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Thermodynamic and Kinetic Aspects Involved in the Development of Nanocarriers and Drug Delivery Systems Based on Cationic Biopolymers

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Abstract: During the last years we have seen an increasing number of reports describing new properties and potential applications of cationic polymers and derived nanostructures. This review gives a summary of their applications in drug delivery, the preparation methods for nano and microstructures and will attempt to give a glimpse on how their structure, chemical composition and properties may be affected or modulated as to make them suitable for an intended application as drug delivery nanocarriers. The compositional complexity with the existence of several reacting groups makes cationic nanostructures critically sensitive to the contribution of thermodynamic and kinetic parameters in the determination of the type and stability of a particular structure and its ability to respond to changes in environmental conditions in the right time frame. Curiously, and contrarily to what could be expected, despite the fact that cationic polymers can form strong electrostatic interactions the contribution of the entropic component has been often found to be very important for their association with negatively charged supramolecular structures. Some general considerations indicate that when considering a complex multimolecular system like a nanocarrier containing an active ingredient it is frequently possible to find conditions under which enthalpic and entropic contributions are compensated leading to stable structures with a marginal thermodynamic stability (free energy change close to zero) which make them able to respond relatively fast to changes in the environmental conditions and therefore suitable for the design of smart drug delivery systems. Like with other nanocarriers, it should always be kept in mind that the properties of cationic nanocarriers will depend not only on their chemical composition but also on the properties of the structures formed by them.

Keywords: Cationic polymers, drug delivery systems, enthalpy, entropy, nanocarriers.

INTRODUCTION

There are three main interrelated aspects that should be kept in mind when designing a nanocarrier for a special application. These are: a) energetics, b) dynamics and c) chemical structure of all the components. The interactions involved ultimately determine the changes in free energy of the chemical processes that are involved in any of the chemical reactions and thus can affect the structure and chemical nature of the components in the nanocarrier. When dealing with biomolecules and especially with macromolecules like cationic polymers, it is clear that the presence of several chemical groups leads to a variety of reacting modes with different kinetics. Therefore, the time dependence of each should be considered when dealing with structures that should have stability during a prolonged period of time that could range from hours to several months and even years. Finally, the chemical structure determines the type and nature of the interactions that can be established. In the case of cationic polymers, more that with other molecules, these include: a) interactions of very different type and strength, b) small contribution to the total energy of any of them, c) strong dependence of the geometry of the interacting groups and d) reduced dynamics due to the covalent attachment of several groups in a single molecule which leads to physical impairment of certain movements.

When dealing with biopolymers, it is often forgotten that the basic concepts of thermodynamics were translated from big machines to simple chemical reactions involving chemical entities with virtually indefinite stability [1]. Polymers have not only a compositional complexity with different chemical groups attached in a single molecule but also display what we may call "conformational complexity". Altogether, these characteristics make the definition of chemical potential anything but straightforward. First of all, the existence of several chemical groups within a single molecule determine that there may be a variety of reactions, therefore we should consider the chemical potentials involved in each of the main reactions that take place within the time frame of interest and by extension the chemical potential of a polymer will be determined by the algebraic sum of them [1]. It should be kept in mind that, for the reasons mentioned above, the idea of equilibrium and thermodynamic parameters associated with it has no practical value. Therefore, with the kinetic characteristics involved in the approach to the "equilibrium state" already mentioned, we could expect to obtain representative thermodynamic parameters of the system under study when they are obtained from measurements that are taken at between 4 and 6 times longer than the time at which the kinetics of the main reaction halves [1]. This somehow theoretical concept can be translated into practical terms by measuring thermodynamic parameters until the same figures are obtained at two different scanning speeds. Although this seems a basic concept it is still frequent to see in the published literature that differential scanning calorimetry is used with polymers and macromolecues at scanning speeds greater than 1 °C.min^{-1} without ever checking if the same values are obtained at slower rates.

The existence of several conformations within a single molecule or within a nanostructure formed by several individual polymer molecules leads inevitably to fluctuations in the structure that take place within the time frame of interest. Therefore, we should always keep in mind that when speaking of chemical potential, conformation or structure of a nanocarrier they will always be represented by an average value that is characteristic of the system in a medium of defined composition. In this context, any molecular

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entity that interacts with the carrier will surely affect its stability. In the more general case of a nanocarrier, if there is a spontaneous binding of an active pharmaceutical ingredient (API), this should be led by a reduction in the free energy of the system which will thus lead to a stabilizing effect of the structure in most of the cases [2-5].

We must also note here that, the differences in the reaction rates of the various processes involved may lead to different metastable states which are dependent on the pathway by which the system arrives to the final compositional state [1, 6]. Furthermore, it has been frequently observed that cyclic operations lead to the creation of different metastable states depending on how the changes are performed on the system [1,6]. This is very important at the moment of standardizing the preparation procedure of a nanocarrier [6].

When dealing with supramolecular structures it is not easy to disclose at the molecular level the contributions of changes in enthalpy and entropy associated with any given phenomena. However, some general considerations indicate that considering a complex multimolecular system and its solvent, it is reasonable to expect that in isothermal processes any formation of bonds led by a decrease in enthalpy will decrease the number of conformations and movements of the system leading also to a decrease in entropy. The same is true when considering the rupture of certain bonds within the structure which will be reasonably linked to an increase in its entropy. Thus, it is reasonable to expect that in most of the situations we will be able to find a link between enthalpy and entropy changes.

As for practical reasons, changes involving a few $Kcal.mol^{-1}$ are those that could participate or be involved in biological applications (i.e. drug release). It has been commonly observed that in the case of complex biological molecules like proteins and polysaccharides enthalpy and entropy changes are compensated. This leads to stable structures with a marginal thermodynamic stability (the free energy change associated to any particular change in structure is close to zero) which make these structures able to respond relatively fast to changes in the environmental conditions [1]. These basic concepts are of fundamental importance when dealing with technological applications using cationic polymers, mainly because they have the chance to form many electrostatic interactions (usually very strong) which could potentially lead to supramolecular structures (i.e. nanocarriers) with a great stability but that lack the potential to respond or be sensitive to environmental changes with at least a moderate displacement in its structure that allow the release of the active components as is required in the case of drug delivery systems (DDS). Curiously, we found that despite the possibility of forming strong electrostatic interactions, the association of chitosan with negatively charged casein micelles is driven by hydrophobic interactions [7]. Furthermore, the contribution of the entropic component has been also shown to be important for the association of other cationic polymers (bearing tertiary amine and quaternary ammonium groups) with casein micelles and cellular membranes [8-11].

CLASSIFICATION OF CATIONIC POLYMERS - CHEMICAL STRUCTURE AND GENERAL PROPERTIES

Although there are several groups of cationic polymers, most of them possess primary, secondary, tertiary amine or quaternary ammonium groups. Here lays a first grouping factor: a) polymers having a group that can bear a positive charge or no charge depending on the protonation state and b) polymers bearing a permanent positive charge (those possessing a quaternary ammonium).

According to their origin cationic polymers can be classified as: a) those derived from natural polymers and b) synthetic. Cationic polymers are rarely found in nature as so. There is just one polysaccharide (chitosan) the others are proteins, which being zwitterions can bear cationic and anionic groups and their net charge will depend on the pH. From the proteins the most widely studied proteins for pharmaceutical and food applications are the collagens and their denatured derivatives the gelatins [12-13].

Chitosan

Chitosan is a linear polysaccharide produced by deacetylation of chitin, the second most abundant polysaccharide in nature, present in the shells of crustaceans and mollusks [14]. This cationic biopolymer comprising units of N-acetylglucosamine and D-glucosamine randomly distributed (Fig. **1**) has received increasing attention due to its biocompatibility, biodegradability, antimicrobial activity, low immunogenicity and non toxicity [15-18]. Furthermore, due to the large amount of reactive amine and hydroxyl groups present in its structure, chitosan has been frequently modified with cationic molecules and other small molecules to achieve the desired function.

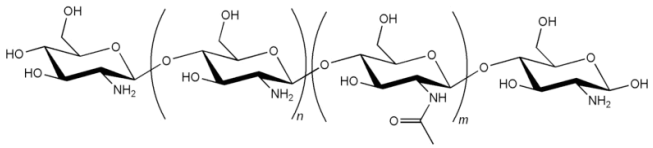

Fig. (1). Chemical structure of chitosan.

Because of these excellent properties, multiple types of Chitosan based drug carriers have been developed for oral, parenteral, nasal, transdermal, vaginal, rectal and cervical administration. There are various methods for preparing chitosan nanoparticles (NPs). The choice of either method depends on the type of particle that is required, in terms of size and shape [19-20]. Among them, it can be mentioned:

- Ionic gelation or ionic cross-linking: This method is the most widely used for generating chitosan NPs because it can be performed at room temperature and the use of solvents is not required; also uniform NPs can be easily obtained with an adjustable size and degree of deacetylation of chitosan. Sodium tri-polyphosphate is generally used as cross-linking agent and when added to a solution of chitosan in acetic acid with constant stirring, electrostatic interactions occur between the amine groups of chitosan and negative groups of the polyanion and NPs are spontaneously formed. This simple method has been widely used for encapsulation of proteins, genes and anticancer drugs.
- Desolvation: In this method a flocculant (usually sodium sulfate or acetone) is added dropwise to an aqueous solution of chitosan. Due to greater affinity of salt to water, there is a decrease in the solubility of chitosan that leads to precipitation of NPs of about 600 nm formed by hydrogen bonds between polymer molecules.
- Covalent cross-linking: This method involves the formation of covalent bonds primarily between amine groups in chitosan and a functional crosslinking agent, such as glutaraldehyde, polyethylene glycol (PEG) dicarboxylic acid or some monofunctional agents such as epichlorohydrin.
- Spray-drying: This method can be used to prepare quickly and in one step chitosan NPs powder; also is a technique that greatly improves the stability of the colloidal nanoparticles.
- Self-assembly: Amphiphilic compounds dispersed in water can form NPs by self-assembly. By this method it is possible to prepare structures with a hydrophilic coating and a hydrophobic core that may be useful to carry both polar and nonpolar drugs.

The small size and large surface area to volume ratio of chitosan NPs gives them unique properties, so that they are being used in many products and the development of new applications in pharma-

ceutical, tissue engineering, enzymes immobilization, food packaging, biosensing, food pres servation, emulsification and stabilization, waste water treatment, among others [21].

Two methods can be used for loading drugs into Nps: incorporating the drug as the particle is being prepared, or after the particle is already formed. In the latter case, the drugs can be added to the matrix of the particle or adsorbed on its surface. Loading reaches generally a greater amount of drug when it is incorporated during the preparation process, but this may entail certain drawbacks related to the final shape and size of the structure [22].

One of the advantages of using chitosan NPs to carry drugs is that by varying the degree of deacetylation and the molecular weight of the polymer it is possible to prepare structures with different release rates of the drug, thus achieving a controlled/sustained release. Furthermore, these NPs can be modified to make them pH sensitive, thermo-sensitive or may be supplemented with various additives conjugates on the surface to achieve greater accuracy in targeting.

Besides the use of chitosan to generate NPs, it has also been described the use of this polymer for the formation of nanocapsules. In a formulation reported by Gnanadhas *et al*., nanocapsules consisting of chitosan and dextran-sulphate were used to transport ciprofloxacin [23]. With these structures, targeting efficiency and death of the pathogen using a lower dose than that used with the antibiotic in the free state was achieved, probably because of an increased circulation time of the nanocapsules compared to the free drug.

Chitosan NPs are those that have received more attention due to their ease of preparation, improved stability, low toxicity and the fact that they can be used on different routes of administration [24]. Also, amine and carboxyl groups in chitosan allow NPs to be combined with mucus glycoprotein, forming hydrogen bonds, and resulting in adhesive effects [25]. Moreover, positive charges present in chitosan NPs give them a strong affinity for negatively charged molecules present in cellular membranes.

Among some of the most important pharmaceutical applications of chitosan NPs can be highlighted: their use in gene delivery (i.e., multidrug resistance gene, MDR1), cancer therapy (i.e., paclitaxel), mucosal delivery (i.e., insulin), topical administration and ocular delivery [24, 26-30].

Collagen - Gelatin

Gelatin is a water soluble protein obtained from the partial hydrolysis of collagen, the most common protein in the animal kingdom, constituent of bone, cartilage and skin (Fig. **2**). The structure and properties of gelatin depend on the source of collagen as well as the preparation technique [31].

There are two types of gelatin which differ in their manufacturing method, type A is obtained by acid hydrolysis of collagen and has an isoelectric point at pH 7-9, while type B gelatin is obtained by basic hydrolysis (isoelectric point at pH 4-5) [32]. Gelatin applications in the areas of food, cosmetics, photography and pharmaceutics mainly rely on their viscoelastic properties and the ability to form gels. In the pharmaceutical industry, most of the production of gelatin is used to form hard and soft capsules gelatin capsules, tabletting and for encapsulation and microencapsulation, preventing oxidation and making formulations most acceptable. It is also widely used in solutions, inhalants, topical, vaginal, dental preparations and injections. Therapeutically, gelatin applications are varied; it has been used in the preparation of dressings for wounds, as a substitute for plasma, as bioadhesive or for devices for sustained release of drugs in living tissues. It has also been extensively studied for use in bone regeneration. In this respect it has been demonstrated that the hydrogel formed by gelatin and TGF beta is a promising tool for surgical repair of the skull [33].

It has been described the preparation of gelatin nanoparticles by the method of desolvation/coacervation, where a homogeneous solution of charged macromolecules is subjected to a liquid/liquid phase separation resulting in a inferior phase rich in polymer and a transparent upper phase. With the subsequent addition of a salt or an alcohol coacervation occurs, thus, controlling the turbidity, the desired degree of crosslinking of the nanoparticle is reached [34]. In this aspect, gelatin nanoparticles have been used to transport non-viral plasmid DNA and for the generation of plasma DNA polyplexes formed by electrostatic interactions between positively charged gelatin and negatively charged DNA [35-36]. Also, it has been described the use of gelatin nanoparticles to transport albumin [37], oligonucleotides [38], anticancer drugs such as paclitaxel [39] and antimalarial drugs like chloroquine phosphate [40].

In recent years the crosslinking of gelatin alone or in combination with other biopolymers has also been extensively evaluated for numerous ocular applications, primarily as bio-adhesives to stabilize retinal tissue [41-42] and as cellularised scaffolds for the repair of the corneal stroma [43].

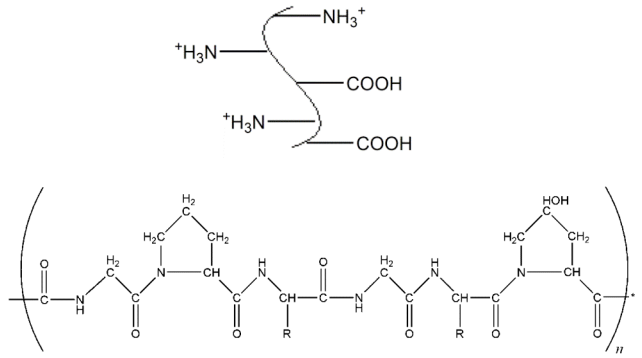

Fig. (2). Scheme of gelatin structure.

Poly-Lysine

Poly-lysine is a polypeptide comprising between 25 and 35 L-lysine residues joined by peptide bonds characterized by having a high number of primary amino groups that allow complexation with polyanions, so it was one of the first cationic polymers studied for the intracellular delivery of nucleic acids (Fig. **3**) [44-45]. However, their high cytotoxicity and poor transfection efficiency, greatly limit its use in this field [46-51].

Various strategies have been attempted to overcome these problems [52]. To reduce toxicity PEG units have been introduced in the structure and to promote cellular uptake various ligands have been incorporated such as antibodies, folate, transferrin, fibroblast growth factor, among others [52]. Nevertheless, the applications of this polymer are limited in this area compared to other polymers like Chitosan, Polyethylenimine and PAMAM dendrimers [53-54]. On the other hand, poly-lysine dendrimers and derivatives with PEG for drug delivery have been prepared [55-57].

Polyethilenimine

For over 20 years, polyethyleneimine (PEI) has been considered the gold standard of polymeric non viral vectors used for gene delivery. This polymer exists in a linear and a branched form (Fig. **4**). The large number of amine groups present in its structure provides PEI a polycation character, with the ability to condense DNA and to mediate gene transfer into mammalian cells.

PEI has been, within the group of cationic polymers, one of the most studied not only by its high capacity for DNA condensation, but also for its intrinsic endosomal activity and buffering capacity,

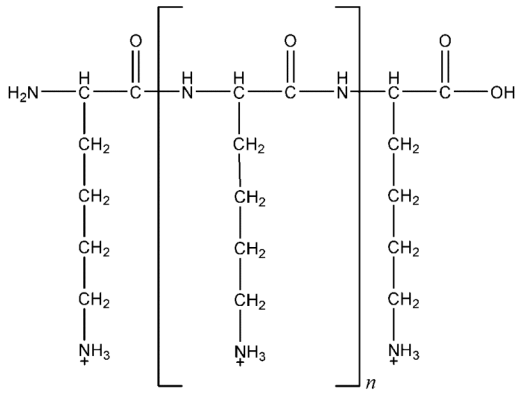

Fig. (3). Chemical structure of poly-Lysine.

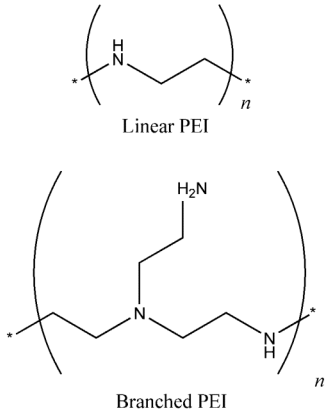

Fig. (4). Chemical structure of polyethyleneimines.

called proton sponge effect. It facilitates the release of the gene associated to the polymer in the cell cytoplasm by osmotic swelling and rupture of endosomes [58-59]. Two strategies have been used to prepare PEI nanoparticles, one is polymer complexation with DNA to form nano-complexes and the other is through the use of cross-linkers to form PEI nanoparticles first followed by the addition of DNA in this pre-formed structure [60-62].

Commercial PEI, called high molecular weight PEI of 25 kDa, has high transfection efficiency, however lacks C-C or C-N degradable linkages, so it is too toxic for therapeutic applications, producing the binding to complement components, aggregation of red blood cells and poor breakdown and subsequent excretion on in-vivo experiments [63-66]. By contrast, the low molecular weight PEI has low cytotoxicity but poor transfection efficiency [67-69].

In order to overcome these limitations, several groups have developed easily degradable PEIs by cross-linking them with various degradable crosslinkers for intracellular degradation either by simple hydrolysis, enzymatic degradation and low endosomal pH-depended hydrolysis, among others [70]. Thus, these new nanoparticles have high transfection efficiency, lower cytotoxicity, high breakdown into water-soluble fragments which can be easily processed and excreted by the cells.

Some authors have also shown that PEI dendrimers can be useful in several biological applications, however, little knowledge of their behavior, mainly related to their genomic and proteomic effects, severely limits the implementation of these systems into in-vivo studies [71].

In addition, like other cationic polymers, PEI has demonstrated significant antimicrobial activity. Some authors have shown that PEI has antibiotics and synergistic effects with some antibacterial agents [72]. Also, certain derivatives of the PEI have been shown to disrupt cell membranes of certain bacteria, so these have also been suggested for antimicrobial coating of devices and surfaces [73].

By contrast, the antiviral activity of the PEIs has not been widely studied. Nevertheless, certain studies have shown that this polymer produces a blockade of the primary binding of HCMV of HPV to their target cells and also inhibit the spread of the latter virus in cell culture [74]. This means that in addition to its antimicrobial effect, this polymer and its derivatives could have a role as prophylactic and therapeutic antiviral agents.

Acrylate Derivatives

Acrylate derivatives are strictly synthetic polymers, but as they are currently used in numerous biotechnological applications, deserve a mention in this review. Poly[2-(N,N-dimethylamino)ethyl methacrylate] (PDMAEMA) is formed by covalently bound 2-(N,N-dimethylaminoethy) methacrylate units and can be synthesized by various methods such as group transfer polymerization (Fig. **5**) [75-79]. In general, the radical polymerization is used for preparing advanced structures of PDMAEMA, because of its robustness and being easily controllable [80]. On the other hand, atom transfer radical polymerization (ATRP) has been frequently used, but needs some precautions in relation to the complexing capacity of the polymer towards the copper catalyst [81-84].

PDMAEMA is a weak polyelectrolyte having ternary amine groups on each monomer and a pKa of about 7 that is why it is fully deprotonated at high pH, partially protonated at physiological pH and fully protonated at lower pH [85]. With these properties, this polymer can act as a proton sponge and destabilize endosomes, achieving an efficient delivery of genes [86-87]. In addition, this polymer suffers conformational changes according to its degree of protonation, as has been observed to be more soluble at low pH where is fully charged. Furthermore, it has been described as a thermo-sensitive polymer, since when it is at high pH, it is soluble at lower temperatures and insoluble at elevated ones [88].

This polymer can be used in many medical applications, but bearing in mind that it has a low degradability, limits its use. For in-vivo application this polymer should be smaller than 30-50 kDa to be excreted by the kidneys [89], otherwise it accumulates in the body and this leads to severe complications [90]. This polymer also can form polyplexes with DNA and make successful gene transfer, but is considered less efficient than polyethyleneimine, the gold standard. Nevertheless, synthetic modifications have been made using DMAEMA monomer to achieve more complex branched structures such as star and bottlebrushes, with which they have enhanced transfection efficiency compared to linear structures [91-102]. Additional, Matyjaszewski group capitalizing on the cytotoxicity of polymers having amine groups, especially quaternary amines, has developed some magnetic nanoparticles coated with the quaternized polymer which function as an antibacterial material which may be recoverable [103]. Furthermore, since the thermo- and pH-sensitive character of the polymer it may be used for the production of reversible hydrogels [104-105].

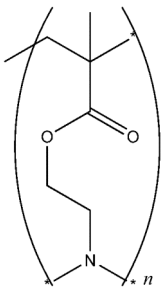

Fig. (5). Scheme of PDMAEMA.

Eudragit E100

Eudragit E100 is a methacrylate copolymer with tertiary amine groups which presents low solubility in aqueous media at pH higher than 5.5-6.0 (Fig. **6**). It is a polymer of high molecular weight, non-toxic and easily absorbed orally. E100 has been widely used in the pharmaceutical industry to improve drug solubility, absorption and also to mask color and smell. On the other hand, it has also been described the viricidal capacity of this polymer [106-107]. These studies have shown that E100 presents destabilizing properties of lipid membranes, together with the ability to form complexes with nucleic acids, and the fact that treatment with the polymer does not affect the majority of plasma protein fractions, make this molecule a potential alternative for viricidal treatment of biological cell-free media [9,11].

In recent years, several nanoparticulate drug delivery systems based on Eudragit E have been developed. There are reports of nanoparticles of alginate with Eudragit E100 as a complexing agent; the gelling versatility of alginate offers the possibility of preparing nano and micro particles with this polymer [106-108].

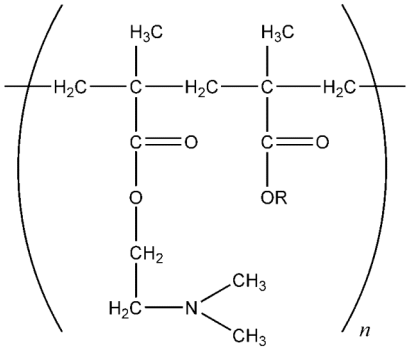

Fig. (6). Chemical structure of Eudragit E100.

Cationic Cellulose

Cellulose is a polysaccharide composed of glucose monomers linked by β (1-4) bonds and the main component of the cell wall of plants, so it is considered as the most abundant organic compound in the world. Despite its abundance, biodegradability, biocompatibility, capacity for renewal and derivatization, this polymer is not soluble in water or common organic solvents because it has strong inter- and intramolecular hydrogen bonds that give it a highly ordered structure [109]. Therefore, chemical modification of cellulose to generate water soluble cationic derivatives is of particular interest. These smart polyelectrolytes, are generally sensitive to changes in pH, temperature and ionic strength, and therefore have the potential to be used in drug [110-112] and gene delivery systems [113-115]. In fact, it has been shown that these cellulose derivatives can be used for delivering acidic drugs. In this case, controlled release of the drug is achieved by the ionic interaction occurring between amine groups of the derivative and the acid groups of the drug [112]. Also it has been seen that these derivatives can be applied as hair conditioners, as thickeners in mineral processing and oil recovery as well as flocculants in wastewater treatment and as additives in papermaking [116]. In particular, water-soluble quaternized cellulose derivatives are among the most studied and have valuable properties (Fig. **7**) [117]. Some examples are PQ-10, SC-230, SC-240C, Sensomer 10M, among others. However, the strong basic character of some of these derivatives has limited its application, as it causes irritation of skin and mucosa. This is why those cationic derivatives with tertiary amine groups with an intermediate basic character are frequently preferred for pharmaceutical, biomedical or cosmetic applications. These polymers are widely used in cosmetics and in topical drugs delivery devices. There are also reports of its use in the food industry, where it is described the precipitation of milk caseins by addition of the polymer [10].

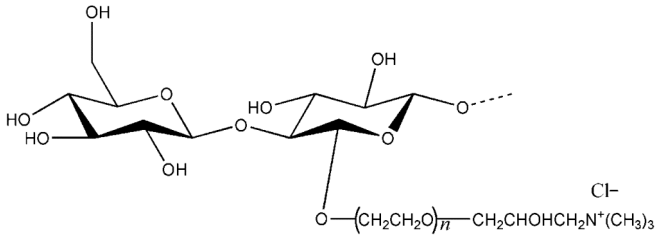

Fig. (7). Chemical structure of cationic cellulose.

Cationic Dextran

Dextran is a linear homopolysaccharide of glucose, composed mainly of 1,6-glycosidic linkages and some lateral branches with 1,3-glycosidic linkages [118]. This polymer is naturally synthesized from sucrose by certain lactic acid bacteria such as *Leuconostoc mesenteroides* and *Streptococcus mutans*. Due to their biocompatibility, biodegradability, immunoneutrality and aqueous solubility regardless of the pH of the solution, dextran is widely used in biomedical science as a bioinert material and in tissue engineering. Moreover it is being actively investigated for use as a delivery system of drugs, proteins, antibiotics, enzymes and anti-diabetic agents [119-127]. Also, by having hydroxyl groups at each glucose unit, this polymer is easily modified by chemical conjugation (Fig. **8**) [128].

One of such modifications may be the incorporation of amine groups, and examples include the (diethylaminoethyl) dextran [129] and dextran-spermine [130] the latter having a high efficiency of DNA transfection [131].

In a study by Stenekes and collaborators the encapsulation of drug-loaded liposomes on a dextran based polymer, used as a drug reservoir material, was achieved [132]. Thus, slow degradation of this material results in a sustained release of loaded liposomes up to 100 days. Moreover, it has been possible to encapsulate recombinant DNA (containing chloramphenicol acetyl-transferase) in cationic liposomes which were then integrated with the dextran. This system could stop transfection efficiency within the colon wall epithelium *in vivo* [133].

It has been also demonstrated the potential use of this polymer for delivery of siRNA [134-136]. In fact, some authors have demonstrated the capacity of cationic dextran hydroxyethyl methacrylate (dex-HEMA) based nanogels to incorporate siRNA efficiently and be taken up by cells *in vitro*, releasing free intact siRNA in the cell cytosol. These nanogels were used as a reservoir to release siRNA at the desired time and prolong the effect of gene silencing. Thus the dose of siRNA was deployed more efficiently [137]. In another study by Yeo and Kohane, it has been shown that dextran hydrogels can be manufactured using derivatives of carboxymethyldextran or carboxymethylcellulose [138]. Also it was demonstrated the formation of polymeric micelles composed of dextran (hydrophilic domain) and PLGA (hydrophobic domain) with the ability to incorporate antitumor agents [139].

Cationic Cyclodextrins

Cyclodextrins (CDs) are sugar derivatives produced by enzymatic degradation of starch [140]. They are cyclic oligomers formed by 6, 7 and 8 glucopyranose units connected by α (1,4) glucosidic bonds that are categorized as α, β, γ-CD, respectively [141-142]. These three major CDs are crystalline, homogeneous,

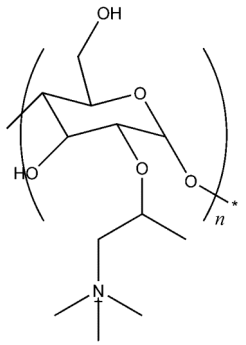

Fig. (8). Chemical structures of cationic dextran.

non-hygroscopic, low-toxic and form a bulbous structure or truncated cone with a hydrophobic cavity surrounded by a hydrophilic exterior, unique structure that allows CDs to include a wide variety of compounds, even polymers.

Nowadays, these structures are used in a wide variety of applications including food processing [143], chemical separations [144], adsorbents [145-146] and as pharmaceutical excipients [147].

Purification of α- and γ-CD significantly increases the production cost, so 97 % of CDs used in the market are the β-CD. Besides this β-CD has been the most widely studied due to its optimal size and their ability to form inclusion complexes with a wide variety of drugs [148]. Thus, such structures increase the aqueous solubility, bioavailability and chemical stability of drugs. However, the presence of intra-molecular hydrogen bonds from the secondary hydroxyl groups in this molecule has been considered as the main cause of the low aqueous solubility of the β-CD [149]. This is why currently much emphasis is being put on the development of CD derivatives through introduction of ionic fragments in order to increase water solubility and decrease toxicity (Fig. **9**) [150]. In addition, modification of CDs with cationic or amphiphilic groups have shown great potential for use as carrier vectors of genes and siRNA [151-153]. In fact, modified β-CD demonstrated to be a successful transport of genes to a variety of cell types in *in-vitro* assays and *in-vivo* tumor models [154-156]. Also, the functionalization of CDs with weak acids or bases can achieve supra-molecular reversible complexation behaviors induced by changes in pH [157-158].

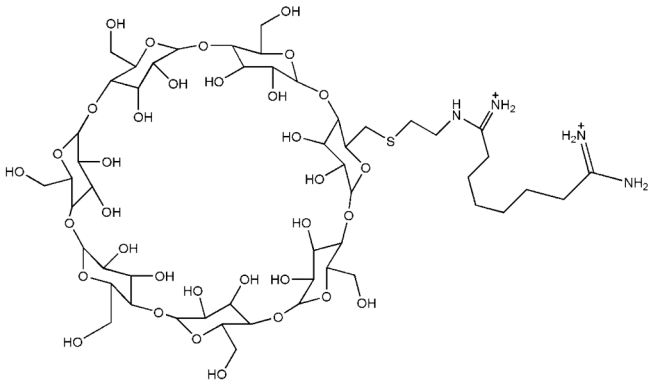

Fig. (9). Structure of cationic cyclodextrin.

HISTORICAL BACKGROUND

Technological Applications of Cationic Polymers and Related Structures

As briefly introduced above, biopolymers and amphiphilic molecules that self-aggregate have found many different applications in the last decades. Based in their interaction with other biomolecules or biological systems cationic polymers have found several technological applications [7-11, 159]. In this context, we found that an amphiphilic cationic polymer (Eudragit E100) can lyse biological membranes and inactivate virus [9, 11] and another (chitosan) can display immunomodulatory activities [160].

In the biomedical field, biopolymers have been studied for their use as space fillers either due to their physical or rheological properties [160, 161]. In the case of cationic polymers, due to their electrostatic interaction with nucleic acids several groups characterized them as delivery vehicles for either DNA or RNA or derivatives [161, 162].

Microbicidal Activity of Cationic Polymers

The interactions and biological effects on microorganisms (bacteria and fungi) range among the most widely studied properties of cationic polymers [162-163]. Several of them have been reported to have antimicrobial activity under different conditions which led several groups to propose their use in sanitation, virus and bacterial inactivation [164-170]. Quaternary ammonium compounds, for example, have been used for a variety of purposes, being as disinfectants and antiseptics the most common. Some of the main reasons why these compounds are becoming increasingly important reside in the advantages that they offer, their high molecular weight, stable chemical structures, long half-life and versatility through modification of their functional groups. Moreover, the growing complications associated with antibiotic resistance have greatly accelerated the development of antimicrobial macromolecular systems. In this context, cationic polymers offer an interesting alternative that, in addition to responding at many of the mentioned issues, provide other features such as the possibility to response to stimuli and to display also antioxidant, antitumor or anti-inflammatory activity.

Advances in research methodologies on macromolecules allowed achieving significant improvements in polymer processes, which enabled more precise synthesis and their comprehensive characterization. Moreover, these advances led to a deeper understanding of the mechanisms of action of these compounds and the management of structural factors affecting their biological activity.

A generally accepted mechanism for interaction of cationic polymers with bacterial membranes involves three basic steps: first an approximation mediated by electrostatic attraction between positive groups of these compounds and the negative charges of the membrane, followed by a second phase of rearrangement that produces a lateral segregation of the components of the membrane that leads to destabilization and subsequent drilling of the same in a third segregation step. The understanding of these basic mechanisms has allowed identifying the structural parameters that determine a higher or lower microbicidal activity of these compounds. Thus, it is known that the presence and balance between hydrophobic regions and the cationic groups is one of the factors that have a great influence on their microbicidal activity. The molecular weight is another critical factor to consider.

As mentioned above, most cationic polymers possess primary, secondary, tertiary amine groups and quaternary ammonium compounds groups. In this respect, the different reactivities observed among them are also reflected in the microbicidal capability that they exhibit. In general, the greatest activity has been observed for compounds with quaternary ammonium groups (QACs).

Technological advances, coupled with the acquired knowledge on structure-function relationship result in a growing tendency in the development of novel synthetic cationic polymeric compounds.

Cationic polymers of natural origin, chitosan, gelatin, dextran, cellulose, etc., have been the subject of several studies, and recently, a lot of them evaluating a wide range of synthetic derivatives of these compounds have also emerged [171-185].

Regarding synthetic cationic polymers, derivatives of polyethyleneimine (PEI) are some of the most commonly used, although PEIs as such are used in various fields, their high cytotoxicity, low hemo-compatibility and the fact of not being biodegradable, hinder their therapeutic use, which has led to the development of biodegradable low toxicity variants of these compounds [186-209]. The high positive charge density of these compounds provided by the presence of primary, secondary and tertiary amine groups, together with various modifications of the molecule give them good biocidal capacity. Furthermore, they have also been investigated as carriers of antimicrobial substances.

One polymer family well-known in the area of dendrimers, the Poly(amidoamine)s (PAMAMs) have also been considered as microbicides based on their structural characteristics. While high generation PAMAM dendrimers have shown a high antimicrobial activity also show high toxicity. In order to overcome this problem, several modifications have been introduced to the original molecules. This has led to new derivatives that are able to avoid these unwanted effects [202-204].

Another families of cationic polymers that have been successfully modified to achieve microbicidal properties include the Poly (amino-co-ester)s (PAEs), Poly(N,N-dimethylaminoethylmethacrylate) (PDMAEMA), Dioctadecyldimethyl ammonium bromide (DODAB), Polyhexamethylenebiguanide (PHMB) and many others.

Among these polymers, QACs deserve a special mention. These have been the most widely used cationic compounds in terms of variety of uses and also in time from being used. Most of the works published on the antimicrobial activity of cationic polymers refer to the activity on bacteria, and some on their antifungal activity, but only a few describe their activity on viruses. Recent reports describe a strong inhibition of PEI on some virus types [205]. In this context, we have recently described that an acrylic polymer with tertiary amine groups, Eudragit E100, displays an important inhibitory activity of on different viral models even in the presence of biological material like human plasma [9, 11]. The results obtained allowed to disclose the mechanism of action of E100 and to design a virus inactivation procedure that involves three sequential steps:

- An approach of the polymer to the virus through electrostatic interactions between the positively charged amine groups of the polymer and the negative charges of the viral membrane. This is a reversible interaction with a low energetic barrier.
- At a second step, the polymer is inserted into the membrane bilayer establishing a strong hydrophobic interaction leading to the irreversible formation of E100-virus multimolecular complexes,
- A third step involves the remotion of the viral particles strongly associated to the polymer either by their interaction to a cation exchange resin or through the precipitation of the complexes via a pH change to neutralize the polymer (and further removal of the precipitate either by filtration or by centrifugation). This is possible because after the interaction with the viral particles the polymer still displays an excess of positive charges [9, 11].

It was also shown that upon treatment of human plasma with E100:

- The total protein concentration remains essentially the same (the polymer does not interact with the major protein components of human plasma),
- The aggregation state of the proteins is not affected,
- The biological activity of antibodies and enzymes is not affected and no neoantigens are formed.

Altogether, these results allowed us to develop a simple and cheap inactivation procedure that is suitable for cell free applications involving the purification of biological molecules [9, 11].

In summary, the use of cationic polymers as microbicides is a field that offers enormous possibilities for research. While there is a general agreement on basic questions of their action mechanisms, there is much to elucidate about these interactions. The deep knowledge of these mechanisms will allow much progress on the development of new and improved compounds.

Oral Drug Delivery and Food Related Applications of Nano- or Microparticles

It is known that many of the active principles and nutraceuticals are hydrophobic, therefore a first challenge when developing a DDS is that as they are, these molecules will have a very low or poor bioavailability. Even after being inserted into a water soluble nanocarrier, their release from the matrix to the biological fluid is generally low and slow. When intended for oral delivery, these factors are also complicated by the interactions with the gastrointestinal fluids and the possibility of chemical modifications by enzymes and acid/base or redox reactions [205].

As reasonably expected, the first encapsulation systems protected by a patent were developed for oral delivery [206]. Since then, many encapsulation technologies and carriers have been developed [207-209].

In this context, the ability of proteins to gellify has been used originally for the development of oral delivery systems and more recently for the fabrication of nano or microparticles [210-215]. For these applications it is extremely important to know precisely the molecular structure, charge distribution, overall polarity and post-transduction modifications because altogether these characteristics determine the solubility profile of the protein under different environmental conditions. The interplay between the combination of variables that affect protein solubility and structural stability (I.e: protein concentration, pH, temperature, salt, solvent polarity) lead to situations under which protein molecules interact forming 3D networks of variable size, frequently known as gels or hydrogels. With adapted fabrication procedures, it has been possible to modulate these properties to prepare protein nano or microparticles [6, 212, 217].

As with many linear polymers, many proteins can also be used as thickening agents. Not only their relative size, much bigger than the size of solvent molecules, but also their hydration makes their hydrodynamic volume much larger. Therefore, it is frequent to find a concentration dependent profile that passes through a region of a viscous liquid before ending in a firm gel. Although there are no particles present, it is important to remember that viscous solutions can also be used to improve the kinetic stability of particle dispersions just slowing down their movement thus retarding their sedimentation by gravitational force and their aggregation.

In the case of hydrogels, they have been used in the development of biomedical applications, including space filling, burn dressings, biosensors, cell encapsulation and for drug delivery applications [212, 217-219]. Cationic polymeric particles based on PEI and cationic inulin have been recently developed for the preparation of tablets for the oral delivery of bis(phosphonates) [220].

First of all, in the situation of oral delivery, it should be remembered that despite the fact that biopolymer-based nano- or microparticles, mainly those based on proteins and/or polysaccharides, can be designed for the controlled delivery of an active compound they can also find applications in the food industry for the modulation of physicochemical or organoleptic characteristics of food products [221-223]. In this context, all the knowledge that has been acquired on fundamental physicochemical properties of biopolymers and biocompatible molecules has been increasingly incorporated in the rational development of innovative food products with improved nutritional properties, from which a growing trend is in foods that also provide health benefits [223]. An issue that has always to be taken into account is the time and cost that has to be invested in the development of new ingredients that pass the ap-

proval processes. For these reasons, there is a growing tendency to search for new applications to very well known biological molecules or those that, although not natural, have been widely tested and approved. Therefore, improvement in the control of structural organization of foods, medicines and related products has been a very important source of new innovations during the last decade [7, 8, 10, 223].

The main uses of nano- or microparticles have been for protection, improvement of solubility in aqueous media and delivery [211]. When incorporated in foods or oral delivery systems, it is always important to remember that the particles should not adversely affect the organoleptic characteristics of the product into which they are incorporated. In this context, it is known from studies on chocolate that sensory perception is highly dependent on particle size distribution [224]. Therefore, at least from the physical characteristics, nanoparticles should be a preferred structure in terms of their neutrality on the physicochemical properties of the product. Of course, and as stated above, nano- or microparticles can also be especially designed as to modulate the physicochemical or sensory properties of foods in which case the same knowledge can be applied to fulfill opposite requirements.

CHEMICAL AND PHYSICOCHEMICAL ISSUES TO BE CONSIDERED FOR THE PREPARATION OF NANOCARRIERS

Physicochemical Principles Involved in the Formation of Structured Polymeric Systems

Different physicochemical principles have been used so far for the preparation of polymeric nano- or microparticles with relatively good stability. These include: a) controlled complexation, b) segregation, c) gelation; d) extrusion, e) size-reduction, f) solvent-removal [225].

From all these methods, the most common structures obtained are:

- Molecular complexes,
- Nanofibres and nanotubes,
- Beads (spherical, filled, core-shell, non-sperical)

It should be kept in mind that the functional properties of a biopolymer nanocarrier ultimately depends not only on its composition but also on its fabrication process which will determine its physicochemical properties, structural characteristics and stability under different environmental conditions.

Chemical Composition

This is by far the most widely recognized factor that is considered, because it is well known that inevitably, the properties of nanoparticles will somehow depend on their chemical composition. Most of the biocompatible cationic nanoparticles studied so far have been fabricated using proteins and polysaccharides and a few approved synthetic polymers. These include the natural or cationic derivative forms of: chitosan, chitin, albumin, gellatin, whey proteins, alginates, starch, soy protein, cellulose, acrylate derivatives, polyethileneimine and poly-Lysine, among others.

Other ingredients that are frequently included and should be considered when establishing a preparation procedure of a nanocarrier are: salts, sugars and lipids. Last but not least, the composition of the solvent will also determine and condition the thermodynamic and kinetic stability of the nanocarrier. All these together with the final purpose of making a suitable vehicle for a particular API or bioactive compound, which, depending on its proportion in the final product could have more or less effect on its structure and properties.

As mentioned above, the sequential order in which the ingredients are incorporated and the physicochemical approach or technique used to prepare the nanoparticle are important aspects to be considered because they will finally determine the spatial organization and distribution of the components. This will thus determine if the distribution of API is homogeneous or not or if we end having a core-shell distribution with something that could be working as an internal compartment or reservoir of the bioactive principle. It is clear that the nature of the interactions involved (physical or chemical) and their magnitude are aspects that can be controlled by changing the chemical composition in order to attain a desired nanoparticle or a particle with specially tuned properties.

The proportion of ingredients as well as their interactions and location within the particles will determine the physicochemical properties like density, refractive index, rheology, environmental sensitivity, enzyme digestibility, dissolution kinetics and release profile of API. Therefore, its functional characteristics will be thus dependent not only on the chemical composition but also on the properties of the structures formed by them.

With all this in mind, selection of components will be determined by: a) their ability to form a particular structure or to assemble into a desired type of particle, b) the functional requirements of the particles to be prepared such as size and size distribution, charge, stability in certain environmental conditions and c) manufacturing and regulatory issues (scalability of the production processes, sterilizability of ingredients and final product, legal status, cost, etc.).

Structure of the Particle - Size, Dimensions and Distribution of Components

The size and size distribution of the particles will determine not only the physicochemical properties but also the immunogenicity and sensory attributes in the case of foods and oral delivery systems. For drug delivery applications the mean diameter of particles that have been studied typically goes from a few nanometers (Abraxane: mean particle diameter 130 nm) to several micrometers (Sandostatin: mean particle diameter 50 μm). It should be kept in mind that when speaking of nanostructured products their size/dimensions are frequently expressed giving the particle size distribution (mean +/- S.D.) or as mean particle diameter with an associated polydispersity index (this is more frequently found when referring to polymers). It is also common to see a reference to the proportion or fraction of the total that fall below a certain size [225].

Although most of the approved particles are spherical or closely spherical in shape, other shapes are also possible including fibers, rods, ribbon-like structures and so on (i.e.: Abelcet is a ribbon like lipidic structure for the controlled delivery of Amphotericin B). Regarding the shape, it is important to consider not only its external appearance but also the internal structure because it is not the same to have a homogeneous particle than to have an internal compartment. Depending on the manufacturing procedure and the composition it is possible to have particles with different internal structures. In this regard, particle interior can be homogeneous or heterogeneous and these last can be core-shell or a continuous phase with an internal discontinuous phase of different composition that is dispersed within.

The physicochemical properties of the API and the pharmacological requirements will determine how it should be associated to the particles (either to the surface or encapsulated inside). The structure of the particles will determine if they have environments with different polarities (this will allow or condition the solubility of certain APIs or more precisely will determine where the API will end located within the particles) [5]. The internal structure and dimensions will determine also the encapsulation efficiency, the amount of API that can be loaded into each particle, the release kinetics and related properties. It is also important to emphasize that new properties of the particles can arise upon the incorporation of API. It has been observed that particle dynamics (for example the

equilibrium between monomers and micelles) is dramatically affected by the association of an active principle [2].

Electrical Properties

When dealing with cationic polymers the presence of net charges is important for several reasons. First of all, it should be kept in mind that most of the surfaces that exist in biological systems are negatively charged and most of the polyelectrolytes present are negatively charged. Therefore, the interactions with other molecules via strong Coulombic forces is an issue that can determine the type and nature of the mixed particles that can be formed and also, the interaction with surrounding molecules or surfaces will condition the stability of particles formed using cationic polymers. Therefore, the possibility to modulate the net electrical charge of the particles could be used to tune or to design them for special applications. For instance, in the case of oral delivery systems, mucoadhesivity via electrostatic interactions between positively charged particles and negatively charged mucus layers could delay the transit through the gastrointestinal tract favoring a more prolonged delivery of a particular active ingredient.

For food applications it is important to keep in mind that positive charges are usually perceived by the tongue as astringent. So, this is a quality of the food that can also be modulated by the presence of particles containing cationic polymers.

The presence of electric charges is also important to confer stability of the particles in solution as the repulsion between them prevents aggregation and clumping. When dealing with ionizable groups, the pH dependency of this ionization and its dependence with the presence of salts, are aspects that should be kept in mind not only for the production process but also for their fate in living systems.

Physicochemical and Dynamical Properties

Altogether and as already mentioned, the chemical composition and preparation procedure determine the physicochemical and dynamical properties of the particles. These are important aspects to consider because these properties will determine:

- viscosity and surface tension of the solutions,
- the way that they interact between them and with other particles or structures,
- their stability upon dilution,
- their phase structure and porosity will determine the partition coefficient of small molecules, which will affect their capacity to load and release APIs.

The dependence of these properties with pH, salt concentration and temperature are aspects that have been used for the design of particles for special applications [226, 227].

When dealing with a functional biological active material, its incorporation into specially designed nanoparticles contributes to its protection not only from degradation but also from inactivation due to denaturation or chemical modification of its structure.

In the case of proteins, the presence of many different functional groups, domains and binding sites makes them attractive for the development of nanoparticles modified as to improve their interaction with specific bioactive compounds (when acting as carriers) or their targetting (when incorporated in nanocarriers but not playing the "transportation" role).

Effect of Surface Free Energy on the Functionality of Nanocarriers

Although most cationic polymers are highly hidrophilic many show important surface activity [7,8]. Furthermore, it has been clearly shown that even upon interaction with negatively charged structures like casein micelles, the main contribution to the overall energetics is due to the hydrophobic effect [7,8]. In this context, it is not surprising to find that several cationic polymers spontaneously self-aggregate leading to nano- or microparticles with an important surface free energy that allows the incorporation of important amounts of highly hydrophobic APIs [9, 10, Bianco, Alasino & Beltramo, unpublished observations]. The modification of their surface free energy under a change in pH or buffer composition has also been the base to aid in the solubilization of hydrophobic APIs [6].

NANOPARTICLE FABRICATION TECHNIQUES

Many different methods have been reported for the fabrication of polymeric nanoparticles [223]. As can be reasonably expected, due to their biodegradability by digestive enzymes most protein nanoparticles have been initially developed for food applications or for oral drug delivery systems. Their natural immunogenicity restricts the use of protein nanoparticles for parenteral drug delivery to human proteins like albumin [6, 228, 229].

Self-Assembly

This is by far the ideal fabrication method. Whenever reproducible conditions can be established for the self-aggregation of a polymer this leads to a fabrication procedure that is very cheap (usually tends to be the cheapest) and to nanoparticles that are thermodynamically very stable [2-5]. If these conditions can be combined as to incorporate the active principle as part of a self-aggregated structure this gives us an excellent start in the development of a drug delivery system.

Evolution has selected a special case of self-aggregated micellar system as the optimum delivery system for calcium for mammals. This is achieved by micellar structures formed by caseins that encapsulate and stabilize calcium phosphate [230, 231]. As reasonably expected, this strategy has also been explored for the oral delivery of hydrophobic compounds. Just as an example, with this in mind Semo and colleagues standarized conditions for the *in vitro* self-assembly of casein micelles incorporating vitamin D2, a highly hydrophobic compound [232]. The idea behind this strategy was to use these micelles to improve the nutritional properties of non-fat or low-fat products. As a bonus of the strategy, the researchers also found that the incorporation of vitamin D into casein micelles provided protection against its UV induced degradation [232]. The strategy developed is based on a combination of salts and pH to establish conditions under which the hydrophobic compound ends incorporated within the micelles [232]. A different strategy is to disassemble the natural structure, either from the micelles or from the protein itself, thus exposing hydrophobic residues or regions onto which the hydrophobic active compound can be bound and afterwards readjust pH to regain the structure or reassemble the micelles [6, 233].

Nanoparticles Formed by Protein Denaturation

It is well known since the beginning of studies on protein stability that many proteins aggregate upon heating. The basics behind this aggregation have been also well characterized for many globular proteins [1]. Briefly, as the structure of the protein opens due to heating, hydrophobic groups normally buried in their interior are exposed to interact with other non polar residues from neighbor protein molecules thus leading to the formation of aggregates. The technological problem of these strategies is that they are not suitable for heat-sensitive active principles.

With studies aimed at elucidation of the molecular structure of proteins we came to know that this opening of the internal structure of proteins can also be attained using special salts, pH and ionic strength. Therefore, this knowledge opened the possibility to standardize methods for the production of protein aggregates with adjusted sizes that can be performed at room temperature or even under refrigeration [234].

A logic follow up of these strategies was to modify the polar nature of the solvent. This so-called anti-solvent strategy has been originally standardized for protein fractionation and purification [235]. The most common procedures involve dissolving the protein and slowly changing the solvent composition by incorporating the anti-solvent.

Spray Drying

By far the most common dehydration method used in the food industries to prepare protein powders and microparticles is spray-drying. The most widespread spray-drying method is based on spraying a protein solution to a current of hot air which leads to a fast evaporation of water with the final result of a fine protein powder. Therefore, contrarily to what could be thought, as the exposure to heat is very short in time, protein denaturation is in some cases low which allowed its broad use in the food industry. However, this technique has been only adapted to the preparation of relatively large microparticles and as far as we are aware there are still not nanocarriers based in this technique.

Emulsion Based Strategies

In these methods, the formation of an emulsion is used as a strategy to put together the polymer with the bioactive compound, reduce the size of the droplets and then harden the microparticles by any known method (chemical crosslinking, heat denaturation, etc.). Therefore, the basics of these methods is to dissolve the polymer and the bioactive compound, then preparing a water in oil (W/O) emulsion, crosslink the polymer within the droplets and finally collect them either by disrupting the emulsion and solvent removal or by a physical method like filtration or centrifugation [241]. Whenever a hydrophobic compound has to be encapsulated, this has been done by preparing a triple emulsion O/W/O. Therefore, in this case the hydrophobic bioactive compound is first dissolved in a suitable organic solvent that is emulsified within a water solution. Afterwards, this emulsion is further emulsified in another non-polar solvent. Upon removal of the internal solvent, this leads to a physical trapping of the hydrophobic active compound within the microparticle [223-236].

Electrospinning

More recently, polymeric nanofibres have been prepared by injecting the solutions through a small nozzle by applying an electric field [237]. Briefly, the polymer and bioactive compound are both dissolved in a suitable solvent, afterwards, the solution is placed in a capillary tube and forced to pass through a nozzle with a selected diameter by a high-voltage electric field. During the time consumed to pass through the capillary tube and the nozzle the solvent evaporates leading to the formation of nanofibres or nanocapsules that can be used to design special 3D structures [169].

Hydrogels

Their importance in medicine was initially established in the 1950s with the development of soft contact lenses based on a hydrogel prepared using PHEMA (poly (2-hydroxy ehtylmethacrylate)) [238].

Main characteristics of biomedical hydrogels:

- Structure, chemical and physical,
- Swelling properties in different media,
- Surface properties, including the presence of ionizable groups, functional moieties, etc.

These together determine other characteristics like:

- diffusion properties of included material,
- reversibility of swelling, which is important for the determination of the release profile under different pHs, the possibility to use them as pulsatile release systems under varying conditions (generally pH or temperature), syneresis.

Polymer Complexation

A large number of structures can be formed by the interactions of polymers with so called "complimentary" molecules. From these, the most widely studied are interpolymer complexes formed by the interaction between at least two different polymers [239-246]. From the thermodynamic point of view, there are two fundamental driving forces that determine the nature, structure and stability of these complexes. If the interactions dominate, this will be reflected in a negative enthaply (exothermic process) whereas when hydrophobic processes contribute with the major driving force this will be reflected as a relatively temperature independent process (minor enthalpic contribution) or a process in which the enthalpy is even positive. Contrarily to what can be expected, it has been found that entropy is the main driving force for the formation of several complexes between cationic polymers and negatively charged structures [7].

From the kinetic point of view, isothermal titration calorimetry and surface plasmon resonance have been recently used to characterize not only the thermodynamic parameters but also the rate and extent of the interactions between a series of polymers leading to the formation of intermolecular complexes [245]. These techniques are complementary and highly sensitive. Isothermal titration calorimetry can provide precise information regarding the thermodynamic parameters like enthalpy and binding affinity constant of the intermolecular interaction. On the other side, surface plasmon resonance measures the change in mass on the surface of the sensor and the rate at which these changes are produced thereby providing data on the kinetics of the process [239].

SELF-ASSEMBLY AS A STRATEGY TO FABRICATE NANOCARRIERS OR OTHER SUPRAMOLECULAR STRUCTURES BASED ON CATIONIC POLYMERS

As they are formed by various molecules, each of which has usually various chemical groups, nanocarriers based on cationic polymers are dynamic structures exhibiting complex functions.

Keeping in mind the considerations mentioned in the introduction, we could essentially find two main types of self-assembly processes: A) Equillibrium based structures (static) and B) Kinetic-based structures (dynamic). In the case of nanocarriers prepared by self-assembly structures, the interactions between the components of the particle dominate and therefore, the energetics involved lead the process towards the formation of a certain structure. In this situation, the chemical composition of the system is determinant to achieve a desired structure and its stability. In the case of kinetic-based structures metastable non-equillibrium states may be stabilized by free energy barriers that lend them kinetically stable under given environmental conditions. It is clear that in these systems the sequence of events in the production method is critical to achieve a desired nanostructure. A very interesting example on this subject can be seen in Ladet *et al* [247]. They were able to produce a sequence of chitosan membranes layered each one on top of the other like onions simply bathing a solution of chitosan in an alkaline medium and interrupting the precipitation of chitosan by changing the aqueous medium. Therefore, concentric membranes of chitosan membranes can be formed just by repeating the cycle. This is a very simple example of "kinetic control" of structuring. In the case of chitosan, although it's chemical structure looks mostly hydrophilic at acidic pH, the presence of intra- and intermolecular hydrogen bonds and its main backbone give it an important surface activity which results in a thermodynamic tendency to self-aggregate [248].

As mentioned above, the dynamic control of the self-assembly can also be attained when there is a supply of potential energy that is released during the fabrication of a nanostructure. An example of this was shown by Capito *et al*., [249] using a positively charged

peptide solution that was mixed with a highly viscous solution of hyaluronic acid. Upon contact of the two solutions a membrane is formed in the interface between the liquids. This membrane acts as a barrier impeding mixing of the solutions and therefore avoiding the dissipation of the chemical potential gradients. The membranes thus formed could trap either the peptide or the hyaluronic acid solution in closed compartments. Furthermore, an interesting property that arouse was that the membranes formed self-healed when broken [249].

CONCLUSION

The first two issues that should be kept in mind when attempting to prepare a nanocarrier based in a cationic polymer are: a) when dissolved they tend to increase the viscosity of the solution, b) the presence of several positive charges within a single molecule lead to strong electrostatic interactions that are established extremely fast. These can lead to problems for assuring the complete mixing of the components. Therefore, these aspects have to be kept in mind when selecting a preparation procedure.

On the other hand, we have found many situations in which entropic changes are the main contributors to the energetics of the process. This has been seen as "detergent like" effects of several cationic polymers.

A very important issue that has to be stressed is that, as shown here, cationic polymers can display an intrinsic bioactivity (i.e.: immunomodulatory). Therefore, it is very important to remember that cationic polymers should not be considered as inert, biocompatible components. In this context, it should be emphasized that their biocompatibility not only depends on their chemical nature but also on the physical properties of the structures into which they are incorporated and presented to living systems.

Improvement in the knowledge of the thermodynamic and kinetic aspects involved in the structuring process of cationic biopolymers is leading a field that is finding many new preparation procedures and applications for cationic nanocarriers. Counterintuitively, entropy has been shown to play a very important role as a driving force for the formation of supramolecular structures or in the biological properties of events in which a cationic polymer is associated with negatively charged molecules. Interestingly, the presence of an important contribution of the entropic component acts frequently as a compensation for the strong enthalpic contribution that is present when dealing with cationic polymers leading to nanostructures that having a marginal stability can respond in a tunable time-frame releasing their content as a response to a subtle change in the environmental conditions making these structures suitable for the preparation of what are known as smart drug delivery systems. Although we are still far from Paul Ehrlich's magic bullet the increasing control of the structure and properties of nanocarriers make them very promising for the design of new and more precise and effective delivery systems.

CONFLICT OF INTEREST

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REFERENCES

[1] Weber G. Protein Interactions. In: Rouletge, Chapman & Hall, Inc. New York 1992; pp. 20-30.

[2] Leonhard V, Alasino RV, Bianco ID, Beltramo DM. Self-assembled micelles of gangliosides as nanodelivery vehicles for taxanes. J Control Rel 2012; 162: 619-627.

[3] Leonhard V, Alasino RV, Bianco ID, Garro AG, Heredia V, Beltramo DM. Paclitaxel and albumin are spontaneously and sequentially loaded into different nano-domains of GM1 ganglioside micelles: a novel strategy for oncologic drug delivery. J Nanomed Nanotechnol 2013; 4: 1-7.

[4] Leonhard V, Alasino RV, Bianco ID, Garro AG, Heredia V, Beltramo DM. Biochemical characterization of the interactions between Doxorubicin and lipidic GM1 micelles with or without Paclitaxel loading. Int J Nanomed 2015; 10: 3377-88.

[5] Leonhard V, Alasino RV, Bianco ID, Garro AG, Heredia V, Beltramo DM. Biochemical characterization of GM1 micelles-amphotericin B interaction. Curr Drug Deliv 2015; 12(4): 406-14.

[6] Garro AG, Beltramo DM, Alasino RV, Leonhard V, HerediaV, Bianco ID. Reversible exposure of hydrophobic residues on albumin as a novel strategy for the formulation of nanodelivery vehicles of taxanes. Int J Nanomed 2011; 6: 1193-200.

[7] Ausar SF, Bianco ID, Badini GD, *et al.* Characterization of Casein Micelle Precipitation by Chitosans. J Dairy Sci 2001; 84: 361-9.

[8] Ausar SF, Bianco ID, Castagna LF, Alasino RV, Beltramo DM. Interaction of a cationic acrylate polymer with caseins: biphasic effect of eudragit E100 on the stability of casein micelles. J Agric Food Chem 2003; 51: 4417-23.

[9] Alasino RV, Ausar SF, Bianco ID, Castagna LF, Contigiani M, Beltramo DM. Amphipathic and membrane-destabilizing properties of the cationic acrylate polymer Eudragit E100. Macromol Biosci 2005; 5: 207-13.

[10] Ausar SF, Bianco ID, Castagna LF, *et al.* Reversible precipitation of casein micelles with a cationic hydroxyethylcellulose. J Agric Food Chem 2005; 53: 9031-8.

[11] Alasino RV, Bianco ID, Vitali MS, Zarzur JA and Beltramo DM. Characterization of the inhibition of enveloped virus infectivity by the cationic acrylate polymer Eudragit E100. Macromol Biosci 2007; 7: 1132-8.

[12] Du, Q, Fu, C, Tie, J, *et al.* Gelatin microcapsules for enhanced microwave tumor hyperthermia. Nanoscale 2015; 7(7): 3147-54.

[13] Khan, SA, Schneider, M. Improvement of nanoprecipitation technique for preparation of gelatin nanoparticles and potential macromolecular drug loading. Macromol Biosci 2013; 13(4): 455-63.

[14] Cao W, Lai JC, Leung SW. Functional enhancement of chitosan and nanoparticles in cell culture, tissue engineering, and pharmaceutical applications. Front Physiol. 2012; 3: 1-13.

[15] Borchard G, Junginger HE. Modern drug delivery applications of chitosan. Adv Drug Delivery Rev 2001; 52: 145-50.

[16] Chandy T, Sharma CP. Chitosan as a biomaterial. Biomater Artif Cells Artif Organs 1990; 1(18): 1-24.

[17] Rinaudo M. Main properties and current applications of some polysaccharides as biomaterials. Polym Int 2008; 3(57): 397-430.

[18] Mourya VK, Inamdar NN. Chitosan-modifications and applications: opportunities galore. React Funct Polym 2008; 6(68): 1013-51.

[19] Wang JJ, Zeng ZW, Xiao RZ, *et al.* Recent advances of chitosan nanoparticles as drug carriers. Int J Nanomed 2011; 6: 765-74.

[20] Kumar N. A review on chitosan nanoparticles for cancer treatment. Int J Nanomat Biostruct 2014; 4(4): 63-5.

[21] Jafarizadeh Malmiri H, Ghaz Jahanian MA, Berenjian A. Potential applications of chitosan nanoparticles as novel support in enzyme immobilization. Am J Biochem Biotechnol 2012; 8 (4): 203-19.

[22] Agnihotri *et. al.* Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J Control Rel 2004; 100: 5-28.

[23] Gnanadhas DV, Thomas MB, Elango M, Raichur AM, Chakravortty D. Chitosan-dextran sulphate nano capsule drug delivery system as an effective therapeutic against intraphagosomal pathogen Salmonella. J Antimicrob Chemother 2013; 68(11): 2576-86.

[24] Rajan M, Raj V. Formation and characterization of chitosan-polylacticacid-polyethylene glycol-gelatin nanoparticles: A novel biosystem for controlled drug delivery. Carbohyd Polymers 2013; 98: 951-8.

[25] Wang JJ, Zhao WZ, Xiao RZ, *et al.* Recent advances of chitosan nanoparticles as drug carriers. Int J Nanomed 2011; 6: 765-74.

[26] Fan J, Chen J, Yang L. Preparation of dual-sensitive graft copolymer hydrogel based on N-maleoyl-chitosan and poly (N-isopropylacrylamide) hydrogels. J Mat Sci Mater Med 2009; 20: 583-90.

[27] Yu H, Chen X, Lu T, *et al.* Poly(L-lysine)-graft-chitosan copolymers: synthesis, characterization, and gene transfection effect. Biomacromol 2007; 8: 1425-35.

[28] Wong K, Sun G, Zhang X, *et al.* PEI-g-chitosan a novel delivery system with transfection efficiency comparable to polyethylenimine *in vivo* and after liver administration *in vivo*. Bioconjugate Chem 2006; 17: 152-8.

[29] Xu T, Xin M, Li M, Huang H, Zhou S. Synthesis, characteristics and antibacterial activity of N,N,N-trimethyl chitosan and its carboxymethyl derivatives. Carbohydr Polym 2010; 81: 931-6.

[30] Sayin B, Somavarapu S, Li XW, Sesardic D, Senel S, Alpar OH. TMC-MCC (N-trimethyl chitosan-mono-N-carboxymethyl chitosan) nanocomplexes for mucosal delivery of vaccines. Eur J Pharm Sci 2009; 38(4): 362-9.

[31] Johnston-Banks FA. Gelatin. In P. Harris (Ed.), Food gels (pp. 233e289). London: Elsevier Applied Science Publishers 1990.

[32] Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food Hydrocoll 2011; 25: 1813-27.

[33] Hong L, Tabata Y, Miyamoto S, *et al.* Regeneration at Rabbit Skull Defects Treated with Transforming Growth Factor-beta 1 Incorporated into Hydrogels with Different Levels of Biodegradability. J Neurosurg 2000; 92(2): 315-25.

[34] Oppenhiem RC. Paclitaxel loaded gelatin nanoparticles for intravesical bladder cancer therapy. Int. J. Pharm. 1981; 8: 217.

[35] Akimoto A, Dorn K, Gros L, Ringsdorf H, Schupp H. Polymer Model Membranes. Angew Chem 1981; 20: 90-1.

[36] Inada S, Fujiwara H, Atsuji K, *et al.* Successful gene transfer into dendritic cells with cationized gelatin and plasmid DNA complexes via a phagocytosis-dependent mechanism. Anticancer Res 2006; 26: 1957-64.

[37] Li JK, Wang N, Wu XS. Gelatin nanoencapsulation of protein/peptide drugs using an emulsifier-free emulsion method. J Microencapsul 1998; 15(2): 163-72.

[38] Jan Zillies CC. Evaluating gelatin based nanoparticles as a carrier system for double stranded oligonucleotides. J Pharm Pharmaceut Sci 2004; 7(4): 17.

[39] Lu Z, *et al.* Paclitaxel-loaded gelatin nanoparticles for intravesical bladder cancer therapy. Clin Cancer Res 2004; 10: 7677-84.

[40] Bajpai AK, Choubey J. Design of gelatin nanoparticles as swelling controlled delivery system for chloroquine phosphate. J Mater Sci Mater Med 2006; 17(4): 345-58.

[41] Chen T, Janjua R, McDermott MK, Bernstein SL, Steidl SM, Payne GF. Gelatin-based biomimetic tissue adhesive. Potential for retinal reattachment. J Biomed Mater Res B Appl Biomater 2006; 77: 416-22.

[42] Yamamoto S, Hirata A, Ishikawa S, Ohta K, Nakamura K,Okinami S. Feasibility of using gelatin-microbial transglutaminase complex to repair experimental retinal detachment in rabbit eyes. Graefe's Arch Clin Exp Ophthalmol 2013; 251: 1109-14.

[43] Lai J-Y. Corneal Stromal Cell Growth on Gelatin/Chondroitin Sulfate Scaffolds Modified at Different NHS/EDC Molar Ratios. Int J Mol Sci 2013, 14: 2036-55.

[44] Zauner W, Ogris M, Wagner E. Polylysine-based transfection systems utilizing receptor-mediated delivery. Adv Drug Deliv Rev 1998; 30: 97-113.

[45] Wu GY, Wu CH. Receptor-mediated *in vitro* gene transformation by a soluble DNA carrier system. J Biol Chem 1987; 10(262): 4429-32.

[46] Wagner E, Cotten M, Foisner R, Birnstiel ML. Transferrin-polycation-DNA complexes: the effect of polycations on the structure of the complex and DNA delivery to cells. Proc Natl Acad Sci USA 1991; 10(88): 4255-9.

[47] Emmrich S, Wang W, John K, Li W, Putzer BM. Antisense gapmers selectively suppress individual oncogenic p73 splice isoforms and inhibit tumor growth *in vivo*. Mol Cancer 2009; 8(61): 1-13.

[48] Thiersch M, Rimann M, Panagiotopoulou V, *et al*. The angiogenic response to PLL-g-PEG-mediated HIF-1α plasmid DNA delivery in healthy and diabetic rats. Biomaterials 2013; 34(16): 4173-82.

[49] Asparuhova MB, Barde I, Trono D, Schranz K, Schumperli D. Development and characterization of a triple combination gene therapy vector inhibiting HIV-1 multiplication. J Gene Med 2008; 10(10): 1059-70.

[50] Deng J, Gao N, Wang Y, *et al*. Self-assembled cationic micelles based on PEG-PLL-PLLeu hybrid polypeptides as highly effective gene vectors. Biomacromol 2012; 13(11): 3795-804.

[51] Morille M, Passirani C, Vonarbourg A, Clavreul A, Benoit JP. Progress in developing cationic vectors for non-viral systemic gene therapy against cancer. Biomaterials 2008; 29(24-25): 3477-96.

[52] Fischer D, Li Y, Ahlemeyer B, Krieglstein J and KisselT. *In vitro* cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. Biomaterials 2003; 24: 1121-31.

[53] Capowski EE, Schneider BL, Ebert AD, *et al.* Lentiviral vector-mediated genetic modification of human neural progenitor cells for ex vivo gene therapy. J Neurosci Methods 2007; 2(163): 338-49.

[54] Okumura K, Nakase M, Nakamura S, Kamei T, Inui M, Tagawa T. Bax gene therapy for human osteosarcoma using cationic liposomes *in vivo*. Oncol Rep. 2007; 4(17): 769-73.

[55] Sideratou Z, Sterioti N, Tsiourvas D, *et al.* Arginine end-functionalized poly (L-lysine) dendrigrafts for the stabilization and controlled release of insulin. J Coll Interface Sci 2010; 351: 433-41.

[56] Fox ME, Guillaudeu S, Frechet JMJ, Jerger K, Macaraeg N, Szoka FC. Synthesis and *In Vivo* antitumor efficacy of PEGylatedPoly(L-lysine) dendrimer-camptothecin conjugates. Mol Pharm 2009; 6: 1562-72.

[57] Kaminskas LM, Kelly BD, McLeod VM, *et al.* Pharmacokinetics and tumor disposition of PEGylated, methotrexate conjugated poly-L-lysine dendrimers. Mol Pharm 2009; 6: 1190-204.

[58] De Laporte L, Rea JC, Shea LD. Design of modular non-viral gene therapy vectors. Biomaterials 2006; 7(27): 947-54.

[59] Brown MD, Schatzlein AG, Uchegbu IF. Gene delivery with synthetic (non-viral) carriers. Int J Pharm 2001; 229(1-2): 1-21.

[60] Dai ZJ, Gjetting T, Mattebjerg MA, Wu C, Andresen TL. Elucidating the interplay between DNA-condensing and free polycations in gene transfection through a mechanistic study of linear and branched PEI. Biomaterials 2011; 33(32): 8626-34.

[61] Nimesh S, Goyal A, Pawar V, *et al.* Polyethylenimine nanoparticles as efficient transfecting agents for mammalian cells. J Control Rel 2006; 110(2): 457-68.

[62] Nimesh S, Aggarwal A, Kumar P, Singh Y, Gupta KC, Chandra R. Influence of acyl chain length on transfection mediated by acylated PEI nanoparticles. Int J Pharm 2007; 337(1-2): 265-74.

[63] Boussif O, Lezoualch F, Zanta MA, *et al*. A versatile vector for gene and oligonucleotide transfer into cells in culture and in-vivo polyethylenimine. Proc Natl Acad Sci USA 1995; 16(92): 7297-301.

[64] Zou SM, Erbacher P, Remy JS, Behr JP. Systemic linear polyethylenimine (l-PEI)-mediated gene delivery in the mouse. J Gene Med 2000; 2(2): 128-34.

[65] Fischer D, Bieber T, Li YX, Elsasser HP, Kissel T. A novel non-viral vector for DNA delivery based on low molecular weight, branched polyethylenimine: effect of molecular weight on transfection efficiency and cytotoxicity. Pharm Res 1999; 8(16): 1273-9.

[66] Merkel OM, Urbanics R, Bedocs P, *et al*. *In vitro* and *in vivo* complement activation and related anaphylactic effects associated with polyethylenimine and polyethylenimine- graft-poly(ethylene glycol) block copolymers. Biomaterials 2011; 21(32): 4936-42.

[67] Godbey WT, Wu KK, Mikos AG. Poly(ethylenimine) and its role in gene delivery. J Control Rel 1999; 2-3(60): 149-60.

[68] Thomas M, Klibanov AM. Non-viral gene therapy: polycation-mediated DNA delivery. Appl Microbiol Biot 2003; 1(62): 27-34.

[69] Kunath K, von Harpe A, Fischer D, *et al*. Low-molecular-weight polyethylenimine as a non-viral vector for DNA delivery: comparison of physicochemical properties, transfection efficiency and *in vivo* distribution with high-molecular-weight polyethylenimine. J Control Rel 2003; 1(89): 113-25.

[70] Jere D, Jiang HL, Arote R, *et al*. Degradable polyethylenimines as DNA and small interfering RNA carriers. Expert Opin Drug Deliv 2009; 6(8): 827-34.

[71] Lungwitz U, Breunig M, Blunk T, Gopferich A. Polyethylenimine-based non-viral gene delivery systems. Eur J Pharm Biopharm 2005; 60(2): 247-266.

[72] Khalil H, Chen T, Riffon R, Wang R, Wang Z. Synergy between polyethylenimine and different families of antibiotics against a resistant clinical isolate of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2008; 52: 1635-41.

[73] Milovic NM, Wang J, Lewis K, Klibanov AM. Immobilized N-alkylated polyethylenimine avidly kills bacteria by rupturing cell membranes with no resistance developed. Biotechnol Bioeng 2005; 90: 715-22.

[74] Spoden GA, Besold K, Krauter S, *et al.* Antimicrobial agents and chemotherapy polyethylenimine is a strong inhibitor of human pa-

pillomavirus and cytomegalovirus infection. Antimicrob Agents Chemother 2012; 56(1): 75-82.

[75] Webster OW. Group transfer polymerization: mechanism and comparison with other methods for controlled polymerization of acrylic monomers. Adv Polym Sci 2004; 167: 1-34.

[76] Pafiti KS, Patrickios CS, Georgiou TK, *et al.* Cationic star polymer siRNA transfectants interconnected with a piperazine-based cationic cross-linker. Eur Polym J 2012; 48: 1422-30.

[77] Themistou E, Patrickios C S. Synthesis and Characterization of Amphiphilic Star Copolymers of 2-(Dimethylamino)Ethyl Methacrylate and Methyl Methacrylate: Effects of Polymer Architecture and Composition. Eur Polym J 2007; 43(1): 84-92.

[78] Georgiou TK, Vamvakaki M, Phylactou LA, Patrickios CS. Synthesis, characterization, and evaluation as transfection reagents of double-hydrophilic star copolymers: Effect of star architecture. Biomacromol 2005; 6: 2990-7.

[79] Schacher F, Müllner M, Schmalz H, Müller AHE. New Block Copolymers with Poly (N, N-dimethylaminoethyl methacrylate) as a Double Stimuli-Responsive Block. Macromol Chem Phys 2009; 210 (3-4): 256-62.

[80] Niskanen J,Wu C, Ostrowski M. *et al.* Thermo-responsiveness of PDMAEMA: Electrostatic and Stereochemical Effects. Macromol 2013; 46(6): 2331-40.

[81] Matyjaszewski K. Atom Transfer Radical Polymerization (ATRP): Current Status and Future Perspectives. Macromol 2012; 45: 4015-39.

[82] Mao BW, Gan LH, Gan YY. Ultra high molar mass poly[2-(dimethylamino)ethyl methacrylate] via atom transfer radical polymerization. Polymer 2006; 47(9): 3017-20.

[83] Amin A. Amphiphilic star homo- and block copolymers of 2-(dimethylamino) Ethyl-methacrylate via atom transfer radical polymerization. J Macromol Sci, Part A: Pure Appl Chem 2007; 44: 329-35.

[84] Mao B, Gan L-H, Gan Y-Y, *et al.* Controlled polymerizations of 2-(dialkylamino)ethyl methacrylates and their block copolymers in protic solvents at ambient temperature via ATRP. J Polym Sci, Part A 2004; 42: 5161-9.

[85] Dubruel P, Schacht E. Vinyl polymers as non-viral gene delivery carriers: current status and prospects. Macromol Biosci 2006; 6: 789-810.

[86] Van de Wetering P, Moret EE, Schuurmans-Nieuwenbroek NME, van Steenbergen MJ, Hennink WE. Structure-activity relationships of water-soluble cationic methacrylate/ methacrylamide polymers for nonviral gene delivery. Bioconjugate Chem 1999; 10(4): 589-97.

[87] Sonawane ND, Szoka FC, Verkman AS. Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. J Biol Chem 2003; 278: 44826-31.

[88] Plamper FA, Ruppel M, Schmalz A, *et al.* Tuning the Thermoresponsive Properties of Weak Polyelectrolytes: Aqueous Solutions of Star-Shaped and Linear Poly(N,N-dimethylaminoethyl Methacrylate). Macromol 2007; 40: 8361-6.

[89] Duncan R. The dawning era of polymer therapeutics. Nat Rev Drug Disc 2003; 2: 347-60.

[90] Fox ME, Szoka FC, Frechet JMJ. Soluble Polymer Carriers for the Treatment of Cancer: The Importance of Molecular Architecture. Acc Chem Res 2009, 42: 1141-51.

[91] Cherng J-Y, van de Wetering P, Talsma H, Crommelin DA, Hennink W. Effect of size and serum proteins on transfection efficiency of poly ((2-dimethylamino) ethyl methacrylate)-plasmid nanoparticles. Pharm Res 1996;13: 1038-42.

[92] Van deWetering P, Cherng JY, Talsma H, Hennink WE. Relation between transfection efficiency and cytotoxicity of poly (2-(dimethylamino) ethyl methacrylate)/plasmid complexes. J Control Rel 1997; 49: 59-69.

[93] Georgiou TK, Vamvakaki M, Phylactou LA, Patrickios CS. Synthesis, characterization, and evaluation as transfection reagents of double-hydrophilic star copolymers: effect of star architecture. Biomacromol 2005; 6: 2990-7.

[94] Newland B, Tai H, Zheng Y, Velasco D, *et al.* A highly effective gene delivery vector--hyperbranchedpoly(2-(dimethylamino)ethyl methacrylate) from in situ deactivation enhanced ATRP. Chem Commun 2010; 46: 4698-700.

[95] Xu F J, Zhang Z X, Ping Y, *et al*. Star-shaped cationic polymers by atom transfer radical polymerization from beta-cyclodextrin cores for nonviral gene delivery. Biomacromol 2009; 10: 285-93.

[96] Alhoranta AM, Lehtinen JK, Urtti AO, *et al.* Cationic amphiphilic star and linear block copolymers: synthesis, self-assembly, and *in vitro* gene transfection. Biomacromol 2011; 12: 3213-22.

[97] Yu S, Chen J, Dong R, *et al.* Enhanced gene transfection efficiency of PDMAEMA by incorporating hydrophobic hyperbranched polymer cores: effect of degree of branching. Polym Chem 2012; 3: 3324-9.

[98] Yang C, Li H, Goh SH, Li J. Cationic star polymers consisting of alpha-cyclodextrin core and oligoethylenimine arms as nonviral gene delivery vectors. Biomat 2007; 28: 3245-54.

[99] Schallon A, Jérôme V,Walther A, *et al*. Performance of three PDMAEMA-based polycation architectures as gene delivery agents in comparison to linear and branched PEI. React Funct Polym 2010; 70: 1-10.

[100] Dai F, Sun P, Liu Y, LiuW. Redox-cleavable star cationic PDMAEMA by arm-first approach of ATRP as a nonviral vector for gene delivery. Biomat 2010; 31: 559-69.

[101] Georgiou TK, Vamvakaki M, Patrickios CS, Yamasaki EN, Phylactou LA. Nanoscopic cationic methacrylate star homopolymers: synthesis by group transfer polymerization, characterization and evaluation as transfection reagents. Biomacromol 2004; 5: 2221-9.

[102] Georgiou TK, Phylactou LA, Patrickios CS. Synthesis, characterization, and evaluation as transfection reagents of ampholytic star copolymers: effect of star architecture. Biomacromol 2006; 7: 3505-12.

[103] Dong H, Huang J, Koepsel RR, *et al.* Recyclable antibacterial magnetic nanoparticles grafted with quaternizedpoly(2-(dimethylamino)ethyl methacrylate) brushes. Biomacromol 2011; 12(4): 1305-11.

[104] Madsen J, Armes SP. (Meth)acrylic stimulus-responsive block copolymer hydrogels. Soft Matter 2012; 8: 592-605.

[105] Tsitsilianis C. Responsive reversible hydrogels from associative "smart" macromolecules. Soft Matter 2010; 6: 2372-88.

[106] Joshi GV, Kevadiya BD, Bajaj HC. Controlled release formulation of ranitidine-containing montmorillonite and Eudragit E-100. Drug Dev Ind Pharm 2010; 36(9): 1046-53.

[107] Sonavane GS, Devarajan PV. Interpolyelectrolyte complexes of Eudragit® EPO with sodium alginate as potential carriers for colonic drug delivery: Monitoring of structural transformation and composition changes during swellability and release evaluating. J Biomed Nanotechnol 2007; 3(2): 160-9.

[108] Moustafine R I, Salachova AR, Frolova ES, Kemenova VA, Guy Van den Mooter. Interpolyelectrolyte complexes of Eudragit® EPO with sodium alginate as potential carriers for colonic drug delivery: Monitoring of structural transformation and composition changes during swellability and release evaluating. Drug Dev Ind Pharmacy 2009; 35(12): 1439-51.

[109] Schurz J. A bright future for cellulose. Prog Polym Sci 1999; 24: 481-3.

[110] Rodriguez R, Alvarez-Lorenzo C, Conheiro A. Cationic cellulose hydrogels: kinetics of the cross-linking process and characterization as pH-/ion-sensitive drug delivery systems. J Control Rel 2003; 86: 253-65.

[111] Pillai O, Panchagnula R. Polymer in drug delivery. Curr Opin Chem Biol 2001; 5(4): 447-51.

[112] Liesiene J. and Matulioniene J. Application of water-soluble diethylaminoethylcellulose in oral drug delivery systems React Funct Polym 2004; 59(2): 185-91.

[113] Song Y, Sun Y, Zhang X, Zhou J, Zhang L. Homogeneous quaternization of cellulose in NaOH/urea aqueous solutions as gene carriers. Biomacromol 2008; 9: 2259-64.

[114] Garnett MC. Gene-delivery systems using cationic polymers. Crit Rev Ther Drug Carrier Syst 1999; 16: 147-207.

[115] Fayazpour F, Lucas B, Alvarez-Lorenzo C, Sanders NN, Demeester J and De Smedt SC. Physicochemical and transfection properties of cationic Hydroxyethylcellulose/DNA nanoparticles. Biomacromol 2006; 7: 2856-62.

[116] Klemm D, Philipp B, Heinze T, Heinze U, Wagenknecht W. Introduction. In: Comprehensive Cellulose Chemistry: Fundamentals and Analytical Methods, Volume 1, Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. 1998; 1-45.

[117] Zhang LM. New water-soluble cellulosic polymer: a review. Macromol Mater 2001; 286(5): 267-75.

[118] Mehvar R. Dextrans for targeted and sustained delivery of therapeutic and imaging agents. J Control Rel 2000; 69(1): 1-25.

[119] Danhauser-Riedl S, Hausmann E, Schick H, *et al*. Phase I clinical and pharmacokinetic trial of dextran conjugated doxorubicin (AD-70, doxorubicin-OXD). Invest New Drugs 1993; 11: 187-95.

[120] Du YZ, Weng Q, Yuan H, *et al*. Synthesis and antitumor activity of stearate-g-dextran micelles for intracellular doxorubicin delivery. ACS Nano 2010; 4: 6894-902.

[121] Feng L, Zhang L, Liu M, *et al*. Roles of dextrans on improving lymphatic drainage for liposomal drug delivery system. J Drug Target 2010; 18: 168-78.

[122] Ichinose K, Tomiyama N, Nakashima M, *et al*. Antitumor activity of dextran derivatives immobilizing platinum complex (II). Anticancer Drugs. 2000; 11: 33-8.

[123] Jeong YI, Choi KC, Song CE. Doxorubicin release from core-shell type nanoparticles of poly(DL-lactide-co-glycolide)-grafted dextran. Arch Pharm Res 2006; 29: 712-9.

[124] Qi J, Yao P, He F, Yu C, and Huang C. Nanoparticles with dextran/chitosan shell and BSA/chitosan core-Doxorubicin loading and delivery. Int J Pharm 2010; 393(1-2): 177-85.

[125] vanTomme SR, Hennink, WE. Biodegradable dextran hydrogels for protein delivery applications. Exp Rev Med Dev 2007; 4(2): 147-64.

[126] Jin T, Zhu J, Wu F, Yuan W, Geng LL, Zhu H. Preparing polymer-based sustained-release systems without exposing proteins to water-oil or water-air interfaces and cross-linking reagents. J Control Rel 2008; 128(1): 50-9.

[127] Bachelder EM, Beaudette TT, Broaders KE, *et al*. *In vitro* analysis of acetylated dextran microparticles as a potent delivery platform for vaccine adjuvants. Mol Pharm. 2010; 7(3): 826-35.

[128] Heinze T, Liebert T, Heublein B, Hornig S. Functional polymers based on dextran. Adv Polym Sci 2006; 205: 199-291.

[129] Kaminski K, Plonka M, Ciejka J, *et al*. Cationic derivatives of dextran and hydroxypropylcellulose as novel potential heparin antagonists. J Med Chem 2011; 54: 6586-96.

[130] Hosseinkhani H, Azzam T, Tabata Y, Domb AJ. Dextran-spermine polycation: an efficient nonviral vector for *in vitro* and *in vivo* gene transfection. Gene Ther 2004; 11: 194-203.

[131] Azzam T, Eliyahu H, Makovitzki A, Linial M, Domb AJ. Hydrophobized dextran-spermidine conjugates as potential vector for *in vitro* gene transfection. J Control Rel 2004; 96: 309-23.

[132] Stenekes RJH, Loebis AE, Fernandes CM, Crommelin DJA, Hennink WE. Controlled release of liposomes frombiodegradable dextran microspheres: a novel delivery concept. Pharm Res 2000; 17(6): 690-5.

[133] Liptay S, Weidenbach H, Adler G, Schmid RM. Colon epithelium can be transiently transfected with liposomes, calcium phosphate precipitation and DEAE dextran *in vivo*. Digestion. 1998; 59(2): 142-7.

[134] Raemdonck K, Naeye B, Buyens K, *et al*. Biodegradable dextran nanogels for RNA interference: Focusing on endosomal escape and intracellular siRNA delivery. Adv Funct Mater 2009; 19: 1406-15.

[135] Raemdonck K, Naeye B, Høgset A, Demeester J, De Smedt SC. Prolonged gene silencing by combining siRNA nanogels and photochemical internalization. J Control Rel 2010; 145: 281-8.

[136] Nagane K, Jo JI, Tabata Y. Promoted adipogenesis of rat mesenchymal stem cells by transfection of small interfering RNA complexed with a cationized dextran. Tissue Eng, Part A. 2010; 16: 21-31.

[137] Raemdonck K, Naeye B, Buyens K, *et al*. Biodegradable dextran nanogels for RNA interference: focusing on endosomal escape and intracellular siRNA delivery. Adv Funct Mater 2009; 19: 1406-15.

[138] Yeo Y, Kohane DS. Polymers in the prevention of peritoneal adhesions. Eur J Pharm Biopharm 2008; 68(1): 57-66.

[139] Jeong YI, Kim DH, Chung CW, *et al*. Doxorubicin-incorporated polymeric micelles composed of dextran-b-poly(DL-lactide-coglycolide) copolymer. Int J Nanomed 2011; 6: 1415-27.

[140] Bonnet V, Gervaise C, Diedaïni-Pilar F, Furlan A, Sarazin C. Cyclodextrin nanoassemblies: a promising tool for drug delivery. Drug Discov Today 2015; 20(9): 1120-6.

[141] Peptu CA, Ochiuz L, Alupei L, Peptu C, Popa M. Carbohydrate based nanoparticles for drug delivery across biological barriers. J Biomed Nanotechnol 2014; 10(9): 2107-48.

[142] Hedges AR. Industrial Applications of Cyclodextrins. Chem Rev 1998; 98: 2035-44.

[143] López-de-Dicastillo C, Gallur M, Catalá R, Gavara R, Hernandez-Muñoz P. Immobilization of β-cyclodextrin in ethylene-vinyl alcohol copolymer for active food packaging applications. J Membr Sci 2010; 353: 184-91.

[144] Chin YP, Mohamad S, Abas MR. Removal of parabens from aqueous solution using cyclodextrin cross-linked polymer. Int J Mol Sci 2010; 11: 3459-71.

[145] Mohamad S, Yusof NHM, Asman S. The effect of bifunctionalisocyanate linker on adsorption of chromium (VI)-diphenylcarbazide complex onto β-cyclodextrin polymer. Asian J Chem 2013; 25: 2213-20.

[146] Li N, Wei X, Mei Z,*et al*. Synthesis and characterization of a novel polyamidoamine-cyclodextrin crosslinked copolymer. Carbohyd Res 2011; 346: 1721-7.

[147] Qian L, Guan Y, Xiao H. Preparation and characterization of inclusion complexes of a cationic β-cyclodextrin polymer with butylparaben or triclosan. Int J Pharm 2008; 357: 244-51.

[148] Hirayama F, Uekama K. Cyclodextrin-based controlled drug release system. Adv Drug Del Rev 1999; 36: 125-41.

[149] Szente L, Szejtli J. Highly soluble cyclodextrin derivatives: chemistry, properties, and trends in development. Adv Drug Del Rev 1999; 36: 17-28.

[150] Arima H, Yunomae K, Miyake K, Irie T, Hirayama F,Uekama K. Comparative studies of the enhancing effects of cyclodextrins on the solubility and oral bioavailability of tacrolimus in rats. J Pharm Sci 2001; 90: 690-701.

[151] Mellet CO, Fernandez JMG, Benito JM. Cyclodextrin-based gene delivery systems. Chem Soc Rev 2011; 40: 1586-608.

[152] Méndez-Ardoy A, Urbiola K, Aranda C, *et al*. Polycationic amphiphilic cyclodextrin-based nanoparticles for therapeutic gene delivery. Nanomedicine 2011; 6: 1697-707.

[153] O'MahonyAM, O'Neill MJ, Godinho BMDC, *et al*. Cyclodextrins for Non-Viral Gene and siRNA Delivery. Pharm Nanotechnol 2012; 1: 6-14

[154] McMahon A, O'Neill MJ, Gomez E, *et al*. Targeted gene delivery to hepatocytes with galactosylatedamphiphiliccyclodextrins. J Pharm Pharmacol 2012; 64: 1063-73.

[155] O'Neill MJ, Guo J, Byrne C, Darcy R, O' Driscoll CM. Mechanistic studies on the uptake and intracellular trafficking of novel cyclodextrin transfection complexes by intestinal epithelial cells. Int J Pharm 2011; 413: 174-83.

[156] Guo J, Ogier JR, Desgranges S, Darcy R, O Driscoll C. Anisamide targeted cyclodextrin nanoparticles for siRNA delivery to prostate tumours in mice. Biomaterials. 2012; 33: 7775-84.

[157] Yang SY, Green MM, Schultz G, Jha SK, Muller AHE. An on/off circular dichroism signal reveals a pH dependent competition between a cyclodextrin and a polyelectrolyte for an atropisomeric aromatic guest. JACS 1997; 119: 12404-5.

[158] Yang SY, Schultz G, Green MM, Morawetz H. Clustering of Poly(methacrylic acid) around Appended Binaphthyl Labels As Reflected by the Disruption of γ-CyclodextrinComplexation and Racemization Kinetics. Macromolecules 1999; 32: 2577-84.

[159] Porporatto C, Canali MM, Bianco ID, Correa SG. Ability of the polysaccharide chitosan to inhibit proliferation of CD4+ lymphocytes from mucosal inductive sites, *in vitro* and *in vivo*. Cell Prolif 2009; 42(6): 780-7.

[160] Zhou, HY, Jiang, LJ, Cao, PP, Li JB, Chen XG. Glycerophosphate-based chitosan thermosensitive hydrogels and their biomedical applications. Carbohydr Polym 2015, 117: 524-36.

[161] Nguyen MK, Alsberg, E. Bioactive factor delivery strategies from engineered polymer hydrogels for therapeutic medicine. Prog Polym Sci 2014; 39(7): 1236-65.

[162] Jin Q, Wang Z, Yan F, *et al*. A Novel Cationic Microbubbles Coated with Stearic Acid-Modified Polyethylenimine to Enhance DNA Loading and Gene Delivery by Ultrasound. PLoS ONE. 2013; 8(9): e76544.

[163] Schlecht U, St. Onge RP, Walther T, Francois JM, Davis RW. Cationic Amphiphilic Drugs Are Potent Inhibitors of Yeast Sporulation. 2012; PLoS ONE 7(8): e42853.

[164] Al-Ahmad A, Laird D, Zou P, *et al*. Nature-Inspired Antimicrobial Polymers - Assessment of Their Potential for Biomedical Applications. PLoS ONE. 2013; 8(9): e73812.

[165] Tashiro T. Antibacterial and bacterium adsorbing macromolecules. Macromol Mater Eng. 2001; 286(2): 63-87.

[166] Kenawy ER, Mahmoud YAG. Biologically active polymers, 6: synthesis and antimicrobial activity of some linear copolymers with quaternary ammonium and phosphonium groups. Macromol Biosci 2003; 3(2): 107-16.

[167] Kenawy E-R, Abdel-Hay FI, El-Magd AA, Mahmoud Y. Synthesis and antimicrobial activity of some polymers derived from modified amino polyacrylamide by reacting it with benzoate esters and benzaldehyde derivatives. J Appl Polym Sci 2006; 99: 2428-37.

[168] Samal SK, Dash M, van Vlierberghe S, *et al.* Cationic polymers and their therapeutic potential. Chem Soc Rev. 2012; 41: 7147-94.

[169] Gilbert P, Moore LE. Cationic antiseptics: diversity of action under a common epithet. J Appl Microbiol 2005; 99: 703-15.

[170] Chen J, Wang F, Liu Q, Du J. Antibacterial polymeric nanostructures for biomedical applications. Chem Commun. 2014; 50: 14482-93.

[171] Kumar MN, Muzzarelli RA, Muzzarelli C, Sashiwa H, Domb AJ. Chitosan chemistry and pharmaceutical perspectives. Chem Rev 2004; 104: 6017-84.

[172] Rabea EI, Badawy MET, Stevens CV, Smagghe G, Steurbaut W. Chitosan as antimicrobial agent: Applications and mode of action. Biomacromol 2003; 4: 1457-65.

[173] Huh MW, Kang IK, Lee DH, *et al.* Surface characterization and antibacterial activity of chitosan-grafted poly(ethylene terephthalate) pre-pared by plasma glow discharge. J Appl Polym Sci 2001; 81: 2769-78.

[174] Belalia R, Grelier S, Benaissa M, Coma V. New bioactive biomaterials based on quaternized chitosan. J Agric Food Chem 2008; 56: 1582-8.

[175] Kim CH, Choi JW, Chun HJ, Choi KS. Synthesis of chitosan derivatives with quaternary ammonium salt and their antibacterial activity. Polym Bull 1997; 38: 387-93.

[176] Pei L, Cai Z, Shang S, Song Z. Synthesis and antibacterial activity of alkylated chitosan under basic ionic liquid conditions. Appl Polym Sci 2014; 131: 40052.

[177] Ahmad M, Benjakul S, Prodpran T, Agustini TW. Physico-mechanical and antibacterial properties of gelatin film from the skin of unicorn leather jacket incorporated with essential oils. Food Hydrocoll 2012; 28: 189-99.

[178] Min BJ, Oh JH. Antimicrobial activity of catfish gelatin coating containing origanum (Thymus capitatus) oil against gram-negative pathogenic bacteria. J Food Sci 2009; 74: M143-8.

[179] Xu J, Zhou F, Ji BP, Pei RS, Xu N. The antibacterial mechanism of carvacrol and thymol against Escherichia coli. Lett Appl Microbiol 2008; 47: 174-9.

[180] Jiang Q, Xu J, Li T, Qiao C, Li Y. Synthesis and antibacterial activities of quaternary ammonium salt of gelatin. J Macromol Sci, Part B: Physics 2014; 53: 133-41.

[181] Nichifor M, Stanciu MC, Simionescu BC. New cationic hydrophilic and amphiphilic polysaccharides synthesized by one pot procedure. Carbohydr Polym 2010; 82: 965-75.

[182] O'Connor NA, Abugharbieh A, Yasmeen F, *et al.* The crosslinking of polysaccharides with polyamines and dextran-polyallylamine antibact e-rial hydrogels. Int J Macromol Sci 2015; 72: 88-93.

[183] Song Y, Zhang J, Gan W, Zhou J, Zhang L. Flocculation properties and antimicrobial activities of quaternized celluloses synthesized in NaOH/urea aqueous solution. Ind Eng Chem Res 2010; 49: 1242-6.

[184] Jia B, Zhou J, Zhang L. Electrospun nano-fiber mats containing cationic cellulose derivatives and poly (vinyl alcohol) with antibacterial activity. Carbohydr Res 2011; 346: 1337-41.

[185] Cen L, Neoh KG, Kang ET. Surface functionalization technique for conferring antibacterial properties to polymeric and cellulosic surfaces. Langmuir 2003; 19: 10295-303.

[186] Lewis K, Klibanov AM. Surpassing nature: rational design of sterile-surface materials. Trends Biotech 2005; 23: 343-8.

[187] Lin J, Qiu S, Lewis K, Klibanov AM. Bactericidal properties of flat surfaces and nanoparticles derivatized with alkylated polyethylenimines. Biotechnol Prog 2002; 18: 1082-6.

[188] Lin J, Qiu S, Lewis K, Klibanov AM. Mechanism of bacteri-cidal and fungicidal activities of textiles covalently modified with alkylated polyethylenimine. Biotechnol Bioeng 2003; 83: 168-72.

[189] Hilal N, Kochkodan V, Al-Khatib L, Levadna T. Surface modified polymeric membranes to reduce (bio)fouling: a microbiological study using E. coli. Desalination 2004; 167: 293-300.

[190] Yudovin-Farber I, Golenser J, Beyth N, Weiss EI, Domb AJ. Quaternary ammonium polyethyleneimine. J Nanomater 2010; 826343: 1-11.

[191] Beyth N, Pilo R, Weiss EI. Antibacterial activity of dental cements containing quaternary ammonium polyethyleneimine nanoparticles. J Nanomater 2012; 814763: 1-6.

[192] Beyth N, Yudovin-Farber I, Bahir R, Domb AJ, Weiss EI. Antibacterial activity of dental composites containing quaternary ammonium polyethyleneimine nanoparticles against *Streptococcus mutans*. Biomaterials 2006; 27: 3995-4002.

[193] Pasquier N, Keul H, Heine E, *et al.* Amphiphilic branched polymers as antimicrobial agents. *Macromol Biosci* 2008; 8: 903-15.

[194] Pasquier N, Keul H, Heine E, Moeller M. From multifunctionalized poly(ethyleneimine)s towards antimicrobial coatings. Biomacromol 2007; 8: 2874-82.

[195] He Y, Heine E, Keusgen N, Keul H, Möller M. Synthesis and characterization of amphiphilic monodisperse compounds and poly(ethyleneimine)s: Influence of their microstructures on the antimicrobial properties. Biomacromol 2012; 13: 612-23.

[196] Chang HI, Yang MS, Liang M. The synthesis, characterization and antibacterial activity of quaternized poly(2,6-dimethyl-1,4-phenylene oxide)s modified with ammonium and phosphonium salts. React Funct Polym 2010; 70: 944-50.

[197] Bansal R, Pathak R, Jha D, Kumar P, Gautam HK. Enhanced antibacterial activity of amine-phosphonium (N-P) hybrid polymers against Gram-negative and Gram-positive bacteria. Int J Polym Mater 2014; 64: 84-9.

[198] Lewis K, Klibanov AM. Surpassing nature: rational design of sterile-surface materials. Trends Biotech 2005; 23: 343-8.

[199] Lin J, Qiu S, Lewis K, Klibanov AM. Bactericidal properties of flat surfaces and nanoparticles derivatized with alkylated polyethylenimines. Biotechnol Prog 2002; 18: 1082-6.

[200] Lin J, Qiu S, Lewis K, Klibanov AM. Mechanism of bacteri-cidal and fungicidal activities of textiles covalently modified with alkylated polyethylenimine. Biotechnol Bioeng 2003; 83: 168-72.

[201] Yudovin-Farber I, Golenser J, Beyth N, Weiss EI, Domb AJ. Quaternary ammonium polyethyleneimine. J. Nanomater. 2010; 826343 (ID).

[202] Jevprasesphant R, Penny J, Attwood D, Mckeown NB, D'Emanude A. Engineering of dendrimer surfaces to enhance transepithelial transport and reduce cytotoxicity. Pharm Res 2003; 20: 1543-50.

[203] Jevprasesphant R, Penny J, Jalal R, Attwood D, Mckeown NB, D'Emanude A. The influence of surface modification on the cytotoxicity of PAMAM dendrimers. Int J Pharm 2003; 252: 263-6.

[204] Xue X, Chen X, Mao X, *et al.* Amino-terminated generation 2 poly(amidoamine) dendrimer as a potential broad-spectrum, non-resistance-inducing antibacterial agent. AAPS J 2013; 15: 132-42.

[205] Spoden GA, Besold K, Krauter S, *et al.* Polyethylenimine is a strong inhibitor of human papillomavirus and cytomegalovirus infection. Antimicrob Agents Chemother 2012; 56: 75-82.

[206] McClements DJ, XiaoH. Excipient foods: designing food matrices that improve the oral bioavailability of pharmaceuticals and nutraceuticals. Food Funct 2014; 5: 1320-33.

[207] Taylor HF. Vitamin preparation and method of making same. US Patent 2,183,053, 1939.

[208] Betz M, Kulozik U. Microencapsulation of bioactive bilberry anthocyanins by means of whey protein gels. 11th international congress on engineering and food (ICEF11) Proc Food Sci 2011; 1: 2047-56.

[209] Davidov-Pardo G, Joye IJ, McClements DJ. Encapsulation of resveratrol in biopolymer particles produced using liquid antisolvent precipitation. Part 1: Preparation and characterization. Food Hydrocoll 2015; j.foodhyd.2014.11.023.

[210] López-Rubio A, Lagaron JM. Whey protein capsules obtained through electrospraying for the encapsulation of bioactives. Innovative Food Sci Emerg Technol 2012; 13: 200-6.

[211] Davidov-Pardo G, Joye IJ, McClements DJ. Food-grade protein-based nanoparticles and microparticles for bioactive delivery: fabrication, characterization and utilization. Adv Prot Chem Struct Biol 2015; 98: 293-325.

[212] Burey P, Bhandari BR, Howes T, Gidley MJ. Hydrocolloid Gel Particles: Formation, Characterization, and Application, Cr Rev Food Sci Nutr 2008; 48(5): 361-77.

[213] Hedayati R, Jahanshahi M, Attar H. Fabrication and characterization of albumin-acacia nanoparticles based on complex coacervation as potent nanocarrier. J Chem Technol Biotechnol 2012; 87: 1401-8.

[214] Khan SA, Schneider M. Improvement of Nanoprecipitation Technique for Preparation of Gelatin Nanoparticles and Potential Macromolecular Drug Loading. Macromol Biosci 2013; 13(4): 455-63.

[215] Langer K, Balthasar S, Vogel V, Dinauer N, von Briesen H, Schubert D. Optimization of the preparation process for human serum albumin (HSA) nanoparticles, Int J Pharm 2003; 257(1-2): 169-80.

[216] Taheri ES, Jahanshahi M, Mosavian MTH. Preparation, characterization and optimization of egg albumin nanoparticles as low molecular weight drug delivery vehicle. Part Part Sys Charact 2012; 29: 211-22.

[217] Zhang Z, Zhang R, Tong Q, Decker EA, McClements DJ. Food-grade filled hydrogels for oral delivery of lipophilic active ingredients: Temperature-triggered release microgels. Food Res Int 2015; 69: 274-80.

[218] Ormaechea M, Paladini M, Pisano R, *et al*. VANTRIS®, a biocompatible, synthetic, non-biodegradable, easy-to-inject bulking substance. Evaluation of local tissular reaction, localized migration and long-distance migration. Arch Esp Urol 2008; 61(2): 263-8.

[219] Peppas NA, Huang Y, Torres-Lugo M. Physicochemical foundations and structural design of hydrogels in medicine and biology. Ann Rev Biomed Eng 2000; 2: 9-29.

[220] Demadis, KD, Paspalaki M, Theodorou J. Controlled Release of Bis(phosphonate) Pharmaceuticals from Biodegradable Polymeric Matrices. Ind Eng Chem Res 2011; 50: 5873-6.

[221] Chen J. Surface texture of foods: perception and characterization. Crit Rev Food Sci Nutr 2007; 47(6): 583-98.

[222] Sala G, de Wijk RA, van de Velde F, van Aken GA. Matrix properties affect the sensory perception of emulsion-filled gels. Food Hydrocoll 2008; 22(3): 353-63.

[223] Jones OG, McClements DJ. Functional Biopolymer Particles: Design, Fabrication and Applications. Compr Rev Food Sci Food Safety 2010; 9: 374-97.

[224] Afoakwa EO, Paterson A, Fowler M, Ryan A. Factors influencing rheological and textural qualities in chocolate-A review. Trends Food Sci Technol 2007; 18: 290-8.

[225] McClements DJ. Food emulsions: principles, practices, and techniques. 2nd ed. Boca Raton, Fla.: CRC Press. 543, 2005.

[226] Al-Ahmad A, Laird D, Zou P, *et al*. Nature-Inspired antimicrobial polymers - assessment of their potential for biomedical applications. PLoS ONE 2013; 8(9): e73812. doi: 10.1371/journal.pone.0073812.

[227] Degner BM, Chung Ch, Schlegel V, Hutkins R and McClements DJ. Factors influencing the freeze-thaw stability of emulsion-based foods. Compr Rev Food Sci Food Safety 2014; 13: 98-113.

[228] Ibrahim NK, Desai N, Legha S, *et al*. Phase I and pharmacokinetic study of ABI-007, a cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel. Clin Cancer Res 2002; 8: 1038-44.

[229] Desai N, Trieu V, Yao Z, *et al*. Increased antitumoractivity, intratumor paclitaxel concentrations, and endothelial celltransport of cremophor-free, albumin-bound paclitaxel, ABI-007,compared with cremophor-based paclitaxel. Clin Cancer Res 2006; 12(4): 1317-24.

[230] Garnier J. Models of casein micelle structure. Neth Milk Dairy J 1973; 27: 240-7.

[231] Walstra P. Casein sub-micelles: do they exist? Int Dairy J 1999; 9: 189-92.

[232] Semo E, Kesselman E, Danino D, Livney YD. Casein micelle as a natural nano-capsular vehicle for nutraceuticals. Food Hydrocoll 2007; 21: 936-42.

[233] Haham M, Ish-Shalom S, Nodelman M, *et al*. Stability and bioavailability of vitamin D nanoencapsulated in casein micelles. Food Funct 2012; 3(7): 737-44.

[234] Bromley EHC, Krebs MRH, Donald AM. Mechanisms of structure formation in particulate gels of β-lactoglobulin formed near the isoelectric point. Eur Phys J 2006; 21(2): 145-52.

[235] Cohn EJ, Strong LE, Hughes WL, *et al*. Preparation and Properties of Serum and Plasma Proteins. IV. A System for the Separation into Fractions of the Protein and Lipoprotein Components of Biological Tissues and Fluids. J Am Chem Soc 1946; 68: 459-75.

[236] Walker R, Decker EA, McClements DJ. Development of food-grade nanoemulsions and emulsions for delivery of omega-3 fatty acids: opportunities and obstacles in the food industry. Food Funct 2014; 6(1): 42-55.

[237] Quynh PP, Sharma U, Mikos AG. Tissue Engineering. Electrospinning of Polymeric Nanofibers for Tissue Engineering Applications: A Review. Tissue Eng 2006; 12(5): 1197-211.

[238] Wichterle O, Lim D. Hydrophilic gels for biological use. Nature 1960; 185: 117.

[239] Bizley SC, Williams AC, Khutoryanskiy VV. Thermodynamic and kinetic properties of interpolymer complexes assessed by isothermal titration calorimetry and surface plasmon resonance. Soft Matter 2014; 10: 8254-60.

[240] Decher G. Fuzzy Nanoassemblies: Toward Layered Polymeric Multicomposites. Science, 1997,277, 1232-7.

[241] Kharlampieva E, Kozlovskaya V, Sukhishvili SA. Layer-by-layer hydrogen-bonded polymer films: from fundamentals to applications. Adv Mater 2009; 21: 3053-65.

[242] Stockton WB, Rubner MF. Molecular-level processing of conjugated polymers. 4. Layer-by-Layer manipulation of polyaniline via hydrogen-bonding interactions. Macromolecules 1997; 30: 2717-2725.

[243] Wang L, Wang Z, Zhang X, Shen J, Chi L, Fuchs H. A new approach for the fabrication of an alternating multilayer film of poly(4-vinylpyridine) and poly(acrylic acid) based on hydrogen bonding. Macromol Rapid Commun 1997; 18: 509-14.

[244] Caruso F, Niikura K, Furlong DN, Okahata Y. Ultrathin multilayer polyelectrolyte films on gold: construction and thickness determination. Langmuir 1997; 13: 3422-6.

[245] Khutoryanskiy VV. Hydrogen-bonded interpolymer complexes as materials for pharmaceutical applications. Int J Pharm 2007; 334(1-2): 15-26.

[246] Khutoryanskaya OV, Williams AC, Khutoryanskiy VV. pH-mediated interactions between poly(acrylic acid) and methylcellulose in the formation of ultrathin multilayered hydrogels and spherical nanoparticles. Macromolecules 2007; 40: 7707-13.

[247] Ladet S, David L, Domard A. Multi-membrane hydrogels. Nature 2008; 452: 76-9.

[248] Yang Y, Wang S, Wang Y, Wang X, Wang Q, Chen M. Advances in self-assembled chitosan nanomaterials for drug delivery. Biotech Adv 2014; 32: 1301-16.

[249] Capito RM, Azevedo HS, Velichko YS, Mata A, Stupp S. Self-Assembly of large and small molecules into hierarchically ordered sacs and membranes. Science. 2008; 319(5871): 1812-6.

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