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On the kinetics of chitosan/tripolyphosphate micro- and nanogel aggregation and their effects on particle polydispersity

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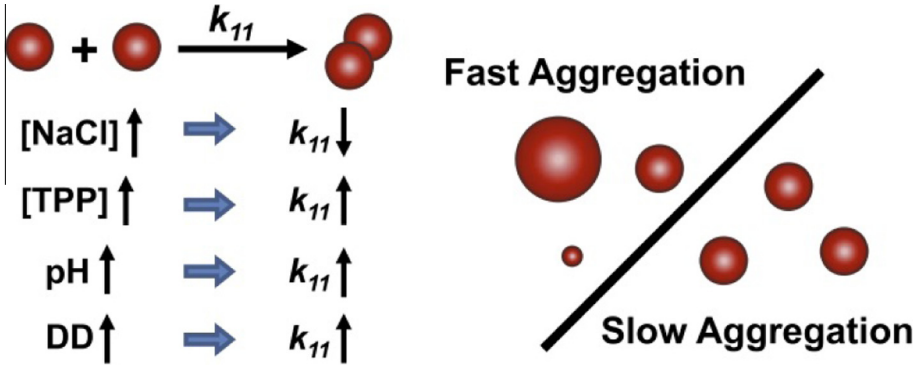
GRAPHICAL ABSTRACT



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ABSTRACT

Submicron chitosan/tripolyphosphate (TPP) particles are widely investigated as nanocarriers for drugs, genes and vaccines. One of the key particle properties that requires control is their size distribution, which depends on the extent of chitosan/TPP primary nanoparticle aggregation into higher-order submicron colloids. To provide a better understanding of this higher-order aggregation process, this study analyzes the factors that control chitosan/TPP particle aggregation kinetics in the presence of free TPP (such as present during particle formation). The aggregation rates exhibit a sharp power-law decrease with the monovalent salt concentration and a power-law increase with the free TPP concentration. Moreover, the aggregation rates increase with the pH and with the chitosan degree of deacetylation (DD). These variations in aggregation rates reflect the effects of monovalent salt, TPP concentration, pH and chitosan DD on particle bridging by the surface-bound TPP. Furthermore, these aggregation rates are much faster than those predicted based on Derjaguin and Landau, Verwey and Overbeek (DLVO) interaction potentials, which might reflect nonuniformities in particle shape and charge, and/or complications caused by particle softness. Finally, implications of the above aggregation kinetics on the uniformity of chitosan/TPP micro- and nanogel size are analyzed, where we: (1) show how particle polydispersity can be diminished by lowering the chitosan DD; and (2) explain the opposing results on how chitosan/TPP particle polydispersity is affected by monovalent salt.

1. Introduction

Submicron particles prepared through the ionic crosslinking of chitosan with tripolyphosphate (TPP) are widely investigated as

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potential vehicles for drug and gene delivery [1–8]. They form under very mild, aqueous conditions (which help preserve the bioactivity of their payloads) [9], are mucoadhesive [10,11] and can facilitate drug transport across epithelial membranes [8,11]. Consequently, they attract special interest for ocular [4,12] and intranasal [8,13] administration of bioactive payloads ranging from small molecules [4,8,14], to protein drugs and vaccines [1,5,6], to polynucleotides [3,15].

To ensure their optimal performance, it is vital to control their size distributions. To this end, numerous experimental studies have been performed [2,14,16,17]. These revealed a variety of guidelines (sometimes conflicting ones) on tuning both the particle average size and polydispersity. The average particle size, for instance, has generally been shown to increase with the concentration of the parent chitosan solution and the chitosan molecular weight [14,17,18]. Likewise, chitosan/TPP particle size and colloidal stability were demonstrated to be sensitive to the TPP:chitosan ratio [14,16,17,19]. When the chitosan monomer (glucosamine) binding sites were in excess, the particle sizes typically decreased with the TPP:glucosamine molar ratio, due to an evident decrease in swelling [16,17,19]. Conversely, when multivalent TPP was present in excess, the particles aggregated and ultimately precipitated [14,16,19].

In addition to controlling their average size, significant attention has been devoted to minimizing chitosan/TPP particle polydispersity [14,16,18–21]. Approaches to doing this have included use of parent chitosan molecules with low molecular weights [14,18], preparation of chitosan samples with highly-uniform molecular weights and degrees of deacetylation (DD) [21], or varying process and formulation parameters such as the chitosan and TPP concentrations [14,18,19], preparation temperature [16] and pH [14,20], and mixing procedures [16,18]. Recently, we and others have shown that (provided that the ionic strength is not high enough to inhibit particle formation) preparation of chitosan/TPP particles in the presence of monovalent salt can drastically reduce particle polydispersity [19,22]. Conversely, Bugnicourt et al., who prepared chitosan/TPP particles with narrow size distributions without the use of salt (by using highly-deacetylated chitosan with a narrow molecular weight distribution [21]), have made opposite observations, where the polydispersities were generally higher at elevated ionic strengths [18]. Such conflicting results have also been reported in studies examining variations in average particle size. Despite studying similar TPP:chitosan ratios, for instance, some groups reported the particle size to increase with the TPP:chitosan ratio [14], others reported the average particle size to decrease with the TPP:chitosan ratio [17,23], and others yet reported non-monotonic TPP:chitosan ratio effects on particle size (where a maximum or minimum particle size occurred at intermediate ratios) [21,22,24]. Consequently, chitosan/TPP micro- and nanogel size is tuned through trial and error, with few reliable guidelines for achieving predictable control, and limited understanding of the underlying molecular and colloidal interactions.

Despite this limited understanding, the dependence of chitosan/TPP particle size on the method by which the chitosan and TPP are mixed [20,25] clearly indicates that particle size distributions are kinetically controlled. To gain further mechanistic insight into their formation kinetics, we have recently shown these particles to form through a two-step mechanism, where primary 20–50 nm nanoparticles form and then aggregate into the larger, higher-order colloids that are obtained at the end of particle formation process [26]. Thus, the ultimate size distributions of chitosan/TPP particles reflect the extent of their secondary/higher-order aggregation [26], whose understanding could enable better control over particle size.

The aggregation events that underlie the ultimate chitosan/TPP micro- and nanogel size require the particles to: (1) collide (i.e., establish surface-to-surface contact); and (2) stick together upon colliding [27]. For lyophobic colloids, it is assumed that the particles always stick (i.e., aggregate) upon colliding due to their strong van der Waals attraction, which dominates at short distances and holds the particles together once they overcome the longer-range electrostatic energy barrier [28]. Conversely, contact between lyophilic particles does not always lead to sticking, due to either a much weaker van der Waals attraction [27,29,30], or repulsive solvation [31] and/or steric forces [32]. This is exemplified in a study by Wu et al., where poly(N-isopropylacrylamide) (pNIPAm) microgels (in their water-swollen states) behaved thermodynamically as hard spheres and did not stick upon colliding [29,33]. This lyophilicity was attributed to the similarity in Hamaker constants between the suspending aqueous medium and water-swollen microgel phases (in other words, negligibly weak van der Waals forces) [29].

Recently, we have postulated that similar phenomena might occur in the case of chitosan/TPP particles [26,27], which are also water-rich [22,34] and have, at least qualitatively, been reported to become more (rather than less) colloidally stable when monovalent salt is added – i.e., increasing their collision frequency does not increase their aggregation rate [19,35]. Even when the van der Waals attraction is weak, however, such lyophilic particles can still flocculate (or aggregate) through bridging and depletion mechanisms [28]. Bridging flocculation occurs when a flocculant (in this case TPP) simultaneously binds to two particles and causes aggregation by "bridging" the particles together. This bridging has been proposed as the dominant aggregation mechanism during the formation of chitosan/TPP micro- and nanogels [14,19]. Still lacking, however, is a quantitative and systematic analysis of the factors that control the kinetics of this aggregation. Indeed, despite the extensive literature on colloidal aggregation rates [36–38], data on the aggregation rate constants of even other types of polymeric micro- and nanogels (e.g., those formed through covalent crosslinking) remains very limited [39].

Here, to gain quantitative insight into how various parameters (i.e., NaCl concentration, TPP concentration, pH and DD-values) affect these aggregation rates, the size evolutions of aggregating chitosan/TPP particles are investigated by light scattering. Though chitosan/TPP particles are typically formed using TPP-limited conditions (so that their aggregation stops once the free TPP is depleted), this study investigates chitosan/TPP particle aggregation in an excess of TPP – i.e., by adding TPP to preformed particles with nearly saturated TPP binding sites. This keeps the free TPP concentration roughly constant throughout each aggregation experiment, so that aggregation rate constants can be obtained from the light scattering data. To further explore the aggregation mechanism, these aggregation rate constants are then compared with the particle collision rates predicted based on Fuchs theory and Derjaguin and Landau, Verwey and Overbeek (DLVO) interaction potentials [28,40,41]. Finally, the impacts of these aggregation rates on chitosan/TPP particle polydispersity are discussed, where we: (1) demonstrate how particle polydispersity can be lowered by reducing the chitosan DD; and (2) explain the conflicting results on how their polydispersity is affected by monovalent salt.

2. Materials and methods

2.1. Materials

Millipore Direct-Q 3 deionized water (18.2 MΩ cm) was used in all experiments. Chitosan (viscosity-average molecular weight, M_v = 80 kDa [42]), TPP and NaCl were purchased from Sigma-Aldrich (St. Louis, USA). The chitosan DD was determined to be 91% by pH titration, as described previously [43]. HCl (6 N) was purchased from Fisher Scientific (Fair Lawn, NJ), while the NaOH