# Application of Nano Drug Delivery System in Mucoadhesive Polymers

Fatemeh Bonyadi

M.Sc. of Medical Nanotechnology, Islamic Azad University, Pharmaceutical Branch, Advance Sciences and Technology College, Tehran, Iran
bonyadi93@gmail.com

**Abstract:** The main purpose of this study is an evaluation the range of use nano drug delivery system in Mucoadhesive polymers. The use of nanotechnology in drug delivery is rapidly increased. Mucus layer that covers the surface of a variety of organs which develop mucoadhesive dosage forms, increasing systemic bioavailability of the administered drug. Performances of intelligent drug delivery systems are continuously improved with the purpose to maximize therapeutic activity and to minimize undesirable side-effects. The present review initially describes the potential of nano-drug delivery systems conceived for mucosal administration. The emergence of micro and nanotechnologies together with the implementation of non-invasive and painless administration routes has revolutionized the pharmaceutical market and the treatment of disease. In addition, the regulatory status of the most extensively used mucoadhesive polymers will be emphasized. Besides, these relatively new and exciting data indicate that the future of nanomedicine is very promising, and that additional preclinical and clinical studies in relevant long-term Mucoadhesive polymers studies, should be conducted.
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## 1. Introduction

The main goal of pharmaceutical research and development is to design products with ensured quality to effectively treat disease. Patient and clinician compliance are crucial to the successful bench-to-bedside translation. Aiming to make this process more rational, coherent, efficient and cost-effective, the field of Pharmaceutical Materials Science (PMS) has emerged as the "study of the physical properties and behaviour of materials of pharmaceutical interest in relation to the product performance". Materials of pharmaceutical interest (MPIs) are classified into two main classes, namely active pharmaceutical ingredients (API) and non-pharmacologically active excipients. The former are entailed to trigger a pharmacological response, while the latter are incorporated into the formulation to improve its (bio) pharmaceutical properties and performance. In this context, PMS embraced the materials science tetrahedron (MST) and pursues the thorough characterization and understanding of the structure-properties relationship of all the components in a pharmaceutical product (including the pure drug) and the development of appropriate processing methods (e.g., micronization, nanonization, freeze-drying and spray-drying) that ensure a predictable performance in vitro (e.g., tablet mechanical properties, disintegration and drug dissolution) and in vivo (e.g., bioavailability).

One of the challenges in early and late PR&D pertains to the poor aqueous solubility and permeability of drugs. This property is common to approximately 50% of the APIs on the market and it represents a crucial hurdle during the stages of drug product development. Moreover, low solubility in biological fluids leads to limited absorption in the gastrointestinal tract (GIT) and limited bioavailability, the oral route being the most popular one. Solubility is an intrinsic property that depends on the nature of the molecule, whereas dissolution is an extrinsic one that can be modified by different means such as reduction of drug particle size and encapsulation in a variety of micro and nanocarriers.

PMS was initially implemented to improve the performance of already approved drugs. However, drug candidates in the pipeline are becoming more complex structures usually highly hydrophobic and this nature jeopardizes not only the conduction of more advanced preclinical and clinical trials but also preliminary high-throughput screening assays *in vitro*. For example, to assess the antiviral activity of a new compound in cell culture, it needs to be soluble in the culture medium. Thus, the early characterization of the solubility and other physicochemical parameters has become a crucial step in the whole PR&D process to increase the translatability of new chemical entities and to reduce the drug attrition rates.

As stated before, most of the pharmaceutical products are solids intended for oral administration. On the other hand, this route is usually associated with hepatic first pass metabolism, chemical and enzymatic degradation in the GIT medium, basolateral-to-apical

efflux by pumps of the ATP-binding cassette superfamily (ABCs) and reduced bioavailability. The most straightforward strategy to circumvent these disadvantages is the parenteral route. However, it provokes tissue damage, pain and patient incompliance. Moreover, systemic exposure often leads to adverse effects that cannot be easily controlled. The oral route is also less feasible when more prolonged release kinetics is demanded owing to the short gastric emptying and intestinal transit times.

Among the different approaches pursued to optimize the physicochemical and (bio)pharmaceutic performance of drugs, the presence of a mucus layer that covers the surface of a variety of organs has been capitalized to develop mucoadhesive dosage forms that remain in the administration site for more prolonged times, increasing the local and/or systemic bioavailability of the administered drug. The emergence of micro and nanotechnologies together with the implementation of non- invasive and painless administration routes has revolutionized the pharmaceutical market and the treatment of diseas. Aiming to overcome the main drawbacks of the oral route and to maintain patient compliance high, the engineering of innovative drug delivery systems (DDS) administrable by mucosal routes has come to light and gained the interest of the scientific community due to the possibility to dramatically change the drug pharmacokinetics. In addition, to achieve the goal of mucosal drug administration, the development of biomaterials has been refined to fit the specific applications.

The present review initially describes the potential of nano- DDS conceived for mucosal administration by diverse non- parenteral routes (*e.g.*, oral, inhalatory, etc.). Then, the benefit of the incorporation of mucoadhesive polymers into the structure of these innovative pharmaceutical products to prolong their residence time in the administration site and the release of the drug cargo will be discussed with focus in the developments of the last decade. In addition, the regulatory status of the most extensively used mucoadhesive polymers will be emphasized. Finally, a thorough overview of the different pharmaceutical applications of mucoadhesive polymers will be addressed.

**Molecular features of mucosae**

Mucosae, and in particular mucosal fluids, are an essential part of mucoadhesive phenomena and thus deserve special attention when addressing the subject. Mucosal tissues cover natural body cavities, providing an epithelial barrier to the external environment. From a histological point of view, the mucosa is composed by (from the lumen to the submucosa) an epithelial layer, which can be of different types, the lamina propria and, at the GIT, the muscularis mucosae. Alongside variations in epithelial type, the presence and/or distribution of carbohydrate moieties at the glycocalyx, and roughness or folding features of mucosal surfaces may also differ, thus influencing the mucoadhesion phenomenon. Another important issue has to deal with shearing at mucosal sites. Moderate values can promote interaction of DDS with mucosae/mucus fluids and favor adhesion but, in cases where shear stress is too high, the time for the consolidation of adhesive bonds may not be long enough. In these cases, rapid washing-out and shear-thinning of mucoadhesive systems is a considerable obstacle to be surpassed when developing drug carriers to be administered in anatomical sites such as the ocular surface.

One defining feature of mucosae is the presence of a protective layer of fluid called mucus, which acts as a physical barrier to chemical and biological insult, as well as a natural lubricant opposing shear damaging. This fluid also plays important homeostatic functions, namely in regulating water balance and ion transport, clearing of cellular debris, mucosal immune-regulation, transporting sperm in the cervicovaginal tract, among others. Mucus is produced by specialized goblet cells or glands at the mucosa/sub-mucosa, except in the case of the stomach (mucus is produced by epithelial cells) and the vagina. In the last case, vaginal fluid results from the mixture of different liquids including mucus produced at the cervix. Mucus is a highly hydrated (≥95% water) non-Newtonian, viscoelastic system comprising a tridimensional network of randomly entangled mucins (2-5% of its mass), and presenting typical viscosity values in the range of 10-103 Pa.s at low shear rate but quite variable depending on particular composition, anatomical site and physiophathological conditions. The width of the mesh spaces delimited by mucin fibers has been previously estimated to be around 20-200 nm, though recent studies indicate that particles as large as 500 nm in diameter can still diffuse through mucus as long as adhesive interactions are minimal. Indeed, a recent study showed that the random distribution and entanglement of mucins leads to substantial heterogeneity in the mucus mesh diameter (50-1800 nm).

Mucins present in mucus are directly involved in adhesion phenomena. The term “mucins” is usually applied in order to include a somewhat heterogeneous group of glycoproteins that are coded by the MUC gene family but differ in their glycosylation and polypeptide sequences.

Secretory (or soluble) mucins are heterogeneous high molecular weight (106-107 g/mol and several micrometers in length) glycoproteins (≈75% carbohydrate/25% amino acid residues linked via O-glycosidic bonds between N-acetylgalactosamine and

serine or threonine residues) with a "bottle brush-like" structure: a long a flexible center protein chain presents regions densely coated with short glycans (the "brush"-like structure with approximately 3-10 nm in diameter and 50-200 nm in length), which alternate with folded "naked" hydrophobic regions that are rich in cysteine residues. Once in aqueous media, mucins entangle and originate heterogeneous, complex jelly-like systems which are stabilized by intra- and intermolecular hydrogen bonding, electrostatic interactions and disulfide bridging between cysteine residues present in non-glycosylated regions. The amount of disulfide bridging confers functionality to mucus and greatly influences its viscoelastic behavior.

Membrane-associated mucins form a tightly packed layer of mucins called the glycocalyx, which is responsible for docking mucus to subjacent epithelia and may also play a role in mucoadhesion, particularly in the specific recognition of extracellular ligands.

In all cases, complete mucus fluids comprise a mixture of secreted mucus and other components resulting from the mucosal environment such as cells and their products (including debris), microbiota and microbiota-produced substances (e.g., lactic acid in the vagina produced by Lactobacilli) or other fluids (e.g., tissue exudates). Thus, besides water and mucin, mucus commonly contains variable amounts of DNA, plasma proteins, immunoglobulins (particularly secretory IgA), lysozyme, lactoferrin, lipids and polysaccharides depending on its anatomical localization, and which may play important biological roles. For example, a thin lipid layer may form on the outer surface of mucus at different sites and act as a barrier to the diffusion of gastric acid in the GIT or water evaporation in the tear film. In the case of immunoglobulins, these biomolecules play an important part in aggregating and trapping pathogens in the mucus mesh, avoiding their spreading and promoting clearance. Mucus from different sites possesses distinct properties. These variations are known to influence significantly mucoadhesion and should be considered when designing anatomical site-specific drug delivery systems. Differences in pH values immediately strike the eye, even for the same mucosal tissue depending on considered site (e.g., in the stomach) or health status (e.g., in the esophagus, the intestine or the vagina). These pH variations may strongly affect the conformation and charge of mucin. Sialic acid (pKa = 2.6) strongly influences the electric charge of mucin (isoelectric point value ≈2); for example, nearly uncharged fluid may be expected in the apical layers of the stomach, while densely negatively charged mucus is present at the eye surface. These features may strongly influence mucoadhesion, particularly when electrostatic forces are involved in mucin/polymer interaction, although the microstructure and bulk rheology of mucus seem to be relatively insensitive to the variation in proton concentration. Further, mucus layer thickness, viscosity and turnover time are variable due to the dynamic structure of the mucin network, and can be altered depending on disease or physiological changes (e.g., higher viscosity/lower clearance of sputum in cystic fibrosis patients, cervicovaginal dryness in menopausal women) and external stimuli (e.g., increased respiratory mucus clearance upon contact with particulate matter, microstructural changes when in contact with different excipients or adhesive particles), with potential consequences in the behavior of mucoadhesive nanosystems.

**Mucoadhesion basics**

Bioadhesion is a particular case of adhesion and can be defined as the state where two materials, of which at least one is biological in nature, come in close contact and stay together for a substantial amount of time due to the establishment of interfacial bonding. If the biological surface is a mucosa, then the phenomenon is usually referred to as mucoadhesion, and interfacial interactions may occur mainly with mucus but also with the epithelial cell lining mucosae. Different materials may be used as mucoadhesives, though these are usually of polymeric or macromolecular nature.

Mucins are the main component of mucus fluids involved in mucoadhesion. Understanding the essential interactions between mucoadhesives and mucosal fluids is vital for the rationale development of mucoadhesive DDS. Different individual theories of adhesion have been suggested in order to explain mucoadhesion but only their combination can provide satisfactory understanding of the occurring phenomena. In the particular case of polymeric mucoadhesives and after initial intimate contact between mucus and polymer, diffusion seems to play an essential role in the establishment of adhesive interactions; polymers diffuse and entangle with mucin fibers, while bonding is simultaneously established/disrupted. Bonding may be either covalent (e.g., disulfide bridging with cysteine residues of mucin) or non-covalent (e.g., electrostatic forces, hydrophobic interactions, hydrogen bonding, van der Waals bonding). The dynamic balance between diffusion, physical entanglement and adhesive/repulsive interactions leads to the consolidation of adhesion.

**Nanosystems/mucus interactions**

Although the basic principles by which intermolecular interactions occur are identical, size matters when considering mucoadhesion. Indeed, polymers can present substantially different

mucoadhesive behavior either at bulk or nanoscale. The high surface-area-to-volume ratio of nanosystems is of utmost importance as the interface available to establish bonding dramatically increases. This usually means that, upon the establishment of adhesive interactions between nanosystems and mucin, bonding endures for longer periods than in larger structures. Counteracting repulsive forces, although present, are of lesser importance and cannot completely disrupt adhesive interactions. Nanosystems can still be transported through the mucin matrix but remain mostly entrapped in it. Besides the influence played on the available surface area, size also governs the ability of particles to fit in the low viscosity aqueous mesh spaces and channels formed within the mucin matrix that composes mucus. In one hand, mucoadhesive particles in the micrometric range tend to remain at the top layers of the mucus due to the inability to fit in these channels; on the other, particles as large as 500 nm have been shown to penetrate the mucin mesh of cervicovaginal mucus, and adhere or diffuse within its interstices, as assessed by multiple-particle tracking (MPT) using video microscopy. The previous upper limit size value may, however, not "fit" all types of mucus fluids present in the human body since the mucin meshes vary according to pathological and non-pathological conditions. For example, cystic fibrosis increases the micro-heterogeneity of sputum and only allows for smaller particles (around 200 nm or less) to be effectively transported, as assessed by MPT. In contrast, Lieleg et al. showed that particles as large as 1 μm can still be transported, even if presenting considerable hindrance, when mixed with reconstituted pig gastric mucus with low mucin concentration (0.25-0.5%). Thus, correct size adjustment seems to be a fundamental feature in the modulation of the mucoadhesive behavior of nanosystems in specific administration sites and pathophysiological conditions. However, the enhanced ability to penetrate mucus does not seem to be linear with size reduction. Again, MPT experiments using different sized mucus-penetrating polymeric nanoparticles (NPs), i.e., polymeric nanosystems in which the surface has been modified in order to minimize adhesive interactions with mucin (see below), showed unexpected diffusion behaviors [46, 98]. Mucus-inert NPs presenting diameters of 200-500 nm were able to show lesser hindrance to transport as compared to 100 nm counterparts. According to the authors of the study, 100 nm particles were capable to access smaller caliber channels within the mucus that often result in dead-end paths, thus reducing mobility of these particles due to physical entrapment rather than adhesive interactions with mucin. Thus, fine tuning of nanosystems size may be an interesting (and relatively simple, scalable and cost-viable) way to modulate mucoadhesion.

Another important aspect has to deal with surface chemistry and, in particular, surface charge. As mentioned above, the low isoelectric point of mucin determines its negative charge at most physiological pH values. This will then effect on the overall balance of adhesion strength as negatively-charged and positively-charged nanosystems will observe repulsive and attractive electrostatic forces, respectively, when in contact with mucus. Thus, positively-charged particles will present the potential to increase adhesion. This effect has been recently demonstrated for various polymeric NPs (size of approximately 200 nm) bearing different surface charge (negative or positive) by MPT using native pig GIT mucus (pH 6.5-7.5), and purified type II mucin reconstituted at different concentrations in different polyelectrolyte solutions with pH ranging from 4.2 to 7.4. Besides charge, chemical moieties present at the surface of nanosystems also impact on the mucoadhesive potential. In general, promoting adhesive interactions with mucus by any of the above mentioned mechanisms will increase the mucoadhesive potential of nanosystems; of particular interest, and the main topic of this review, is of course the presence of polymers at the surface.

**Modulation and assessment of the mucoadhesion of nanosystems**

As detailed above, the surface chemistry, charge and size of nanosystems determine their mucoadhesive behavior. These properties are tunable in order to maximize or minimize interactions with mucus fluids present at different mucosae, as summarized. When mucoadhesive systems are required, this can be simply achieved by using mucoadhesive polymers as matrix-forming materials, alone or in mixtures. However, not all the available mucoadhesive polymers can readily or easily used to produce NPs or, for instance, other nonpolymeric systems may be preferable (e.g., lipid-based nanosystems). In the last case, surface modification can be an alternative, either by attaching mucoadhesive polymers on preformed nanosystems (covalently or by simple adsorption) or by conjugating polymers with other matrix-forming materials. Another important aspect is the charge at the surface; positively-charged systems are preferred in order to maximize mucoadhesion. Chitosan (CS)-based NPs, in particular, are often considered as the typical example of highly mucoadhesive nanosystem. Also, hydrophobic nanosystems may possess high ability to establish adhesive interactions with hydrophobic domains of mucin, namely by promoting hydrophobic bonding. In contrast with the previous, mucus-inert nanosystems that avoid interaction with mucus may

also be desirable. Thus, mucus-penetrating NPs may be generally obtained by conferring a hydrophilic uncharged surface. The group of Justin Hanes at Johns Hopkins University (Baltimore, MD, USA) demonstrated this by modifying the surface of different polymeric NPs (100-500 nm) with a dense layer of low molecular weight poly (ethylene glycol) (PEG; 2-10 kg/mol). For example, MPT experiments showed that 200 nm PEG-modified NPs diffused in cervicovaginal mucus at rates near the ones predicted for the same sized nanospheres in water (up to around onelog hindrance), while non-PEGylated ones were nearly immobile (diffusion rates reduced by at least 3-log). The hydrophilic and nonionic nature of PEG avoid the establishment of hydrophobic and ionic bonding, respectively, while the short chain of the polymer diminishes mucoadhesive entanglement with mucin fibers. These observations for densely, short chain PEG-modified nanosystems have also been confirmed by other groups. For instance, when tested in vivo and after vaginal delivery to mice, densely PEG-modified NPs were shown able to readily distribute throughout the cervicovaginal tract and provide enhanced drug delivery to the underlying mucosa due to their ability to tackle the mucus barrier. Moreover, this strategy was proved advantageous in a mice model of vaginal herpes simplex virus type 2 (HSV-2) as evidenced for acyclovir-loaded PEGylated mucus-inert NPs.

As for size, depending on the properties of mucus present at different anatomical sites, particular size ranges may be preferable either to increase or decrease diffusion along the aqueous channels formed by the mucin matrix. In this case, adhesion is based on physical hindrance rather than on interfacial interaction, but it still presents substantial influence on the overall mucoadhesiveness of the nanosystems. One important thought to keep in mind is that the structure of mucus fluids is dynamic and can be highly variable on the surrounding conditions.

Besides pH, the possible presence of other substances (e.g., electrolytes, chemicals, pharmaceutical excipients) may impact the arrangement of mucin fibers and, thus, the mucoadhesive behavior of NPs. Moreover, the simple establishment of adhesive interactions of NPs with mucin can impact on the structure and the viscoelastic properties of mucus. Different experimental methods can be used for the characterization of the mucoadhesive potential of nanosystems. These can be classified as indirect or direct. The former are based on the evaluation and balance of contributing and detrimental interactions between nanosystems and mucins or other mucosal components (tissue or mucus), while the latter are performed in vivo (animal or humans) or in close proximity to the in vivo situation (ex vivo settings). A summary of different direct and indirect methods. The detailed description of each is out of the scope of this manuscript but readers are referred to a recent review by the authors on the subject.

**Mucoadhesive polymers**

**Synthetic polymers**

The mucoadhesive properties of PEG/PEO are controversial because of the lack of side functional groups (e.g., amine, carboxylic acid) that can specifically interact with components of mucin, though the mechanism would rely on the fast interpenetration of PEG chains with the lining mucus layer. The performance also depends on the administration route and the flux of biological fluids. Some groups have compared its performance with more popular mucoadhesive polymers such as CS [306]. In addition, there have been several attempts to improve the adhesiveness of PEG by its modification with PAA [307-308]. Cu et al. modified the surface of poly (lactic-co-glycolic acid) (PLGA) acid NPs with PEG to increase the retention time upon vaginal administration with positive results. On the other hand, none of the marketed vaginal mucoadhesive gels contains PEG.

Following this rationale, other copolymers that contain PEG and display additional features such responsiveness to environmental stimuli have been explored. This is the case of poly (ethylene oxide)-co-poly (propylene oxide) (PEO-PPO) block copolymers. Aqueous solutions of these biomaterials form gels upon heating and produce only minor irritation following administration by different parenteral routes. These biomaterials are commercially available in two architectures, the linear poloxamers and the branched poloxamines and a broad spectrum of molecular weight and hydrophilic-lipophilic balances. The former are only thermo-responsive, while the latter are also pH-sensitive.

Poloxamer and poloxamine gels usually display poor physical stability in contact with fluids due to the low microviscosity of the generated networks. Thus, the group of Cohn investigated different chemical modifications that increased the performance of PEO-PPO copolymers as matrices for drug delivery. To the abovementioned, work of Bromberg that modified different linear derivatives with PAA blocks, other approaches that include chemical modification and blending with have been introduced to optimize the mucoadhesiveness of these copolymers. These systems were envisioned for different administration routes. Huang et al. used a different approach and modified poloxamers with 3,4-dihydroxyphenyl-L-alanine (DOPA), an amino acid found in mussel adhesive proteins. Assays of interaction of the modified copolymer with bovine submaxillary mucin

showed the sharp increase of the viscosity, indicating the ability of the new material to interact with the glycoprotein.

Blends are a relatively simple strategy to combine the properties of different polymers. Bilensoy developed a vaginal gel of Pluronic® F127 (20%) with low concentrations of mucoadhesive polymers (e.g., poly (acrylate) and HPMC) for the localized release of the antifungal drug clotrimazole. However, mucoadhesion was not tested. Majithiya et al. used a similar composition for the nasal delivery of sumatriptan. The mucoadhesive force estimated as detachment stress was determined using sheep nasal mucosal membrane and increased with growing concentrations of poly (acrylate). More recently, others used similar approaches for the mouth.

As mentioned above, PEG has been also used to confer mucus-penetration properties to different types of nanocarriers. The low molecular weight and the high surface-modification density are fundamental to minimize the electrostatic and the hydrophobic interactions with mucus, the phenomenon being more remarkable in larger NPs. These studies support the improved transport of tetanus toxoid encapsulated within poly (lactic acid) (PLA) NPs coated with PEG across intestinal and nasal mucosae. This approach was useful to prepare non-viral gene vectors that penetrate human mucus barriers (e.g., sputum).

**Poly (acrylic acid) and poly (methacrylic acid) derivatives**

PAA, also known as carbomer, is a high molecular weight polymer of acrylic acid used as a viscosity modifier in semi-solids. Due to the good biocompatibility, they have been approved for use in non-parenteral pharmaceutical products.

Due to the presence of pendant carboxylic acid units (one per repeating unit), PAA exhibits very high adhesive bond strength in contact with tissues, enhancing the mucosal penetration of drugs; the presence of a unionized carboxyl group is critical in the formation of a strong interaction with mucus. These interactions are thought to be a result of the hydrogen bonds between PAA and the proton-accepting groups in mucin. Due to the presence of numerous carboxyl groups, PAA likely adopts a more favorable macromolecular conformation and an increased accessibility of its hydrogen-bonding groups when compared to other polymers. Additionally, due to its ability to control the release of drugs, PAA and, mainly its derivatives combining several substituted repeating units [e.g., poly(methylacrylate)], have been extensively exploited as polymeric excipients for the development of conventional DDS for non-parenteral. However, their specific application for NPs is still limited, being now under consideration for the production of colloidal carriers.

To fine tune the properties of PAA, some research groups have synthesized copolymers of PAA and PEG. PEG has been reported to act as an adhesion promoter between PAA and mucin by linear diffusion of the PEG chains into the acrylic networks and the mucin layer. In this context, PAA-PEG NPs have been reported to improve the transcorneal diffusion of pilocarpine and possess excellent in vitro antitumor activity to drug-sensitive as well as drug-resistant cancer cells. In another work, papain/PAA blend NPs were used to study the transport through intestinal porcine mucus compared to unaltered PAA NPs. Results demonstrated a strongly enhanced permeation performance with respect to pure PAA counterparts owing to the local disruption of mucus by papain. Improved transport rates, reduction in mucus viscosity and the retarded release of hydrophilic macromolecular compounds make proteolytic enzyme functionalized NPs very promising to improve the targeted drug delivery of drugs at different mucosal surfaces. Later, it was demonstrated that the majority of the papain functionalized PAA NPs were able to cross the mucus layer and remained in the duodenum and jejunum, where the absorption of the drug primarily occurs. In a similar approach, cysteine/PAA microparticles increased the permeation of vitamin B12 across the intestinal mucosa, taking advantage of the thiolated particles compared with unmodified PAA ones. The group of Bromberg has extensively investigated the modification of linear poly (ethylene oxide-co-propylene oxide) block copolymers, commercially known as Pluronic®, with PAA terminal segments to confer the copolymer mucoadhesive features and assessed these new materials in different DDS, including gels, matrices and polymeric micelles. Poly(methacrylates) are hydrophobic synthetic polymers composed of pristine and modified methacrylic acid repeating units with good degree of biocompatibility, though very limited biodegradability. In this context, a broad variety of derivatives commercially available as Eudragit® have been developed. These copolymers have been approved by regulatory agencies for use in medical devices and usually nonparenteral pharmaceutical products. For example, the main components of hard and soft contact lenses are poly (methyl methacrylate) (PMMA) and poly (hydroxyethylmethacrylate) (PHEMA), respectively. Thus, these copolymers in general and PMMA in particular have been widely employed as excipients in the development of classical controlled release pharmaceutical dosage forms. More recently, some authors have suggested their biocompatibility for parenteral routes. In fact, PMMA is the main component of conventional and medicated bone cements used in hip replacement interventions. In more recent past, they have been

explored for the production of NPs, although due to its bio-inertness the use as colloidal carrier has been somehow neglected. PMMA-based NPs can be prepared either by the direct polymerization of the MMA monomer or from pre-formed polymers of different molecular weight by emulsion/solvent evaporation or nanoprecipitation techniques.

Despite the advantageous mucoadhesive properties, PMMA has been reported to show an incomplete drug release, possibly due to its hydrophobic nature. To improve drug release, recent strategies are focusing on increasing polymer hydrophilicity by synthesizing functionalized PMMA with carboxylic functional groups or by formulating PMMA composites with hydrophilic polymers. An alternative approach consists in enhancing drug diffusion within the polymer matrix by including plasticizers in the formulations. So far, there have been few attempts to encapsulate proteins or DNA in PMMA NPs. The incorporation of bovine para-influenza type 3 virus (BPI-3) was reported using PMMA NPs as vaccine carriers. Higher levels of virus-specific antibody have been reported in comparison to soluble viral proteins alone. Cationic aminoalkylmethacrylate copolymer NPs were evaluated for their use as potential anionic antisense oligonucleotide carriers. A significant portion of adsorbed oligonucleotides were protected from enzymatic degradation. The cellular uptake of oligonucleotides into Vero cells was significantly enhanced. To further adjust the features of the delivery system to the application, NPs of the amphiphilic poly (methacrylic acid) (PMAA)-grafted-PEG were prepared by dispersion polymerization and assessed for the oral administration of calcitonin. These NPs exhibited pH-sensitivity release, suitable for gastrointestinal administration and demonstrated to be safe to the intestinal mucosa. Similar NPs were prepared by emulsion polymerization for DNA vaccine applications. The NPs reversibly adsorbed large amounts of DNA, mainly through electrostatic interaction, preserved its functional structure, efficiently delivered it intracellularly, induced significant antigen-specific humoral and cellular responses and greatly increased Th1-type T cell responses and CTLs against HIV-1 Tat and were non-toxic both in vitro and in vivo. The combination of poly (metahcrylate) with other polymers is another approach to develop nanocarriers with more tuned properties. For example, PMMA NPs surrounded by a cationic branched poly (ethyleneimine) (PEI) shell were synthesized via a graft co-polymerization of methyl methacrylate from branched PEI to encapsulate plasmid DNA. PEI is able to condense DNA into compact particles and protect it from enzymatic degradation. Those NPs internalized and released the plasmid DNA into HeLa cells very efficiently, with less toxic effects than DNA associated with PEI alone.

More recently, Seremeta et al. encapsulated the antiretroviral efavirenz within NPs of pure polycationic poly (methacrylate), a derivative that binds to mucin through electrostatic interactions, and blends with poly (epsilon-caprolactone). Overall these copolymers have become key players in the development of mucoadhesive nano-DDS.

**Inhalator administration**

Thus, the airways provide a very large absorption bed that can be advantageous in the treatment of pulmonary diseases (e.g., asthma) and overcoming local infectious diseases due to the restriction of systemic exposure and adverse effects but also for the systemic delivery of drugs in the so-called transpulmonary route. At the same time, the potential of this alternative route has promoted the development of novel aerosol technologies that the administered dose and the deposition level. Inhalation pharmaceutical products must fulfill a number of features that include aerosol particles with mean aerodynamic diameter between the 0.5 and 5 μm to favor deposition in the deep lung, aerosol particles with low size distribution and high reproducibility, dissolution or adhesion to the lining mucosa and appropriate drug release and permeability. In this context, different nano-DDS have been conceived for inhalatory administration. Surprisingly, the research at the interface of nano-DDS for inhalation and mucoadhesion is elusive and the reports countable. In two different works, the group of Lehr showed the beneficial effect of lecithin to increase the adhesion of liposomes to alveolar macrophages. Others coated different nanocarriers with PAA, CS [399] and HPC. Since tuberculosis (TB) is primarily an infection localized in the lungs, Khuller extensively assessed the potential of the inhalation route to treat the pulmonary for of TB by employing different inhalable dry powders loaded with first- line anti- TB drugs. This approach was also advantageous to actively target alveolar macrophages, the intracellular TB reservoir of the mycobacterium. Following this trend, our group has successfully nanoencapsulated the anti-TB drug rifampicin within “flower-like” polymeric micelles and used this platform to develop a liquid rifampicin/isoniazid combination that showed improved oral bioavailability of rifampicin. The coating of these polymeric micelles with CS and hydrolyzed GalM conferred those recognition and mucoadhesive properties. These novel nano-DDS showed significantly greater uptake by macrophages in vitro and good aerosolization ability, thus opening new therapeutic opportunities to treat this global health threat.

**Ocular administration**

The eye is a complex and sensitive organ, consisting of three main layers, the outer coat or the sclera and cornea, a middle layer or uveal coat and the inner coat or retina. The sclera is made of fibrous tissues shaped as segments of two spheres, the sclera and cornea. From the drug absorption point of view, cornea and conjunctiva represent the two major mucosal barriers that drugs must cross to reach the possible local of actions. The cornea is a clear, transparent, avascular tissue to which nutrients and oxygen are supplied by the lachrymal fluid and aqueous humour. The corneal epithelium consists of 5 to 6 layers of columnar cells squeezed forward by the new cells. Replacement of the epithelial cells occurs by mitotic division of the basal layer every 4 to 8 days. The conjunctiva is a thin transparent membrane, which lines the inner surface of the eyelids and is reflected onto the globe. At the corneal margin, it is structurally continuous with the corneal epithelium.

Conjunctival epithelium is composed by 5 to 7 cell layers connected by tight junctions, which render the conjunctiva relatively impermeable. The membrane is vascular and moistened by the tear film. Despite the apparent easy accessibility, the eye is well protected from foreign materials by several efficient mechanisms forming a physical-biological barrier, such as blinking, induced lacrimation, tear turnover, nasolacrimal drainage, which cause rapid removal of drugs from the eye surface and from the back cornea. Additionally, the blood-retinalbarrier (BRB) and the extra ocular epithelia represent the obstacle in the drug delivery to the choroid, retina, and vitreous. Only a fraction of the drug administered orally or by subcutaneous or intramuscular routes reaches the retina, requiring large doses to be therapeutically effective. Moreover, approximately 95% of the administered drug is removed by the tears and does not reach the site of action or, conversely, is absorbed in to the systemic circulation leading to adverse effects. In this context, DDS for topical ocular administration are an interesting and promising way to treat eye diseases, especially because they are a non-invasive way of releasing drugs in a controlled fashion directly to a specific compartment of the eye and because they prolong the residence time of the drug in the site of action and reduce the amount of drug that is absorbed by alternative routes. Among the possible strategies for ocular drug delivery, which include biocompatible viscous solutions and film-forming gels, liposomes, solid lipid NPs (SLNs), microspheres and medicated-contact lenses, the use of biodegradable nanocarriers has been considered a very promising system, though scarcely capitalized until now. In ophthalmic applications, it is convenient that particulate systems have an appropriate size, preferably within the nano-range, in order to avoid irritation, foreign body sensation, and discomfort to the patients. Other factors depending on the success of NP systems for ocular drug delivery lays on optimizing lipophilic-hydrophilic properties of the polymer-drug system, optimizing rates of biodegradation, and safety. However, the highly sensitive corneal/conjunctival tissues require great caution in the selection of the carriers towards eye penetration to maximize drug transport.

Different biomaterials have been used to prepare NPs, such as poly (acrylates), PLA, PLGA, dextran, ALG, collagen, hyaluronic acid and CS and their ocular application evaluated. CS has been investigated as a superior mucoadhesive cationic polymer due to its ability to develop molecular attraction forces by electrostatic interactions with the negative charges of mucin, as mentioned before. CS NPs may encapsulate a wide range of drugs for ocular purposes, maintaining their biological activity as antibacterial or anti-inflammatory agents. CS NPs are also able to interact and remain associated to the ocular mucosa for extended periods of time and after inoculation with rabbit ocular surface, no signs of inflammation or alteration were observed. Simultaneously, it was confirmed that CS NPs are up-taken by conjunctival and corneal epithelia in vivo. CS NPs cross linked with sulfobutylether-cyclodextrin were developed to encapsulate econazole, presenting sustained drug release and better in vivo antifungal effect in rabbits compared to the free drug for 8 hours. CS NPs have also been exploited to develop gene delivery systems to the eye, taking advantage of the synergic mucoadhesive and transfection enhancing properties of the polymer. As example, to determine whether CS NPs would be suitable for intraocular use, pDNA carrying the ubiquitously expressed CBA-eGFP expression cassette was compacted administered to adult wild-type albino mice. At day 14 post-injection, substantial green fluorescent protein expression was observed exclusively in the retinal pigment epithelium in eyes treated with GCS NPs but not in those treated with pDNA or the vehicle. Moreover, no signs of gross retinal toxicity were observed, and there was no difference in electro-retinogram function between NPs, pDNA, or vehicle-treated eyes. In a similar approach, formulations of CS-DNA NPs were administered to rat corneas as model animal resulting in luciferase gene expression 5 times greater than following administration of PEI-DNA NPs [460]. Even though these formulations were not assessed in topical administration, they open new research avenues towards less invasive ophthalmic therapies. PLGA and PLGA-PEG NPs were used to encapsulate melatonin, a neuro-hormone secreted by the pineal gland able to modulate intraocular pressure [461].

Topical application of melatonin formulations caused ocular hypotension in rabbit eyes, thus emerging as an alternative approach to treat glaucoma. The maximum effect (5 mmHg), which was obtained with the PLGA-PEG formulation, occurred at 2 h and persisted up to 8 h, with a significant difference compared to melatonin aqueous solution and PLGA NPs, showing that mucoadhesion generally prolongs the contact time of a formulation with the eye surface. PLGA NPs were also used to deliver cyclosporine A to the eye, for the treatment of inflammation of the rabbit eye surface as model animal. The cytotoxic effect of NPs was found to be time and concentration dependent and also showed significantly higher degree of cellular uptake, tear film concentration of the drug and double bioavailability values in comparison with the drug emulsion.

Another polymer used to develop NPs for drug delivery was PAA. Cross-linked particles based on PAA and PEG with nanometer size and spherical shape loading pilocarpine demonstrated enhanced drug release and permeability into the corneal mucus stratum because of NP assembly and mucoadhesion.

Recently, polymeric micelles of PEO-PPO have been evaluated for the encapsulation of the anti-glaucoma agent ethoxzolamide [463]. However, this delivery system is not mucoadhesive what represents a limitation for this administration route. On the other hand, the use of higher concentrations of these thermo-responsive copolymers would enable both the nanoencapsulation of the drug and the formation of a gel upon contact with the ocular mucosa.

It is now well-established that polymeric mucoadhesive NPs are able to deliver any drug at the right time in a safe and reproducible manner to a specific anterior and posterior segment of eye at required level. In this scenario, the exploration of more sophisticated mucoadhesive nano-DDS in the coming years is ensured.

**Intranasal administration**

The nose is a complex organ entailed to perform a variety of functions that range from olfaction to humidification, warming and filtering of the inhaled air before it reaches the trachea and the lungs [489]. The nasal mucosa comprises two layers, the luminal epithelium containing goblet cells that produce the mucus that covers the epithelium and the underlying lamina propria that is rich in blood and lymphatic vessels, nerves, glands and cells of the immune system. Due to the high surface area offered by the nasal mucosa, the high irrigation and the presence of lymphocytes and mast cells, it has been capitalized for the local and systemic drug delivery employing different products and devices. However, the small dimensions and the great sensitivity to xenobiotics impose limitations to the kind of drug and DDS that can be implemented; usually, drugs administered by the nasal route must be very potent to attain therapeutic concentrations in very small administered volumes. The design of nano-DDS could expand the applicability of this route to other drugs. However, reports on mucoadhesive nano-DDS are almost unavailable. One of the few works was published by Jain et al. that developed mucoadhesive multivesicular liposomes (26-34 μm) coated with CS and Carbopol® for the transmucosal (systemic) delivery of insulin. The carriers contained high protein payloads between 58-62%. Furthermore, administration of the mucoadhesive liposomes to streptozocin-induced diabetic rats reduced plasma glucose levels in 35% for 2 days, a better performance than the uncoated ones that reduced them to a similar extent though for only 12 h. It is worth noting that this DDS was also administered by the ocular route with even more promising results; the hypoglycemic effect was observed for 72 h.

The transport of drugs from the systemic circulation into the CNS is constrained by the presence of the blood-brain barrier (BBB) and the bloodcerebrospinal fluid barrier (BCSFB). For decades, these barriers prevented the use of therapeutic agents for the treatment of Alzheimer's disease, stroke, brain tumor, head injury, spinal cord injury, depression, anxiety and other CNS disorders. The greatest challenge faced by the development of new therapeutics for the treatment of diseases of the CNS is overcoming these barriers and the achievement of therapeutic concentrations in the cerebral parenchyma. Direct injection of therapeutic agents into the brain by stereotaxis is possible though this practice entails serious drawbacks associated with the invasiveness of the procedure and the emergence of immunological side effects that limit its application in clinics. Attempts to transiently increase the permeability of the BBB (e.g., with mannitol) were also assessed. However, opening the barrier allows the entry of toxins, undesirable molecules and eventually pathogens to the CNS, resulting in potentially significant damage. The capitalization of anatomical pathways represents an appealing approach to localize the drug release and minimize systemic exposure.

The exposure of the intranasal mucosa to environmental NPs and contaminants and their effect on the CNS revealed the presence of a direct noseto-brain transport pathway that bypasses the BBB and the BCSFB. It is interesting to note that the olfactory region is contiguous to the cerebrospinal fluid (CSF) tracts around the olfactory lobe. Drug transport to the brain would be possible through delivery into the olfactory CSF, providing that the molecule is transported across the nasal epithelium and subsequently transported across the arachnoid

membrane that separates the sub-mucosal space of the nose and the olfactory CSF. For example, Wang et al. reported on the significant increase of the bioavailability of methotrexate in the CSF with respect to plasma after the intranasal (i.n.) administration. Different mechanisms have been proposed for this direct passage though the transcellular one appears as the most relevant, while the paracellular one has been less investigated. In the case of drug- loaded nanocarriers, they would be internalized by the neuronal terminals of the olfactory nerve system that emerge in the brain and end at the olfactory neuronal epithelium. Thus, the i.n. administration of nano- DDS enhances the bioavailability of the cargo in the CNS. Initially, the upper limit for efficient transport across the i.n. mucosa was reported to be 100 nm though, though more recently NPs as large as 300 nm were also shown to reach the CNS. Sosnik and coworkers exploited efavirenz- loaded polymeric micelles developed for oral administration to target the CNS, one of the most challenging HIV reservoirs, employing the i.n. route. The relative exposure index in CNS was increased up to 3 times with respect to plasma. In contrast, the i.v. administration resulted in CNS concentrations significantly smaller than in plasma.

On one hand, the in. route presents remarkable advantages such as (i) minimal invasiveness, (ii) painlessness, (iii) self- administration and (iv) high patient compliance. On the other hand, only small volumes could be administered per nostril at each administration time and only highly concentrated systems could enable the attainment of therapeutic doses. This disadvantage has most likely precluded the bench- to- bedside translation of intranasal products.

**Future perspectives**

The capitalization of mucosal tissues has emerged as a promising and solid strategy to improve the bioavailability of drugs, to reduce systemic exposure and to subsequently increase the therapeutic index by means of the design of mucoadhesive nano-DDS. On the other hand, the variability of mucosae and their properties challenge the design of versatile platforms. The broad spectrum of natural, synthetic and semisynthetic polymers commercially available and (in many cases) approved by regulatory agencies for use in pharmaceuticals enable the adjustment of the properties to the specific properties of the specific mucosa to be addressed and, at the same time, increase the possiblity of technology transfer. However, regardless of the variety of mucoadhesive nanotechnology platforms that have been investigated in academia and the richness of the intellectual property derived from these works, mucoadhesive nano-DDSs have not reached the market yet. This situation reveals the difficulties faced to conduct clinical trials, even for more advanced products of already-approved drugs. In this scenario, the coming years will be crucial to consolidate the field and to place the first pharmaceutical products with such features that will support the valuable contribution of these unique nanosystems to treat disease.

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