides a non-invasive method for monitoring total GI residence time without affecting normal GI motility.

11.3.3. Swelling index

Swelling index enumerates the ability of the mucoadhesive microcarriers to get swelled at the absorbing surface by absorbing fluids available at the site of absorption, a primary requirement for initiation of mucoadhesion (Rajput G *et al*, 2010). From the size of dried microcarriers (D_0) and those after incubation (D_T), in suitable fluid for stipulated period of time, the percent swelling value can be determined using following equation.

$$\text{Percent swelling} = [D_T - D_0] / D_0 \times 100$$

11.3.4. Particle size and size distribution, and flow property study

Particle size and size distribution study was done by photon correlation spectroscopy with the dispersions of microcarriers, in suitable non-solvent system (Vyas TK et al. 2006). Particle size and size distribution governs the performance and fate of mucoadhesive microcarriers. Flow property of the mucoadhesive microcarriers was determined from the result of study parameters namely Angle of repose, Carr's index, and Hausner ratio (Alli SMA et al. 2010).

11.3.5. Surface charge study

The surface charge (zeta potential) of the mucoadhesive microcarriers can be determined from photon correlation spectroscopy data by relating measured electrophoretic mobility into zeta potential with an in-built software based on the Helmholtz–Smoluchowski equation (Vyas TK et al. 2006). Zeta potential was an indicator of particle surface charge, which can be used to predict and control the stability; and the adhesive strength as well as the mechanisms of mucoadhesion. Process of mucoadhesion was a corollary of interactions between the mucus layer on mucosa and mucoadhesive polymers, and was influenced by mucus and polymer structure including their charge. Measurement of zeta potential of microcarriers and mucosal homogenates can be an insight into electrostatic interactions during mucoadhesion (Bogataj M et al. 2003).

11.3.6. Surface characterisation of the mucoadhesive microcarriers

The scanning electron microscopy, the electron microscopy, and the scanning tunnelling microscopy data provides insight to the surface morphology of microcarriers and the morphological changes produced through polymer degradation. The surface morphology changes occurring through polymer degradation can be studied by incubating the microcarriers in the phosphate buffer saline at different intervals of time (Mathiowitz E et al. 1999b). The coarser surface texture improves the adhesion through stronger mechanical interactions, while smooth texture of the microcarrier surface leads to weak mucoadhesive properties (Peppas NA

and Buri PA, 1985; Chowdary KPR and Rao YS, 2004).

11.3.7. *In vitro* release study

Standard IP/BP/USP dissolution apparatus had been used to study *in vitro* release profile in the dissolution media that was similar to the fluid present at the absorption site, using rotating basket or paddle (Sonani NG et al. 2010).

11.3.8. *In vitro* release kinetic studies, statistical evaluation, and data fitting

The kinetic model describes drug dissolution from the solid dosage form, where the released amount of drug as a function of test time was studied. Under appropriate test conditions, a dissolution profile could distinguish the product more precisely than a single point dissolution test. A mean value of three determinations at each time point was used to fit an *in vitro* drug dissolution profile of all formulation batches to different kinetic models so as to uncover the best fit kinetic model and to find out their release exponents, while the mean value of twelve determinations at each time point was used to calculate the factors of the model-independent approach (Alli SMA et al. 2010).

11.3.9. Stability studies

The success of an effective formulation could be evaluated only through stability studies that were aimed to obtain a stable product which assures its safety and efficacy, and peak profile up to the end of shelf life, at defined storage conditions (Sonani NG et al. 2010; Chi N et al, 2009). Amongst all ICH guidelines were followed mostly as was internationally recognised.

12. CONCLUSION

Mucoadhesive microcarrier system had potentiality to improve GI residence time, performance and patient compliance vis-à-vis could be used for controlling release, enhancing bioavailability, and for drug targeting. Basing upon the potential site of application/absorption; and polymer characteristics, adhesive strength, biocompatibility and safety; suitable mucoadhesive polymer should be selected and microcarrier preparation techniques should be adopted. However, much more work was needed on these novel mucoadhesive microcarrier formulations for eliciting its clinical utility.

REFERENCES

1. Allen HJ and Johnson EA. The isolation of lectins on acid-treated agarose. Carbohydr Res. 1976;50(1):121-131.
2. Alli SMA, Samanta A, Mukherjee B, Ali SMA, Dehury G and Kanungo S. Hydrophilic polymeric matrix tablet for sustained delivery of levofloxacin. Int J Pharm Sci Tech. 2010;5(2):40-55.
3. Arya RKK, Singh R and Juyal V. Mucoadhesive microspheres of famotidine: preparation characterization and in vitro evaluation. Int J Eng Sci Tech. 2010;2(6):1575-1580.
4. Asane GS, Nirmal SA, Rasal KB, Naik AA, Mahadik MS and Rao YM. Polymers for mucoadhesive drug delivery system: a current status. Drug Dev Ind Pharm. 2008;34(11):1246-1266.
5. Avichezer D and Arnon R. Differential reactivities of the *Arachis hypogaea* (peanut) and *Vicia villosa* B4 lectins with human ovarian carcinoma cells, grown either *in vitro* or *in vivo* xenograft model. FEBS Lett. 1996;395(2-3):103-108.
6. Banker GS and Anderson NR. Tablets. In: Lachman L, Lieberman HA, Kanig JL, eds. The theory and practice of industrial pharmacy, 3rd edn. India: Varghese Publishing House; 1987:296-303.
7. Belgamwar V, Shah V and Surana SJ. Formulation and evaluation of oral mucoadhesive multiparticulate system containing metoprolol tartarate: an *in vitro-ex*

vivo characterization. Curr Drug Deliv. 2009;6(1):113-121.
8. Benita S. Microencapsulation Methods and Industrial Applications. New York: Marcel Dekker; 1996:1-8.
9. Bernkop-Schnürch A, Schwarz V and Steininger S. Polymers with thiol groups: a new generation of mucoadhesive polymers? Pharm Res. 1999;16(6):876-881.
10. Bodmeier R and Chen HG. Preparation of biodegradable poly(+/-)lactide microparticles using a spray-drying technique. J Pharm Pharmacol. 1988;40(11):754-757.
11. Bogataj M, Mrhar A and Korosec L. Influence of physicochemical and biological parameters on drug release from microspheres adhered on vesical and intestinal mucosa. Int J Pharm. 1999;177(2):211-220.
12. Bogataj M, Vovk T, Kerec M, Dimnik A, Grabnar I and Mrhar A. The correlation between zeta potential and mucoadhesion strength on pig vesical mucosa. Biol Pharm Bull. 2003;26(5):743-746.
13. Brandsch M, Ramamoorthy S, Marczin N, Catravas JD, Leibach JW, Ganapathy V and Leibach FH. Regulation of taurine transport by Escherichia coli heat-stable enterotoxin and guanylin in human intestinal cell lines. J Clin Invest. 1995;96(1):361-369.
14. Carino PG, Jacob JS, Chen CJ, Santos CA, Hertzog BA, Mathiowitz E. Bioadhesive, bioerodible polymers for increased intestinal uptake. In: Mathiowitz E, Chickering DE, Lehr CM, eds. Bioadhesive Drug Delivery Systems: Fundamentals, Novel Approaches and Development. New York: Marcel Dekker; 1999:459-475.
15. Chang RK and Robinson JR. Sustained drug release from tablets and particles through coating. In: Lieberman HA, Lachman L, Schwartz JB, eds. Pharmaceutical Dosage Form-Tablets, 1st edn, Vol- 3. New York: Marcel Dekker Inc; 1982:156-157.
16. Chi N, Tang X and Xu H. Development and optimization of chemically stable lipid microspheres containing flunarizine. Drug Dev Ind Pharm. 2009;35(6):738-745.
17. Chickering D, Jacob J and Mathiowitz E. Poly(fumaric-co-sebacic) microspheres as oral drug delivery systems. Biotechnol Bioeng. 1996;52(1):96-101.
18. Chickering DE and Mathiowitz E. Fundamentals of bioadhesion. In: Mathiowitz E, Chickering DE, Lehr CM, eds. Bioadhesive Drug Delivery Systems: Fundamentals, Novel Approaches and Development. New York: Marcel Dekker; 1999:1-85.
19. Chickering DE, Santos CA and Mathiowitz E. Adaptation of a microbalance to measure bioadhesive properties of microspheres. In: Mathiowitz E, Chickering DE, Lehr CM, eds. Bioadhesive Drug Delivery Systems: Fundamentals, Novel Approaches and Development. New York: Marcel Dekker; 1999:131-45.
20. Chowdary KPR and Rao YS. Mucoadhesive microspheres for controlled drug delivery. Biol Pharm Bull. 2004;27(11):1717-1724.
21. Chowdary KPR and Srinivas L. Mucoadhesive drug delivery systems: A review of current status. Indian Drugs. 2000;37(9):400-406.
22. Cleary GW, Feldstein MM, Singh P and Plate NA. A new polymer blend adhesive with combined properties to adhere to either skin or mucosa for drug delivery. Submitted for presentation at the 30th Annual Meeting and Exposition of the Controlled Release Society. Glagsow, Scotland, July 2003; 19-23.
23. Costa MS and Margarida Cardoso MM. Effect of uniform sized polymeric microspheres prepared by membrane emulsification technique on controlled release of anthracycline anti-cancer drugs. Desalination. 2006;200:498–500.
24. de Oliveira BF, Santana MHA and Ré MI. Spray-dried chitosan microspheres cross-linked with d,l-glyceraldehyde as a potential drug delivery system: preparation and characterization. Submitted for presentation at the 14th International Drying Symposium (IDS 2004). São Paulo, Brazil, August 2004; B1166-1173.

25. Derjaguin BV and Smilga VP. Electronic theory of adhesion. J Applied Physics. 1967;38(12):4609-4616.
26. Donnelly RF, McCarron PA, Zawislak A and Woolfson AD. Photodynamic therapy of vulval neoplasias and dysplasias: Design and evaluation of bioadhesive photosensitiser delivery systems. In: Verlag M, ed. Saarbrücken: VDM Publication; 2009:78–400.
27. Duchěne D, Touchard F and Peppas NA. Pharmaceutical and medical aspects of bioadhesive systems for drug administration. Drug Dev Ind Pharm. 1988;14(2-3):283-318.
28. Gabor F, Wirth M, Jurkovich B, Haberl I, Theyer G, Walcher G and Hamilton G. Lectin-mediated bioadhesion: proteolytic stability and binding-characteristics of wheat germ agglutinin and *Solanum tuberosum* lectin on Caco-2, HT-29 and human colonocytes. J Control Release. 1997;49(1):27-37.
29. Gercken J and Renwrantz L. A new mannan-binding lectin from the serum of the eel (*Anguilla anguilla* L.): isolation, characterization and comparison with the fucose-specific serum lectin. Comp Biochem Physiol Biochem Mol Biol. 1994;108(4):449-461.
30. Goldstein IJ, Blake DA, Ebisu S, Williams TJ and Murphy LA. Carbohydrate binding studies on the *Bandeiraea simplicifolia* I isolectins. Lectins which are mono-, di-, tri-, and tetravalent for N-acetyl-D-galactosamine. J Biol Chem. 1981;256(8):3890-3893.
31. Good RJ. On the definition of adhesion. J Adhes. 1976;8:1-9.
32. Haas J and Lehr CM. Developments in the area of bioadhesive drug delivery systems. Expert Opin Biol Ther. 2002;2(3):287-298.
33. Heel KA, McCauley RD, Papadimitriou JM and Hall JC. Review: Peyer's patches. J Gastroenterol Hepatol. 1997;12(2):122-136.
34. Helliwell M. The use of bioadhesives in targeted delivery within the gastrointestinal tract. Advanced Drug Delivery Reviews. 1993;11(3):221-251.
35. Hertzog BA and Mathiowitz E. Novel magnetic technique to measure bioadhesion. In: Mathiowitz E, Chickering DE, Lehr CM, eds. Bioadhesive Drug Delivery Systems: Fundamentals, Novel Approaches and Development. New York: Marcel Dekker;1999:147-171.
36. Hoffman A and Stepensky D. Pharmacodynamic aspects of modes of drug administration for optimization of drug therapy. Crit Rev Ther Drug Carrier Syst. 1999;16(6):571-639.
37. Huang Y, Leobandung W, Foss A and Peppas NA. Molecular aspects of muco- and bioadhesion: tethered structures and site-specific surfaces. J Control Release. 2000;65(1-2):63-71.
38. Hui HW and Robinson JR. Ocular delivery of progesterone using a bioadhesive polymer. Int J Pharm. 1985;26(3):203-213.
39. Ikeda K, Murata K, Kobayashi M and Noda K. Enhancement of bioavailability of dopamine via nasal route in beagle dogs. Chem Pharm Bull (Tokyo). 1992;40(8):2155-2158.
40. Ilium L, Farraj N, Critchley H and Davis SS. Nasal administration of gentamicin using a novel microsphere delivery system. Int J Pharm. 1988;46(3):261-265.
41. Inoue T, Chen G, Nakamae K and Hoffman AS. An AB blocks copolymer of oligo(methyl methacrylate) and poly(acrylic acid) for micellar delivery of hydrophobic drugs. J Control Release. 1998;51(2-3):221-229.
42. Jacob J, Santos C, Carino G, Chickering D and Mathiowitz E. An *in vitro* bioassay for quantification of bioadhesion of polymer microspheres to mucosal epithelium. Submitted for presentation in at the 22nd International Symposium on Controlled Release of Bioactive Materials. Seattle, WA, July 30-August 2 1995; 312-313.
43. Jasti B, Li X and Cleary G. Recent advances in mucoadhesive drug delivery systems. Business briefing: Pharmtech. 2003:194-197.
44. Jepson MA, Clark MA and Hirst BH. M cell targeting by lectins: a strategy for mucosal vaccination and drug delivery. Adv Drug Deliv Rev. 2004;56(4):511-525.

45. Jiménez-castellanos MR, Zia H and Rhodes CT. Mucoadhesive drug delivery systems. Drug Dev Ind Pharm. 1993;19(1-2):143-194.
46. Kamath KR and Park K. Mucosal adhesive preparations. In: Swarbrick J, Boylan JC, eds. Encyclopedia of Pharmaceutical Technology, Vol-10. New York: Marcel Dekker; 1992:133-136.
47. Khan GM. Controlled release oral dosage forms: some recent advances in matrix type drug delivery systems. J Med Science. 2001;1(5):350-354.
48. Kruszewska D, Kiela P, Ljungh Å, Erlwanger KH, Weström BR, Linderoth A and Pierzynowski SG. Enteral crude red kidney bean (*Phaseolus vulgaris*) lectin – phytohemagglutinin – induces maturational changes in the enterocyte membrane proteins of suckling rats. Biol Neonate. 2003;84:152-158.
49. Kunisawa J, Okudaira A, Tsutusmi Y, Takahashi I, Nakanishi T, Kiyono H and Mayumi T. Characterization of mucoadhesive microspheres for the induction of mucosal and systemic immune responses. Vaccine. 2000;19(4-5):589-594.
50. Lavelle EC. Targeted delivery of drugs to the gastrointestinal tract. Crit Rev Ther Drug Carrier Syst. 2001;18(4):341-386.
51. Lee JW, Park JH and Robinson JR. Bioadhesive-based dosage forms: the next generation. J Pharm Sci. 2000;89(7):850-866.
52. Lee TW and Robinson JR. Controlled release drug delivery systems. In: Gennaro AR, ed. Remington Pharmaceutical Sciences, 20th edn. Philadelphia: Lippincott Williams and Willkins; 2000:903-929.
53. Lehr CM, Bouwstra JA, Kok W, De Boer AG, Tukker JJ, Verhoef JC, Breimer DD and Junginger HE. Effects of the mucoadhesive polymer polycarbophil on the intestinal absorption of a peptide drug in the rat. J Pharm Pharmacol. 1992a;44(5):402-407.
54. Lehr CM, Bouwstra JA, Kok W, Noach AB, de Boer AG and Junginger HE. Bioadhesion by means of specific binding of tomato lectin. Pharm Res. 1992b;9(4):547-553.
55. Lehr CM. From sticky stuff to sweet receptors--achievements, limits and novel approaches to bioadhesion. Eur J Drug Metab Pharmacokinet. 1996;21(2):139-148.
56. Lele BS and Hoffman AS. Mucoadhesive drug carriers based on complexes of poly(acrylic acid) and PEGylated drugs having hydrolysable PEG-anhydride-drug linkages. J Control Release. 2000;69(2):237-248.
57. Leung SHS and Robinson JR. Polymer structure features contributing to mucoadhesion II. J Control Release. 1990;12(3):187-194.
58. Lim F and Moss RD. Microencapsulation of living cells and tissues. J Pharm Sci. 1981;70(4):351-354.
59. Liu Q, Shao X, Chen J, Shen Y, Feng C, Gao X, Zhao Y, Li J, Zhang Q and Jiang X. *In vivo* toxicity and immunogenicity of wheat germ agglutinin conjugated poly(ethylene glycol)-poly(lactic acid) nanoparticles for intranasal delivery to the brain. Toxicol Appl Pharmacol. 2010;251(1):79-84.
60. Lueßen HL, Lehr CM, Rentel CO, Noach ABJ, de Boer AG, Verhoef JC and Junginger HE. Bioadhesive polymers for the peroral delivery of peptide drugs. J Control Release. 1994:29(3):329-338.
61. Mathiowitz E, Kreitz MR and Peppas LB. Microencapsulation. In: Mathiowitz E, ed. Encyclopedia of Controlled Drug Delivery, Vol. 9. New York: John Willey & Sons; 1999a:493-504.
62. Mathiowitz E, Chickering D, Jacob JS and Santos C. Bioadhesive drug delivery systems. In: Mathiowitz E, ed. Encyclopedia of Controlled Drug Delivery, Vol. 9. New York: John Willey & Sons; 1999b:9-44.
63. Mathiowitz E, Chickering DE and Jacob JS. Mucoadhesive drug delivery system, US Patent number 6197346. 2001.
64. Mathiowitz E and Langer R. Polyanhydride microspheres as drug carriers I. Hot-melt microencapsulation. J Control Release. 1987;5(1):13-22.
65. McCarron PA, Woolfson AD, Donnelly RF, Andrews GP, Zawislak A and Price JH. Influence of plasticizer type and storage

conditions on properties of poly(methyl vinyl ether-co-maleic anhydride) bioadhesive films. J Applied Polymer Sci. 2004;91(3):1576-89.
66. Mikos AG and Peppas NA. Bioadhesive analysis of controlled-release systems. IV. An experimental method for testing the adhesion of microparticles with mucus. J Control Release. 1990;2(1):31-37.
67. Morimoto K, Yamaguchi H, Iwakura Y, Morisaka K, Ohashi Y and Nakai Y. Effects of viscous hyaluronate-sodium solutions on the nasal absorption of vasopressin and an analogue. Pharm Res. 1991;8(4):471-474.
68. Nagai T and Machida Y. Mucosal adhesive dosage forms. Pharm Int. 1985;6:196-200.
69. Nagai T, Nishimoto Y, Nambu N, Suzuki Y and Sekine K. Powder dosage forms of insulin for nasal administration. J Control Release. 1984;1(1):15-22.
70. Nagda C, Chotai NP, Patel U, Patel S, Soni T, Patel P and Hingorani L. Preparation and characterization of spray-dried mucoadhesive microspheres of aceclofenac. Drug Dev Ind Pharm. 2009;35(10):1155-1166.
71. Nur AO and Zhang JS. Recent progress in sustained/controlled oral delivery of captopril: an overview. Int J Pharm. 2000;194(2):139-146.
72. Ogawa Y, Yamamoto M, Okada H, Yashiki T and Shimamoto T. A new technique to efficiently entrap leuprolide acetate into microcapsules of polylactic acid or copoly(lactic/glycolic) acid. Chem Pharm Bull (Tokyo). 1988;36(3):1095-1103.
73. Olsnes S, Saltvedt E and Pihl A. Isolation and comparison of galactose-binding lectins from *Abrus precatorius* and *Ricinus communis*. J Biol Chem. 1974;249(3):803-810.
74. Ozdemir N, Ordu S and Ozkan Y. Studies of floating dosage forms of furosemide: *in vitro* and *in vivo* evaluations of bilayer tablet formulations. Drug Dev Ind Pharm. 2000;26(8):857-866.
75. Park H and Robinson JR. Physico-chemical properties of water insoluble polymers important to mucin/epithelial adhesion. J Control Release. 1985;2:47–57.
76. Park K. Lectin-immobilized nanospheres for GI tumor targeting. J Control Release. 2009;134(1):1.
77. Peppas NA and Buri PA. Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues. J Control Rel. 1985;2:257-275.
78. Peppas NA, Little MD and Huang Y. Bioadhesive Controlled Release Systems. In: Wise DL, ed. Handbook of Pharmaceutical Controlled Release Technology. New York: Marcel Dekker; 2000a:255-269.
79. Peppas NA, Bures P, Leobandung W and Ichikawa H. Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm. 2000b;50(1):27-46.
80. Pinto VP, Debray H, Dus D, Teixeira EH, de Oliveira TM, Carneiro VA, Teixeira AH, Filho GC, Nagano CS, Nascimento KS, Sampaio AH and Cavada BS. Lectins from the red marine algal species *Bryothamnion seaforthii* and *Bryothamnion triquetrum* as tools to differentiate human colon carcinoma cells. Adv Pharmacol Sci. 2009;2009:862162.
81. Poiroux G, Pitié M, Culerrier R, Ségui B, Van Damme EJ, Peumans WJ, Bernadou J, Levade T, Rougé P, Barre A and Benoist H. Morniga G: a plant lectin as an endocytic ligand for photosensitizer molecule targeting toward tumor-associated T/Tn antigens. Photochem Photobiol. 2011;87(2);370-377.
82. Pritchard WH. Aspects of adhesion 6. In: Alder D, ed. 3rd edn. London: London University Press; 1970:11-23.
83. Rajput G, Majmudar F, Patel J, Thakor R and Rajgor NB. Stomach-specific mucoadhesive microsphere as a controlled drug delivery system. Sys Rev Pharm. 2010;1(1):70-78.
84. Richardson JL, Whetstone J, Fisher AN, Watts P, Farraj NF, Hinchcliffe M, Benedetti L and Illum L. Gamma-scintigraphy as a novel method to study the distribution and retention of a bioadhesive vaginal delivery system in sheep. J Control Rel. 1996;42(2):133-142.
85. Robinson JR and Lee VH. Controlled Drug Delivery: Fundamentals and Applications, 2nd edn. New York: Marcel Dekker; 1987:8.
86. Roth-Walter F, Schöll I, Untersmayr E, Fuchs R, Boltz-Nitulescu G, Weissenböck A,

Scheiner O, Gabor F and Jensen-Jarolim E. M cell targeting with *Aleuria aurantia* lectin as a novel approach for oral allergen immunotherapy. The J Allergy Clinical Immunology. 2004;114(6):1362-1368.
87. Roy S, Pal K, Anis A, Pramanik K and Prabhakar B. Polymers in mucoadhesive drug-delivery systems: a brief note. Designed Monomers and Polymers. 2009;12(6):483-495.
88. Santos CA, Jacob JS, Hertzog BA, Freedman BD, Press DL, Harnpicharnchai P and Mathiowitz E. Correlation of two bioadhesion assays: the everted sac technique and the CAHN microbalance. J Control Release. 1999;61(1-2):113-122.
89. Shaikh R, Raj Singh TR, Garland MJ, Woolfson AD and Donnelly RF. Mucoadhesive drug delivery systems. J Pharm Bioallied Sci. 2011;3(1):89-100.
90. Shivanand P. Different techniques of formulation and evaluation of mucoadhesive microspheres. Int J Pharma Bio Sciences. 2010;1(2):1-7.
91. Shojaei AH and Li X. Mechanisms of buccal mucoadhesion of novel copolymers of acrylic acid and polyethylene glycol monomethylether monomethacrylate. J Control Release. 1997;47(2):151-161.
92. Singh BN and Kim KH. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J Control Release. 2000;63(3):235-259.
93. Singh M, Briones M and O'Hagan DT. A novel bioadhesive intranasal delivery system for inactivated influenza vaccines. J Control Release. 2001;70(3):267-276.
94. Sonani NG, Hiremath SP, Dasankoppa FS, Jamakandi VG and Sreenivas SA. Design and evaluation of gastroretentive mucoadhesive cephalexin tablets. Pharm Dev Technol. 2010;15(2):178-183.
95. Sparnacci K, Laus M, Tondelli L, Bernardi C, Magnani L, Corticelli F, Marchisio M, Ensoli B, Castaldello A and Caputo A. Core-shell microspheres by dispersion polymerization as promising delivery systems for proteins. J Biomater Sci Polym Ed. 2005;16(12):1557-1574.
96. Takeuchi H, Yamamoto H and Kawashima Y. Mucoadhesive nanoparticulate systems for peptide drug delivery. Adv Drug Deliv Rev. 2001;47(1):39-54.
97. Teng CLC and Ho NFH. Mechanistic studies in the simultaneous flow and adsorption of polymer-coated latex particles on intestinal mucus I: Methods and physical model development. J Control Release. 1987;6(1):133-149.
98. Vasir JK, Tambwekar K and Garg S. Bioadhesive microspheres as a controlled drug delivery system. Int J Pharm. 2003;255(1-2):13-32.
99. Vyas SP and Khar RK. Controlled Drug Delivery: Concepts and Advances, 1st edn. India: Vallabh Prakashan; 2002:345-376.
100. Vyas TK, Babbar AK, Sharma RK, Singh S and Misra A. Intranasal mucoadhesive microemulsions of clonazepam: preliminary studies on brain targeting. J Pharm Sci. 2006;95(3):570-580.
101. Wake WC. Adhesion and the formulation of adhesives, 2nd edn. London: Applied Science Publishers; 1982.
102. Wang SY, Yu QJ, Bao JK and Liu B. Polygonatum cyrtonema lectin, a potential antineoplastic drug targeting programmed cell death pathways. Biochem Biophys Res Commun. 2011;406(4):497-500.
103. Woodley JF. Lectins for gastrointestinal targeting--15 years on. J Drug Target. 2000;7(5):325-333.
104. Woolfson AD, McCafferty DF and Moss GP. Development and characterisation of a moisture-activated bioadhesive drug delivery system for percutaneous local anaesthesia. Int J Pharm. 1998;169(1):83–94.
105. Wright S and Huang L. Antibody-directed liposomes as drug-delivery vehicles. Adv Drug Deliv Rev. 1989;3(3):343-389.
106. Yassin AE, Alanazi FK, El-Badry M, Alsarra IA, Barakat NS and Alanazi FK. Preparation and characterization of spironolactone-loaded gelucire microparticles using spray-drying

technique. Drug Dev Ind Pharm. 2009;35(3):297-304.
107. Yu L, Fernig DG, Smith JA, Milton JD and Rhodes JM. Reversible inhibition of proliferation of epithelial cell lines by *Agaricus bisporus* (edible mushroom) lectin. Cancer Res. 1993;53(19):4627-4632.
108. Yuehuei H and An Friedman JR, eds. Hand Book of Bacterial Adhesion: Principles, Methods and Applications. New Jersey: Humana Press. 2000:644-48.
109. Zhang L, Liu Y, Wu Z and Chen H. Preparation and characterization of coacervate microcapsules for the delivery of antimicrobial oyster peptides. Drug Dev Ind Pharm. 2009;35(3):369-278.
110. Zhao X, Singh S, Pardoux C, Zhao J, His ED, Abo A and Korver W. Targeting C-type lectin-like molecule-1 for antibody-mediated immunotherapy in acute myeloid leukemia. Haematologica. 2009;95(1):71-78.