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Review article

Colloidal polyelectrolyte complexes of chitosan and dextran sulfate towards versatile nanocarriers of bioactive molecules

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ABSTRACT

Nanomedicine is an emerging field and requires new tools to achieve its goals, such as nanomaterials capable of performing various functions as bioactive (macro)molecule delivery in a spatio- and time-controlled manner, biofeedback as for instance imaging the course of a therapeutic treatment, active and controlled interaction with the biological environment as in vaccine applications. Obviously, these nanomaterials should be non-toxic, biocompatible, bioresorbable, which means also that the materials and the manufacturing processes should meet these requirements. This review is focused on colloidal polyelectrolyte complexes of chitosan and dextran sulfate, (i) because these polysaccharides comply with the above specifications; (ii) because chitosan is a complex polysaccharide whose physicochemical properties depend on the molar mass and the fraction of N-acetyl glucosamine moieties within the chain; (iii) to underline the impact of the physicochemical properties of chitosan on the performances of the colloidal complexes; (iv) to establish the versatility of the coarcervation, a mild, energy-sparing, environment friendly, straightforward to set-up manufacturing process.

1. Introduction

The development of new approaches in life sciences, like nanomedicine, a cellular level concept of medicine, requires new tools to address the challenges of a safer, less invasive, cheaper and better performing medical practice. For instance, carriers of bioactive molecule are under intense research work to improve the bioavailability of the drug or reduce its toxicity by appropriate targeting of cells/tissues/organs or to increase interactions with the biological environment, as in the vaccine delivery domain. In this latter case, the carrier should not only deliver the vaccine but also interfere with the immune system to help induce an appropriate immune response (adjuvant effect). There are new needs also in imaging to visualize tissue alteration and also to monitor locally the impact of a therapeutic protocol. As new tools for nanomedicine, colloidal carriers tend to be multifunctional that is be capable of performing several functions like targeting cellular compartment to allow their imaging concomitantly with the release of an active substance and observe the local effect of the drug [1–3].

To be successful in this domain, the carriers should be biocompatible, bioresorbable, non-toxic, which seems straightforward for the medical application field, but also the manufacturing aspects should be taken into consideration. Indeed, the carriers will need to be produced in high volume at low cost and these aspects should be taken into account early in the design of the carriers. Obviously, the manufacturing process should comply with regulatory requirements; hence, the use of toxic chemicals, chemical reactions and chemical solvents should be avoided without negatively impacting the performances of the carriers. In other words, elaboration processes should be simple to implement, use only water as solvent, be energy efficient (for instance, the process should take place at room temperature) and with polymer materials originating from natural products. The elaboration of colloidal carriers by the formation of polyelectrolyte complexes, from aqueous solutions of polysaccharides of opposite charges, meets the above requirements and thus is a very promising technology as reviewed globally by Hartig et al. in 2007 [3] and more specifically for chitosan by Berger et al. in 2004 [4].

Colloidal polyelectrolyte complexes can be obtained with a great variety of naturally occurring polymers. Nucleic acids like DNA [5] or siRNA [6] have formed nanoparticles used in molecular strategies, but the most often reported polyanions associated with chitosan are polysaccharides and to a lesser extent polypeptides like poly(glutamic acid) [7] or poly(aspartic acid) [8]. As examples of polysaccharides, carboxymethyl cellulose [9], alginates [10], carboxymethyl konjac glucomannan [11], hyaluronan and heparin [12] were used. But the most widely used polysaccharide is dextran sulfate because it is cheap (when compared to hyaluronan or heparin), easily available (when compared to glucomannans for instance) and the presence of the sulfate groups ensures strong electrostatic interactions with the ammonium groups of chitosan.

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Therefore, the present paper focuses on the state of the art of the knowledge on the elaboration and applications of chitosan and dextran sulfate (DS) colloidal PECs (Fig. 1), with an emphasis on the direct link between the physicochemical properties of polymer solution, the structure and the colloidal properties of resulting particles and, finally, the performances of the obtained nanocarriers. Chitosan is a polycation, a copolymer of N-acetyl glucosamine and glucosamine linked by a β $1 \rightarrow 4$ glycosidic linkage. Dextran sulfate and chitosan are both biocompatible and bioresorbable polymers with safe safety profiles; therefore, they both constitute excellent candidates for the production of multifunctional nanoparticles for nanomedicine according to the requirements defined above.

2. Elaboration of the colloidal polyelectrolyte complexes

2.1. Colloids from polyelectrolyte neutralization

Polyelectrolyte complexes (PECs) can be obtained in aqueous solutions by electrostatic interactions between charged domains of at least two oppositely charged polyelectrolytes. At low ionic strength, the process is entropy driven, thanks to the release of small counterions initially bound to the polyelectrolytes. But other types of interactions can favor the ion-pairing process such as hydrogen bonding or van der Waals interactions [13]. The formation of PECs has been extensively studied with synthetic polymers by the groups of Kabanov [14,15] and Tsuchida and Abe [16]. The complexes obtained from polyelectrolytes having significantly different molar masses, mixed in non-stoichiometric ratios, were water-soluble aggregates at the molecular scale. Complexation between polymers with comparable large and/or strong ionic groups led to phase separation. To obtain a colloidal dispersion, high dilution and non-stoichiometric charge ratio conditions are required [17–19]. Other factors also impact the course of the particle formation process like the order of reactant addition [20], the addition rate [21], the respective molar mass of the two counterparts [17,20,21], their relative (and absolute) concentration [13,22], the presence of salt and also the pH of the medium[17,23] that can alter the ionization degree of the polymers.

In 2003, Chen et al. first reported the elaboration of submicrometric particles by adding an aqueous solution of dextran sulfate (Mw 12,750 g mol^{-1}) to a chitosan (Mw 400,000 g mol^{-1}; DA 15%) solution dissolved in acetic acid [24]. In these experiments, DS was in excess and the particle size decreased with the chitosan/dextran sulfate weight ratio. In 2007, these results were correlated to the n^+/n^- charge ratio: as the charge ratio decreased (n^+/n^- of 0.89 and lower) on increasing the amount of DS in the reaction mixture, the particle size and polydispersity index decreased, the excess DS improving the colloidal stability of the dispersion [25]. The presence of excess negative charges was evidenced by zeta potential measurements. Another important factor impacting the particle size was the initial concentration of the polyelectrolyte solutions: lower concentrations led to smaller particle sizes. Interestingly, this trend was not observed in a follow-up work [26], when the molar mass of DS was reduced from 12,759 g mol^{-1} down to 5000 g mol^{-1}. The authors attributed this to the increased capacity of the lower molar mass DS to diffuse within the core of the particles.

2.2. Conformational aspects

The formation of the particles starts with polyelectrolytes in solution; hence, the polymer conformation in solution is a pertinent parameter to investigate, so as to understand and control the particle formation process. Domard and collaborators have extensively investigated the impact of DA on the physicochemical properties of chitosan in aqueous solution, and they have established a general law of behavior [27–29]. Three regions in the DA range were identified (Fig. 2):

- $0\% \leqslant DA \leqslant 25\%$ in which chitosan, having a high charge density, behaves as a strong polyelectrolyte (with, for instance, a high intrinsic viscosity, high second virial coefficient). In this DA range, the screening of repulsive electrostatic forces by an electrolyte can alter the conformation of the chain;
- $25\% \leqslant DA \leqslant 50\%$ where the physicochemical properties of chitosan remain constant due to balanced hydrophilic and hydrophobic interactions;
- $DA \geqslant 50\%$ for which hydrophobic interactions predominate, favoring polymer chain association.

The stiffness of the polymer chain was assessed by measuring the persistence length (Lp) of chitosan. Lp varied from 4.5 to 8.8 nm according to DA and Mw. When compared, Lp of DS (1.5×10^6 g/mol) was 1.6 nm [13]. The conformation in solution of polyelectrolytes depends on the intensity of electrostatic repulsive forces which expand and stiffen the macromolecules, as also shown by Hlady [30] for dextran sulfate. He found that the intrinsic viscosity of dextran sulfate decreased from 550 to 100 mL g^{-1} when the sodium chloride concentration increased from 1 mM to 100 mM.

2.3. Impact of internal parameters on the formation of colloidal polyelectrolyte complexes

Internal parameters are the DA and the degree of polymerization (Dp) of chitosan and also the chitosan-to-DS DP ratio. The particle formation can be detected via the Tyndall effect, which occurred very early in the addition process, for charge ratios of 20 i.e. in the presence of low amounts of dextran sulfate. This

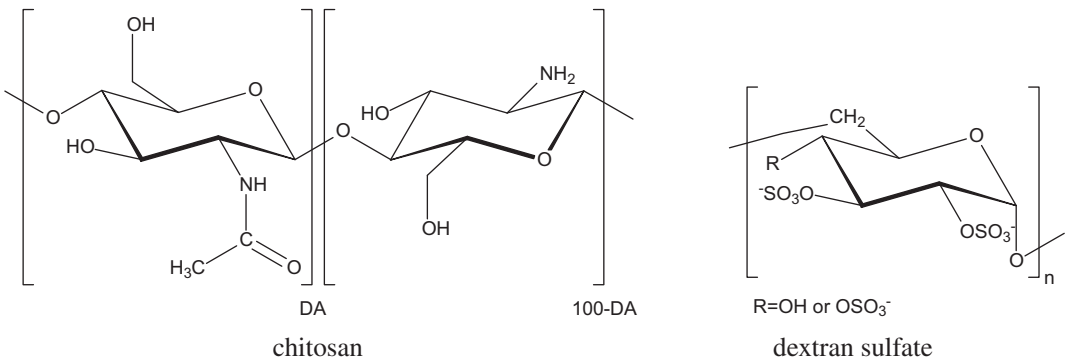

Fig. 1. Chemical structures of the polysaccharides chitosan and dextrans sulfate (DS). DA (%) refers to the degree of acetylation of chitosan.

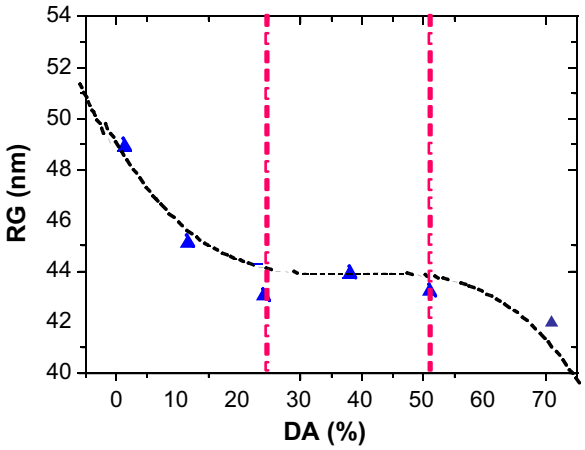

Fig. 2. Variations of the solution properties of chitosan (here the radius of gyration) with the degree of acetylation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

suggests a kinetically controlled formation process, attributed to the difference in pKa_0 of the two polyelectrolytes. As the addition of the reactant proceeded, the solutions became more turbid, suggesting the formation of new particles. The particle size of the polyelectrolyte complexes increased with the DA of chitosan and also its molar mass (Fig. 3) [13,22]. Interestingly, the particle formation process depended also on the nature of the polymer in excess. Similarly, with chitosan, an increase in the molar mass of the polyanion, when used in excess, resulted in larger particles. But low molar mass DS always led to aggregated material, conversely to high molar mass DS and to the fact that this low molar mass DS, used with excess chitosans, led to well-defined colloids. This can be understood by taking into account two factors: (i) the mode of formation of the polyelectrolyte complexes and (ii) the 'reactivity' of the polymers. According to Tsuchida and Abe [16] and Kabanov and Zezin [14], the formation of PECs in the presence of a high molar mass polymer in excess (host) and a default of lower molar mass counterpart (guest) takes place via guest–host complexes in which neutral segments are formed by charge neutralization. These neutral segments can segregate and form the core of the particles, the excess polymer forming the outer shell. This model applies well for cases (a and d) of Fig. 4. But, when the low molar mass polymer is in excess, this model is no longer valid, so the

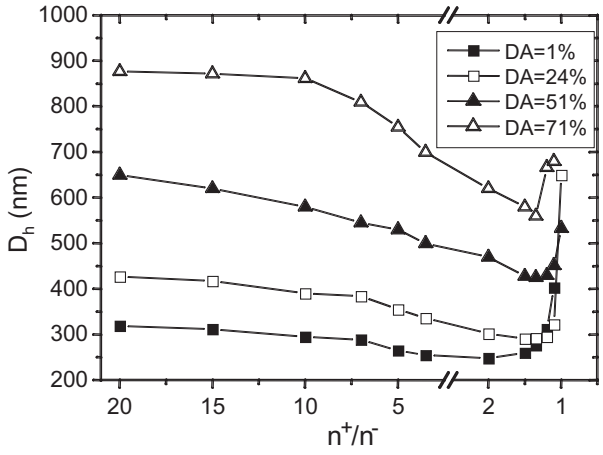

Fig. 3. Influence of the degree of acetylation of a medium molecular weight chitosan (130,000 g mol^{-1}) on the particle size of colloidal PECs. Adapted from [13] with permission.

reactivity of the low molar mass polyion should be considered. The reactivity means the capability of the low molar mass polyion to neutralize the charges of the higher molar mass counterpart. The low molar mass chitosan is more rigid (due to the β, $1 \rightarrow 4$ glycosidic bond) than dextran sulfate (a α $1 \rightarrow 6$ glycosidic linkage) and so less capable of the conformation reorganisations maximizing the charge neutralization. Hence, low molar chitosan can be used in excess to lead to stable colloids (Fig. 4 case b). Conversely, low molar mass dextran sulfate in excess can neutralize the charges of the high molar mass chitosan to such an extent that the PECs aggregate, because of the absence of residual charges to ensure the colloidal stability (Fig. 4 case c).

The presence of the excess polymer at the interface was evidenced by the values of zeta potentials, which were negative or positive when DS or chitosan were respectively in excess. The organization of the interface could be observed by small-angle X-ray scattering (SAXS) on conditions where the DPs of each counterpart were quite different, which corresponds to the guest–host model mentioned earlier (see Fig. 4) [31].

So, to summarize the effect of the polymer solution properties on the formation of the PECs particles, all experimental conditions favoring the ionic neutralization process, leading to segments with a high density of neutral ion pairs, resulted in highly neutralized complexes, hence to small particle sizes. But, if the neutralization is maximal, particles flocculate due to a lack of electrostatic repulsive forces, and this is achieved when the charge ratio is close to unity or during the formation process when the experimental conditions are such that highly neutralized complexes are formed (in that case, we referred that as the reactivity of the polyions, *i.e.* conditions when low molar mass dextran in excess is titrated with high molar mass chitosan).

2.4. Impact of external parameters and mode of addition of reactants on the formation of colloidal polyelectrolyte complexes

Dextran sulfate, as a salt of a strong acid, is always dissociated, thus the pH of the medium is determined by the fact that the primary amines of chitosan should be protonated to allow electrostatic interactions. Hence, the pH limit is below the pKa_0 of chitosan.

The ionic strength of the mixture impacts the final size of the colloids as follows: an increase in ionic strength induced a decrease in the average diameter, which could be related to the increase in chain flexibility reported in Section 2.2.

Finally, the mode of addition of the reactants and the order of addition were investigated. When adding the titrant solution dropwise to the starting solution, the order of mixing was essential and the polymer in default had to be added to the one in excess to avoid the formation of aggregates. In other words, when the charge ratio reached unity, the system irreversibly aggregated in contrast with what was reported for synthetic polymers [20]. Conversely, the one-shot addition of the titrant solution was insensitive to the order of addition, confirming that the particle formation process is kinetically controlled. Hence, a very simple production method was developed relying on the rapid addition of the titrant to reach the desired charge ratio [13,22].

Another interesting aspect is the impact of the composition of the colloidal PECs on their colloidal stability and hence, later, on the performances of the carriers. Weber et al. investigated the colloidal stability, in the presence of a physiological salt concentration, of cationic particles obtained with chitosans of various degrees of acetylation and molar masses, and DS of two distinct molar masses [31]. Particles obtained from chitosan samples of DA inferior to 30% immediately aggregated in 150 mM NaCl. This probably relates to the solution behavior of chitosan, as it has been shown that for DA < 30%, chitosan behaved as a strong

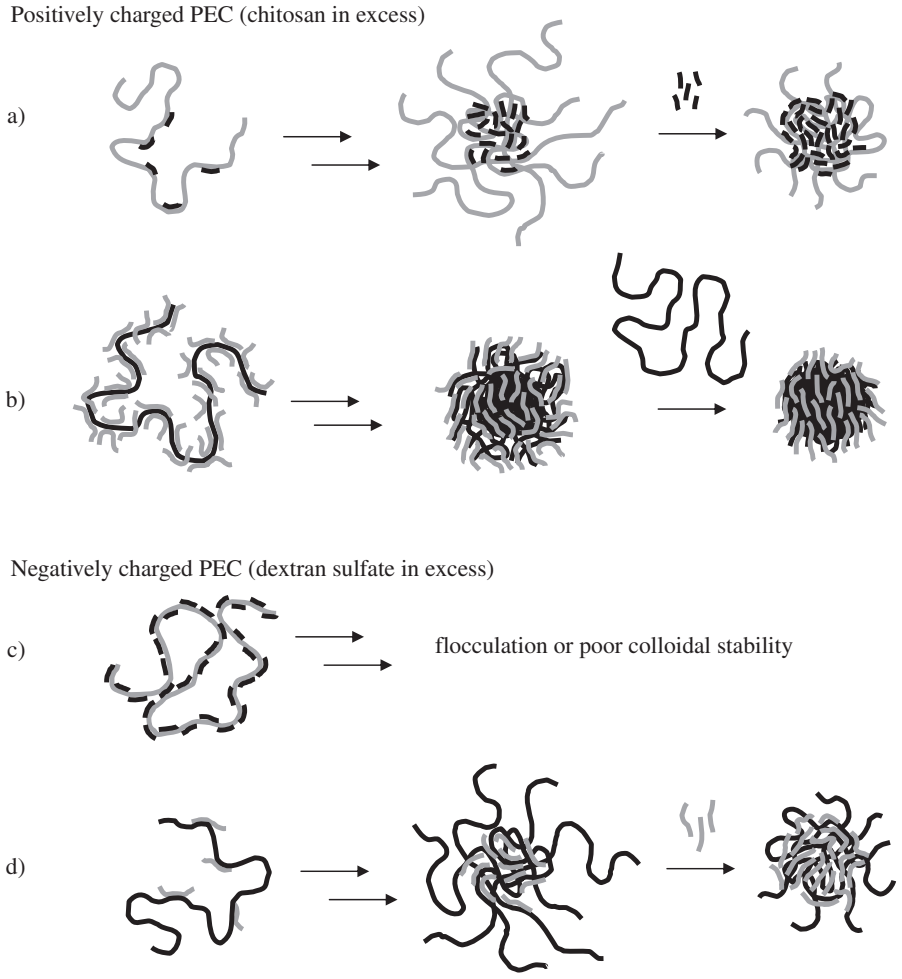

Fig. 4. Formation of colloidal polyelectrolyte complexes based on chitosan (■) and dextran sulfate (■) through the hydrophobic segregation of complexed segments. Four limit cases are considered according to the charge of the colloid and the molecular weight of components (schemes are not to scale). (a and c) high Mw chitosan versus low Mw dextran sulfate (b and d) low Mw chitosan versus high Mw dextran sulfate. From [22] with permission.

polyelectrolyte with a high charge density [27–29]. Thus, the colloidal stability relied mainly on repulsive electrostatic forces. For DAs equal or above 30%, the aggregation kinetics of the colloidal PECs depended on the degree of acetylation and also on the DP ratio of the two polymers. The optimal colloidal stability at physiological salt concentration was obtained for a chitosan sample of DA = 51% with a molar mass of 150 kg mol^{-1} and a DS of 10 kg mol^{-1}. This composition matches the guest–host model in which a high DP polymer used in excess hosts low DP guests, the DP ratio between the host (chitosan) and guest (DS) being 30. The interfacial chitosan chains stabilize the colloid by electrostatic repulsive interactions, but also as the charge density is greatly reduced, steric repulsion occurred. Worth noticing is that for DA = 70%, a poor stabilization was observed, probably because as seen above for the behavior of chitosan in solution, hydrophobic interactions predominate and lead to the aggregation of the particles.

3. Characterization

3.1. Particle size and morphology

The most common and routine method for the determination of the mean hydrodynamic diameter of the particle is quasielastic light scattering or photon correlation spectroscopy. The time-dependent fluctuations in scattering intensity, due to the Brownian motion of the particles, can be derived via an autocorrelation function, which is an exponential decay as the correlator time delay τ increases. The most commonly used algorithm for data analysis is the Cumulant method [32] in which in autocorrelation function, $g(\tau)$ was expanded in a power series:

$$g(\tau) = \exp[-\langle\Gamma\rangle t + (\mu_2/2)\tau^2 - \mu_3/3!)\tau^3 + \cdots]$$

where $\langle\Gamma\rangle$ is the mean decay rate (because particle dispersions are often polydisperse).

From these data, the average diffusion coefficient D is deduced from

$$\langle\Gamma\rangle = q^2\mathrm{D}$$

with q the scattering vector.

Hence, the mean hydrodynamic diameter D_h can be obtained using the Stokes–Einstein equation:

$$D_h = k_B T/3\pi\eta D$$

where k_B is the Boltzmann constant, T the absolute temperature and η the dynamic viscosity of the solvent.

The second cumulant algorithm allows the determination of the polydispersity index PDI of the particle dispersion PDI = $\mu_2/\langle\Gamma\rangle^2$. For a monodisperse colloid, the PDI should remain below 0.05, but values up to 0.5 can be used for comparison purposes [33].

Particles in the submicrometer range were obtained on condition that the polymer concentrations were low enough, around

0.1–0.3% (w/v). As noted previously, the particle size increased with the molar mass of the chitosan and so did the PDI. Schatz et al. obtained particles ranging in the 200–1000 nm range, with a concomitant increase in PDI from 0.2 up to 0.6 [13,22]. In general, negative particles, obtained with an excess of DS, are smaller and less polydisperse than positive ones [22,25,34]. Anyway, the polyelectrolyte complexation process leads to dispersions that cannot be regarded as isodisperse, with polydispersity indexes always above 0.05.

The morphology of the colloidal PECs can be observed by transmission or scanning electron microscopy. The general main feature from a variety of investigators is that rather polydisperse quasi-spherical particles can be observed [34–36]. But one has to be careful about the purity of the samples as the excess polymer can alter the observation: by SEM, Schatz et al. observed smooth spherical particles obtained with an excess of chitosan (whose average diameter was well above the value measured by QELS) but after removing the excess polymer, the particles appeared less smooth with an average diameter closer to the one obtained by QELS [13]. With particles obtained with excess DS, Sarmento et al. [36] could also observe the excess polyanion by TEM, after staining by uranyl acetate. Colloids of chitosan need to be stained for observation by TEM, as this material poorly adsorb the incident beam.

3.2. Complex characterization

3.2.1. IR spectroscopy

Chitosan is characterized by bands at 1651 cm^{-1} and 1596 cm^{-1} corresponding, respectively, to the Amide I band and the NH_3^+ deformation [37]. DS features a characteristic major band at 1229 cm^{-1}, corresponding to the sulfate asymmetric stretching [38] and a minor one at 1638 cm^{-1}.

The formation of the complex was evidenced by the appearance of a specific band at 1522 cm^{-1}, which was observed for both the soluble chitosan DS complexes [39] and colloidal ones [37]. The more complex was formed, the more intense this band, and, moreover, the band at 1230 cm^{-1} broadened and separated into two peaks along with the complexation process as already observed by [38]. Tyaboonchai et al. evidenced the complex formation by a change in the 1241/1251 cm^{-1} region [40] of asymmetric stretching to a new band at 1263 cm^{-1} and a shift of the NH bending adsorption at 1652 cm^{-1} and 1599 cm^{-1} for chitosan to 1623 cm^{-1} in the complex, despite DS having a band at 1642 cm^{-1} that could interfere. Sarmento et al. [41]attributed the formation of the complex to the appearance of bands at 1413 cm^{-1} and 1259 cm^{-1}, though these bands are respectively very close to the one of chitosan at 1411 cm^{-1} and 1261 cm^{-1} of DS.

3.2.2. Differential scanning calorimetry

The change in thermal properties is also a means of characterizing the formation of the polyelectrolyte complex as shown by Sarmento et al. [41]. The parent polymers exhibit endothermic peaks at respectively at 62 °C and 60.6 °C, attributed to the elimination of the water associated with the polymers and exothermic peaks at respectively 311 °C and 210 °C, corresponding to the degradation of the polymers. The DS/chitosan colloids exhibited a broad endothermic peak, whose minimum decreased from 106.5 to 84.9 when the weight ratio of DS increased. The exothermic peak was more defined and value decreased from 232 °C down to 213.5 °C, thus getting close to the value of pure DS, with an increase in the DS/chitosan weight ratio.

3.2.3. Stoichiometry

The complex formation relies on charge neutralization, and the end point of the titration can be detected by various techniques as viscosimetry [42,43], corresponding to the viscosity minimum, potentiometry or conductometry with variations induced by the change in nature of the free ions in solutions. These methods, based on the alteration of a physicochemical property of the continuous phase, provide a global assessment of the complexation reaction and always yield to a 1:1 charge to charge neutralization, but they are not a direct evidence of the complex composition. The complex composition can be obtained by elemental analysis [44,45], solid-state NMR of the complex [44], X-ray photocorrelation spectroscopy [37] or the depletion method [43,46,47]. The latter approach depends on the determination of the amount of polyelectrolyte effectively involved in the complex, by assaying for the residual free polymers. Knowing the original input, one can deduce the composition of the colloidal polyelectrolyte complexes. But discrepancies were reported between the global methods, like potentiometry, and the depletion method, proving that the determination of the real stoichiometry of the complexes cannot be obtained by a global method.

When the depletion method was applied to complexes obtained from chitosan and dextran sulfate, the composition was markedly different according to the charge ratio and to the nature of the polymer in excess. When DS was in excess to yield negative particles, the stoichiometry of the titration was fixed at about 1.7 sulfates per amino group. But when chitosan was the polymer in excess and dextran sulfate was added dropwise to this solution, the stoichiometry of the complex varied from 6 amino groups down to 1 per sulfate moiety, when the charge ratio decreased from 20 down to 1 [37]. This unusual behavior shows, at least with these polysaccharides, that the complexation process was influenced by parameters other than electrostatic interactions and that one had to take into account the flexibility of the polymer and the nature of the ion species (weak for the ammonium, strong for the sulfate group).

4. Chitosan/dextran sulfate colloidal complexes in nanomedicine

4.1. Chitosan/dextran sulfate colloidal complexes as drug delivery systems

This concept comes from the pioneer work of Janes et al., who were facing problems in incorporating doxorubicin, a well-known anticancer drug, into chitosan nanoparticles for the controlled release of the active molecule over an extended period of time [35]. Doxorubicine bears a primary amine, which upon protonation can generate electrostatic repulsive forces preventing an efficient microencapsulation of the drug. To increase the encapsulation efficiency, their idea was to neutralize the positive charge of the drug by using a negatively charged polymer, namely dextran sulfate. This strategy allowed an increase in the encapsulation of the drug by a factor 2 when compared to without any polyanion. But in fact, the particles were obtained by ionic gelation of chitosan with TPP, a method widely used by the group of Santiago de Compostela, and DS was used as a minor component, around 10% w/w in comparison with chitosan. This strategy was also used to encapsulate aminoglycosides like streptomycin, gentamycin and trobramycin in chitosan particles obtained by ionic gelation with TPP [48]. The cross-linking of chitosan was achieved by adding TPP, and the major parameter controlling the particle formation and stability was the chitosan-to-TPP ratio. Excess TPP causing instability and aggregation of the colloids and default TPP leading to poorly defined particles.

Another approach consisted in making chitosan/DS polyelectrolyte complex nanoparticles with DS as the excess polymer and ionically cross-linking the DS chains with zinc sulfate. This time, negatively charged particles were obtained, and the encapsulation

of poorly water-soluble amphotericin B could take place during the mixing of the DS solution containing the drug and the chitosan solution [40].

The first example of purely polyelectrolyte complex as drug delivery systems was reported by Tan et al. [49] who investigated various parameters impacting the elaboration of the microparticles such as the molar mass and concentration of DS, the drug concentration and the pH of the formulation medium. In contrast to the work of Janes et al. [35] and Lu et al. [48], doxorubicin was added to the chitosan solution and not to the DS solution. The authors obtained loading efficiency up to 99% even at a low dose of doxorubicin of 100 nM. A similar approach was used to encapsulate hydralazine, a compound capable of complexing with acrolein, an endotoxin produced after cell injury. An initial burst of 15–30% of the total load was evidenced after 5 h, which decreased to a slow but constant release rate for several days [50]. An initial burst was also observed by Janes et al. [35], with a much lower constant rate, though.

4.2. Chitosan/dextran sulfate colloidal complexes as peptides and protein carriers

Peptides or proteins are fragile natural molecules that can be damaged during formulation, storage and also can be quickly degraded by enzymes after administration. Hence, it is a challenge to develop carriers whose production process is not deleterious to these molecules and which still allow a controlled release of their payloads. The formation of colloidal polyelectrolyte complexes is very mild; it occurs in water and does not require any surfactant. Hence, this encapsulation strategy was used in 2003 by Chen et al., with DS and chitosan as polyions [24]. Their model hexapeptide, arginine-rich (so positively charged), is capable of blocking the growth and metastasis of human colon carcinoma cells, by interacting with a vascular endothelial growth factor. The particles were obtained by adding an excess amount of DS solution in water (containing or not the peptide) to a solution of chitosan. The authors evidenced that too high an excess of DS led to a poor incorporation of the peptide, which was unexpected. The authors explained this result on the basis of the formation of water-soluble complexes of DS and peptide not incorporated within the particles. So, chitosan-to-DS weight ratios above 0.6. were optimum for the incorporation, but the greater the ratio, the larger the particles. In 10 mM phosphate-buffered saline pH 7.4, the release of the peptide depicted a burst up to 24 h (corresponding to 40–60% of the initial payload) and then it levelled off to a very slow release. This result suggests that a fraction of the peptide was not bound to the matrix of the particles via electrostatic interactions. In a latter study, Chen et al. optimized the encapsulation of Rhodamine 6G (R6G, a positively charged dye) as a model water-soluble drug and bovine serum albumin (BSA) as a model protein [25]. The loaded nanoparticles formed spontaneously upon mixing 0.1 w% of DS solution (containing eventually R6G) with 0.1 w% of chitosan solution (containing eventually BSA). The presence of the protein or dye in the nanoparticle matrix led to a marked increased in diameter when compared with the so-called empty particles. The main parameter affecting the entrapment of BSA was the n^+/n^- charge ratio: the lower the ratio, the better the entrapment, which suggests the entrapment efficiency to be directly related to the fraction of DS in the particle formulation recipe. Moreover, the maximum incorporation of BSA was achieved when the protein was below its isoelectric point (i.e. when it was globally positively charged). All these arguments enlighten the major role of electrostatic interactions between the protein and the polyanion. A similar trend was observed for the incorporation of R6G. The release of incorporated molecules was directly related to the ionic strength of the buffer; the higher the salt concentration, the more molecules were desorbed. As seen for small molar mass drugs, a burst effect at day 1 was observed (concerning 40% of the total payload of BSA), which decreased to a constant release rate over several days. The release profile of R6G was much faster, the timescale of the experiment was in hours and the burst release concerned ca. 50% of the payload in 1 h.

Insulin injections are a current means to treat diabetes, but the development of oral formulations would be more convenient on a day-to-day basis. The gastrointestinal uptake of insulin can be improved by the use on nanoparticles whose role would be to protect the protein from degradation from low pH and protease hydrolysis that are the major limitations to intestinal adsorption of intact insulin and transport into systemic circulation. Sarmento et al. have incorporated insulin into colloidal PECs of chitosan and DS [41], and the main parameter impacting insulin incorporation was the chitosan-to-DS weight ratio: high incorporation yields of 80% and higher were obtained for values of the chitosan-to-DS weight ratio of 0.7 and lower. In other words, as we have seen for BSA, DS appears to be responsible for the association of insulin with the nanovectors. The incorporation yields slightly increased when the pH of the preparation medium was decreased from 4.8 down to 3.2, with no alteration in the particle size, an improvement that the authors attributed to the higher charge density of insulin with decreasing pH. As the chitosan-to-DS weight ratio was the major parameter controlling the association of insulin, it was logically an important parameter on the release of insulin; the lower the ratio, the less protein was released. But interestingly, in the ratio range investigated, there was no impact of the chitosan-to-DS weight ratio on the kinetics, nor on the mode of release of insulin. The release profiles were highly dependent on the pH of the medium in which the experiments were carried out: at pH 6.8, a burst release over 30 min was observed leading to a release of ca. 40% of the total insulin payload, whereas at pH 5.2 and lower, no insulin was released whatsoever. Consequently, the authors could show that in simulated gastric fluid at pH 1.2, no release took place, whereas in intestinal simulated fluids at pH 6.8, insulin could be released [51]. A burst over the 30 first minutes, concerning 50% of the payload, was observed, then the release took place at a slower rate over 6 h. Diabetic rats were fed with 50 and 100 UI/kg of insulin incorporated within colloidal PECs, and hypoglycemia was analyzed in comparison with 2.5 UI/kg of insulin injected subcutaneously. The effect of free insulin at reducing plasma glucose levels was instantaneous, and within 2 h, the glucose level dropped at 50% of its original value. The glucose plasma concentration of rats treated with 100 UI/kg of insulin incorporated within colloidal PECs was only reduced to 80% of its original value after 2 h and that of rats treated with 50 UI/kg of insulin incorporated within colloidal PECs was statistically similar to that of non-treated rats. But, interestingly, whereas the glucose plasma level returned to the original value of the rats treated subcutaneously 8 h post-injection, the glycemia of rats fed with encapsulated insulin remained at 70% of its original value up to 24 h. It is interesting to note that there is a delay between the glycemia effect time frame, maximal effect at 8 h post-ingestion and the rapid release of insulin in simulated intestinal fluid: ca. 60% release within 1 h. The difference in kinetics of pharmaceutical effect between free insulin and particle-loaded insulin is explained by the differences in modes of administrations. By subcutaneous injections, insulin is readily available in the blood circulation. By oral administration, the authors show that insulin is transported through the intestinal mucosa via a two-step process involving binding of the nanoparticles to mucosa and, then, internalization via special cells of the intestine, the Peyers's patches or diffusion of insulin through the tissue. Very promisingly, the authors showed that the pharmaceutical availability was increased by a factor 3, thanks to the colloidal PECs [51].

Growth factors are proteins that are involved in the development of many tissues, like in arterio or angiogenesis, by favoring cell proliferation and differentiation. . .These growth factor should be delivered at a specific location in the body, where needed for the regeneration of the right organ, in a sustained way to allow the correct development of the tissue. Growth factors are labile proteins with a very short half-life and so delivery systems should also allow the stabilization of the growth factor in their active forms. Taking these specifications into account, Min et al. investigated the encapsulation of two different growth factors in colloidal PECs based on chitosan and excess DS [34,52]. Growth factors are known to interact with polyanions; hence, the protein was premixed with the DS solution to which the chitosan solution was added. At the end of the elaboration process, zinc sulfate was added to harden the colloids of mean diameter around 250 nm. The entrapment efficiencies were in the 80% range. No burst effect was observed in the release profiles in PBS buffer, suggesting that the interactions with DS were stronger than for BSA and insulin, probably because these proteins bear a cationic pocket [52]. The controlled release persisted for nearly 10 days, and the activities of these growth factors were maintained in comparison with the free protein. These investigations underlie the advantage of excluding harsh conditions to the profit of mild formulation processes (no emulsification, no chemicals) that preserve the integrity of labile proteins and are environment friendly.

An alternative to the encapsulation of proteins is the surface adsorption of the biological macromolecules onto preformed colloids. This strategy was selected by Drogoz et al. and Weber et al. for the delivery of sub-unit vaccines [31,53]. The development of safe, though efficient, vaccines is based on the use of one, or several, protein(s) constituting the pathogen, rather than the whole pathogen itself. Proteins alone do not trigger the immune system efficiently enough and they must be associated with adjuvants, compounds whose role is to stimulate the immune system. For this particular application, colloidal carriers have been under intense investigation [54], and particles obtained from safe, biodegradable materials via a mild formulation process are quite attractive. The coacervation approach is quite versatile for controlling the particle size and interfacial properties by playing with the polymer concentration and the n^+/n^- charge ratio, as we have seen in the 'Elaboration Section'. Drogoz et al. showed that, according to the global surface charge of the colloidal PECs, negative or positive, the adsorption kinetics of a model antigen, the p24 capsid protein from the HIV-1 virus, were quite different: less than 2 h were necessary to reach surface saturation of the negative particles, whereas more than 20 h were required for the positively charged ones. The reasons for these differences were not clearly understood; apparently, electrostatic attractive forces were not responsible for these differences since, in the experimental conditions, the global charge of both the carriers and p24 were of similar nature. The maximal binding capacity of the particles, i.e. the maximal amount of proteins loaded per g of carrier, was of 600 mg g^{-1} and 120 mg g^{-1}, respectively, for the negative and the positive colloidal PECs [53]. Such a high loading capacity was attributed to the (at least) partial diffusion of the protein within the shell, constituted by the excess polymer at the interface of the colloids.

Theses carriers were assessed in mice by subcutaneous injections in blab-C mice and compared with Freund's adjuvant, a bacterial extract regarded as a gold standard but too toxic to be used in humans. The specific antibody titers obtained with either positively or negatively charged colloids were similar to those resulting for immunizations with Freund's adjuvant and were dose dependant on the amount of injected protein (see Fig. 5).

4.3. Imaging applications

Min Huang et al. have obtained gadolinium-loaded colloidal PECs, for magnetic resonance imaging (MRI) using a chitosan sample covalently grafted with Gd diethylenetriaminepentaacetic acid (DPTA) [55]. Particles around 300 nm in diameter, with a negative global charge, were formed using the polyelectrolyte complexation strategy, by adding dropwise an aqueous solution of chitosan–Gd derivative into DS. The coacervation strategy was also used to ionically entrap Gd during the particle formation process, and finally, a combination of both strategies was tested. After formation, the colloids were hardened with zinc sulfate. Particles with chemically bound Gd did not release the gadolinium, whereas a burst effect was observed on day one, concerning 50% of the total payload of the ionically entrapped Gd. The enhancement of MRI signals obtained with the colloidal PECs was similar in peak intensity and trend to Magnevist, a contrast agent commonly used in humans. But, to obtain this result, much larger amounts of PECs were necessary. In vivo, the particles were rapidly cleared by the kidney, as readily evidenced by MRI.

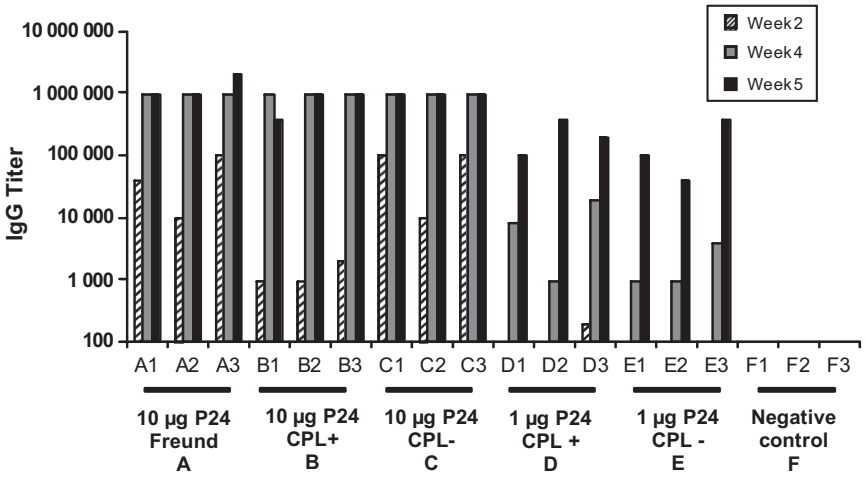

Fig. 5. Antibody titers in mice immunized with p24/particles and in comparison with Freund's adjuvant. Animals, 3 per group, received 3 injections of 10 or 1 µg of p24 protein mixed with Freund's adjuvant or sorbed onto cationic or anionic particles based on chitosan Mw = 136,000 g mol^{-1}, DA = 11.5% and DS Mw = 1.5 × 10^6 g mol^{-1}. For each animal, the IGg titers of serum sample taken on days 14, 28 and 35 was determined individually. CPL stands for colloidal polyelectrolyte complexes the + or − represents the global charge of the particles. From [53] with permission.

5. Conclusions

Many investigations reported here show that the formation of colloids from oppositely charged polysaccharides is a method that meets the requirements for the developments of carriers for nanomedicine.

First, considering the materials used, chitosan is a well-known and characterized polymer, the only existing cationic polysaccharide and is widely available from biomass as it is obtained from chitin, one of the most abundant polymers on Earth. Dextran sulfate is a biodegradable negatively charged polysaccharide widely used for pharmaceutical applications [40].

Second, the particle formation process can be regarded a typical green manufacturing approach: it is environment friendly, using only water as solvent and no toxic chemicals, and energy efficient, the reaction takes place at room temperature with a moderate stirring rate. Moreover, this process consists in mixing two solutions and thus is simple enough to be potentially scaled up.

Third, the particle formation process is mild, by essence, as seen above, thus it can be used to encapsulate fragile molecules like proteins with preservation of their functionality, as we have seen for the growth factors for instance.

Fourth, the particle formation process is versatile, low or high molecular weight (macro)molecules can be entrapped, either water soluble (positively or negatively charged, hence they can interact with one of the counterparts in the manufacturing process) or non-water soluble (in that case a polar water-soluble solvent can be used to dissolve the molecule see [40]). Very interestingly, molecules of biological interest can be loaded at the interface of the colloidal PECs, post-synthesis. Finally, MRI agents can be incorporated within the core of the matrix, opening thus the field of biological imaging to this technology.

Fifth, the colloidal PECs are mucoadhesive, are pH responsive [51], allow the sustained release of low and high molar mass bioactive substances, interact positively with the immune system, can be internalized by various cells, display a very low cytotoxicity, are biodegradable and bioresorbable.

To come back to the versatility of the particle formation process, the properties of the final colloids can be varied by the relative polymer concentration and the charge ratio, but also by their intrinsic properties, that is, the DP and also for chitosan, the DA. As the formation of the particles begins with polymers in solution, every factor affecting the conformation of the polymer chains in solution will have an impact of the final properties of the colloidal PECs. Thus, the modulation of the performances of the final nanocarriers is feasible and requires a good understanding of the physicochemical properties of the polymer solutions.

To be complete, one has to address the limitations of dextran sulfate/chitosan colloidal complexes. The first limitation could probably be the cost of the polymers when compared to the synthetic ones, which would limit the applications to the high-added value ones. As for any polymers obtained from biomass, there is a need for well-standardized production/purification procedures, in particular for chitosan as the impact of the DA on its conformational behavior is essential. For the colloidal chitosans obtained with an excess of polycation, the colloidal stability should be maintained in physiological media. This is a crucial issue as chitosan, with a pKa_0 around 6.5, is poorly charged at pH7.4 and thus the stabilization of the colloidal chitosans should be achieved by other means than purely electrostatic phenomena. The positive charge of the colloidal chitosans obtained with an excess of polycation may have a thrombogenic effect that will need to be taken into consideration prior systemic injections of these carriers. But these limitations will also fuel new investigations for the improvement of this type of carriers.

6. Prospects

The versatility of the particle formation process is another means of fuelling investigations for the future. Hence, nanocoacervation of chitosan and dextran sulfate should be an effective tool for the elaboration of multifunctional nanomedical systems, as suggested in the introduction of this paper, to challenge the issues of targeting the delivery of drugs to localized areas in the body, not only organs but also tissues, cells and subcellular compartments; of getting a feedback control of the therapy by including biomolecular sensors, like in vivo imaging probes; and allowing, more than a therapeutic medicine, a regenerative nanomedicine by the spatiotemporal delivery of appropriate cues for the reconstruction of locally damaged organs.

On a more down-to-earth aspect, to become a true industrial product, the scaled-up manufacturing of colloidal PECs will need to be fully addressed.

The numerous results obtained from various teams working on the nanocoacervation of chitosan and dextran sulfate underline the high potential of this technology for both the short- and long-term development of human medicine.

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References

[1] M.E. Gindy, R.K. Prud'homme, Multifunctional nanoparticles for imaging, delivery and targeting in cancer therapy, Expert Opinion on Drug Delivery 6 (8) (2009) 865–878.
[2] E. Haglund, M.-M. Seale-Goldsmith, J. Leary, Design of multifunctional nanomedical systems, Annals of Biomedical Engineering 37 (10) (2009) 2048–2063.
[3] S. Hartig, R. Greene, M. Dikov, A. Prokop, J. Davidson, Multifunctional nanoparticulate polyelectrolyte complexes, Pharmaceutical Research 24 (12) (2007) 2353–2369.
[4] J. Berger, M. Reist, J.M. Mayer, O. Felt, R. Gurny, Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications, European Journal of Pharmaceutics and Biopharmaceutics 57 (1) (2004) 35–52.
[5] W.G. Liu, S.J. Sun, Z.Q. Cao, Z. Xin, K.D. Yao, W.W. Lu, K.D.K. Luk, An investigation on the physicochemical properties of chitosan/DNA polyelectrolyte complexes, Biomaterials 26 (15) (2005) 2705–2711.
[6] K.A. Howard, U.L. Rahbek, X.D. Liu, C.K. Damgaard, S.Z. Glud, M.O. Andersen, M.B. Hovgaard, A. Schmitz, J.R. Nyengaard, F. Besenbacher, J. Kjems, RNA interference in vitro and in vivo using a chitosan/siRNA nanoparticle system, Molecular Therapy 14 (4) (2006) 476–484.
[7] Y.H. Lin, C.K. Chung, C.T. Chen, H.F. Liang, S.C. Chen, H.W. Sung, Preparation of nanoparticles composed of chitosan/poly-gamma-glutamic acid and evaluation of their permeability through Caco-2 cells, Biomacromolecules 6 (2) (2005) 1104–1112.
[8] Y.L. Zheng, W. Yang, C.C. Wang, J.H. Hu, S.K. Fu, L. Dong, L. Wu, X.Z. Shen, Nanoparticles based on the complex of chitosan and polyaspartic acid sodium salt: preparation, characterization and the use for 5-fluorouracil delivery, European Journal of Pharmaceutics and Biopharmaceutics 67 (2007) 621–631.
[9] S. Ichikawa, S. Iwamoto, J. Watanabe, Formation of biocompatible nanoparticles by self-assembly of enzymatic hydrolysates of chitosan and carboxymethyl cellulose, Bioscience Biotechnology and Biochemistry 69 (9) (2005) 1637–1642.
[10] K.L. Douglas, M. Tabrizian, Effect of experimental parameters on the formation of alginate–chitosan nanoparticles and evaluation of their potential application as DNA carrier, Journal of Biomaterials Science – Polymer Edition 16 (1) (2005) 43–56.
[11] J. Du, R. Sun, L.F. Zhang, L.F. Zhang, C.D. Xiong, Y.X. Peng, Novel polyelectrolyte carboxymethyl konjac glucomannan–chitosan nanoparticles for drug delivery. I. Physicochemical characterization of the carboxymethyl konjac glucomannan–chitosan nanoparticles, Biopolymers 78 (1) (2005) 1–8.
[12] S. Boddohi, N. Moore, P.A. Johnson, M.J. Kipper, Polysaccharide-based polyelectrolyte complex nanoparticles from chitosan, heparin, and hyaluronan, Biomacromolecules 10 (6) (2009) 1402–1409.
[13] C. Schatz, J.M. Lucas, C. Viton, A. Domard, C. Pichot, T. Delair, Formation and properties of positively charged colloids based on polyelectrolyte complexes of biopolymers, Langmuir 20 (18) (2004) 7766–7778.

[14] V. Kabanov, A.B. Zezin, A new class of complex water-soluble polyelectrolytes, Macromolecular Chemistry and Physics (Suppl. 6) (1984) 259–276.
[15] V.A. Kabanov, A.B. Zezin, Soluble interpolymeric complexes as a new class of synthetic poly-electrolytes, Pure and Applied Chemistry 56 (3) (1984) 343–354.
[16] E. Tsuchida, K. Abe, Interactions between macromolecules in solution and intermacromolecular complexes, Advances in Polymer Science 45 (1982) 1–119.
[17] H. Dautzenberg, Polyelectrolyte complex formation in highly aggregating systems. 1. Effect of salt: polyelectrolyte complex formation in the presence of NaCl, Macromolecules 30 (25) (1997) 7810–7815.
[18] H. Dautzenberg, J. Hartmann, S. Grunewald, F. Brand, Stoichiometry and structure of polyelectrolyte complex particles in diluted solutions, Berichte Der Bunsen-Gesellschaft – Physical Chemistry Chemical Physics 100 (6) (1996) 1024–1032.
[19] H. Dautzenberg, N. Karibyants, Polyelectrolyte complex formation in highly aggregating systems. Effect of salt: response to subsequent addition of NaCl, Macromolecular Chemistry and Physics 200 (1) (1999) 118–125.
[20] H.M. Buchhammer, M. Mende, M. Oelmann, Formation of mono-sized polyelectrolyte complex dispersions: effects of polymer structure, concentration and mixing conditions, Colloids and Surfaces A – Physicochemical and Engineering Aspects 218 (1–3) (2003) 151–159.
[21] E.S. Dragan, S. Schwarz, Polyelectrolyte complexes. VI. Polycation structure, polyanion molar mass, and polyion concentration effects on complex nanoparticles based on poly(sodium 2-acrylamido-2-methylpropanesulfonate), Journal of Polymer Science Part A – Polymer Chemistry 42 (10) (2004) 2495–2505.
[22] C. Schatz, A. Domard, C. Viton, C. Pichot, T. Delair, Versatile and efficient formation of colloids of biopolymer-based polyelectrolyte complexes, Biomacromolecules 5 (5) (2004) 1882–1892.
[23] T. Etrych, L. Leclercq, M. Boustta, M. Vert, Polyelectrolyte complex formation and stability when mixing polyanions and polycations in salted media: a model study related to the case of body fluids, European Journal of Pharmaceutical Sciences 25 (2–3) (2005) 281–288.
[24] Y. Chen, V. Mohanraj, J. Parkin, Chitosan–dextran sulfate nanoparticles for delivery of an anti-angiogenesis peptide, Letters in Peptide Science 10 (5) (2003) 621–629.
[25] Y. Chen, V.J. Mohanraj, F. Wang, H.A.E. Benson, Designing chitosan–dextran sulfate nanoparticles using charge ratios, AAPS PharmSciTech 8 (4) (2007) E98.
[26] Y. Chen, B. Siddalingappa, P.H.H. Chan, H.A.E. Benson, Development of a chitosan-based nanoparticle formulation for delivery of a hydrophilic hexapeptide, dalargin, Biopolymers 90 (5) (2008) 663–670.
[27] G. Lamarque, J.M. Lucas, C. Viton, A. Domard, Physicochernical behavior of homogeneous series of acetylated chitosans in aqueous solution: role of various structural parameters, Biomacromolecules 6 (1) (2005) 131–142.
[28] A. Montembault, C. Viton, A. Domard, Rheometric study of the gelation of chitosan in aqueous solution without cross-linking agent, Biomacromolecules 6 (2) (2005) 653–662.
[29] C. Schatz, C. Viton, T. Delair, C. Pichot, A. Domard, Typical physicochemical behaviors of chitosan in aqueous solution, Biomacromolecules 4 (3) (2003) 641–648.
[30] V. Hlady, Adsorption of dextran and dextran sulfate on precipitated calcium oxalate monohydrate, Journal of Colloid and Interface Science 98 (2) (1984) 373–384.
[31] C. Weber, A. Drogoz, L. David, A. Domard, M.-H. Charles, B. Verrier, T. Delair, Polysaccharide-based vaccine delivery systems: macromolecular assembly, interactions with antigen presenting cells, and in vivo immunomonitoring, Journal of Biomedical Materials Research Part A 93A (4) (2010) 1322–1334.
[32] D.E. Koppel, Analysis of macromolecular polydispersity in intensity correlation spectroscopy – method of cumulants, Journal of Chemical Physics 57 (11) (1972) 4814–4820.
[33] A.G.A. Coombes, P.D. Scholes, M.C. Davies, L. Illum, S.S. Davis, Resorbable polymeric microspheres for drug-delivery – production and simultaneous surface modification using Peo–Ppo surfactants, Biomaterials 15 (9) (1994) 673–680.
[34] H. Min, S.N. Vitharana, L.J. Peek, T. Coop, C. Berkland, Polyelectrolyte complexes stabilize and controllably release vascular endothelial growth factor, Biomacromolecules 8 (5) (2007) 1607–1614.
[35] K.A. Janes, M.P. Fresneau, A. Marazuela, A. Fabra, M.J. Alonso, Chitosan nanoparticles as delivery systems for doxorubicin, Journal of Controlled Release 73 (2–3) (2001) 255–267.
[36] B. Sarmento, A. Ribeiro, F. Veiga, D. Ferreira, Development and characterization of new insulin containing polysaccharide nanoparticles, Colloids and Surfaces B – Biointerfaces 53 (2) (2006) 193–202.
[37] A. Drogoz, L. David, C. Rochas, A. Domard, T. Delair, Polyelectrolyte complexes from polysaccharides: formation and stoichiometry monitoring, Langmuir 23 (22) (2007) 10950–10958.
[38] W. Tiyaboonchai, J. Woiszwillo, C.R. Middaugh, Formulation and characterization of amphotericin B-polyethylenimine–dextran sulfate nanoparticles, Journal of Pharmaceutical Sciences 90 (7) (2001) 902–914.
[39] A.I. Gamzazade, S.M. Nasibov, Formation and properties of polyelectrolyte complexes of chitosan hydrochloride and sodium dextransulfate, Carbohydrate Polymers 50 (4) (2002) 339–343.
[40] W. Tiyaboonchai, N. Limpeanchob, Formulation and characterization of amphotericin B-chitosan–dextran sulfate nanoparticles, International Journal of Pharmaceutics 329 (1–2) (2007) 142–149.
[41] B. Sarmento, A. Ribeiro, F. Veiga, D. Ferreira, Development and characterization of new insulin containing polysaccharide nanoparticles, Colloids and Surfaces B – Biointerfaces 53 (2) (2006) 193–202.
[42] K.Y. Lee, W.H. Park, W.S. Ha, Polyelectrolyte complexes of sodium alginate with chitosan or its derivatives for microcapsules, Journal of Applied Polymer Science 63 (4) (1997) 425–432.
[43] T. Takahashi, K. Takayama, Y. Machida, T. Nagai, Characteristics of polyion complexes of chitosan with sodium alginate and sodium polyacrylate, International Journal of Pharmaceutics 61 (1–2) (1990) 35–41.
[44] W.B. Chen, L.F. Wang, J.S. Chen, S.Y. Fan, Characterization of polyelectrolyte complexes between chondroitin sulfate and chitosan in the solid state, Journal of Biomedical Materials Research Part A 75A (1) (2005) 128–137.
[45] Y. Kikuchi, H. Fukuda, Polyelectrolyte complex of sodium dextran sulfate with chitosan, 2, Macromolecular chemistry 178 (1977) 2895.
[46] A. Hugerth, N. Caram-Lelham, L.O. Sundelof, The effect of charge density and conformation on the polyelectrolyte complex formation between carrageenan and chitosan, Carbohydrate Polymers 34 (3) (1997) 149–156.
[47] N. Karibyants, H. Dautzenberg, H. Colfen, Characterization of PSS/PDADMAC-co-AA polyelectrolyte complexes and their stoichiometry using analytical ultracentrifugation, Macromolecules 30 (25) (1997) 7803–7809.
[48] E. Lu, S. Franzblau, H. Onyuksel, C. Popescu, Preparation of aminoglycoside-loaded chitosan nanoparticles using dextran sulphate as a counterion, Journal of Microencapsulation 26 (4) (2009) 346–354.
[49] M.L. Tan, A.M. Friedhuber, D.E. Dunstan, P.F.M. Choong, C.R. Dass, The performance of doxorubicin encapsulated in chitosan–dextran sulphate microparticles in an osteosarcoma model, Biomaterials 31 (3) (2010) 541–551.
[50] Y. Cho, R. Shi, R. Ben Borgens, Chitosan nanoparticle-based neuronal membrane sealing and neuroprotection following acrolein-induced cell injury, Journal of Biological Engineering 4 (2) (2010) 1611–1754.
[51] B. Sarmento, A. Ribeiro, F. Veiga, D. Ferreira, R. Neufeld, Oral bioavailability of insulin contained in polysaccharide nanoparticles, Biomacromolecules 8 (10) (2007) 3054–3060.
[52] H. Min, B. Cory, Controlled release of Repifermin(R) from polyelectrolyte complexes stimulates endothelial cell proliferation, Journal of Pharmaceutical Sciences 98 (1) (2009) 268–280.
[53] A. Drogoz, S. Munier, B. Verrier, L. David, A. Dornard, T. Delair, Towards biocompatible vaccine delivery systems: interactions of colloidal PECs based on polysaccharides with HIV-1 p24 antigen, Biomacromolecules 9 (2) (2008) 583–591.
[54] M. Koping-Hoggard, A. Sanchez, M.J. Alonso, Nanoparticles as carriers for nasal vaccine delivery, Expert Review of Vaccines 4 (2) (2005) 185–196.
[55] H. Min, L.H. Zhixin, B. Mehmet, B. Cory, Magnetic resonance imaging of contrast-enhanced polyelectrolyte complexes, Nanomedicine: The Official Journal of the American Academy of Nanomedicine 4 (1) (2008) 30–40.