Preparation, stabilization, and self-assembly of gold nanoparticles by Chitosan derivatives

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Abstract

Gold nanoparticles (GNPs) are of unique and interesting materials being firstly reported 100 years ago. They are one of the most widely studied nanomaterials potential for disease cure. To improve the colloidal stability, biocompatibility, and hemocompatibility of GNPs, chitosan (CS), a naturally produced polysaccharide with excellent biocompatibility and biodegradation, has been modified to generate water-soluble derivatives and used as the stabilizing agent of GNPs. In the presence of these derivatives, GNPs are stabilized, functionalized, and assembled via electronic static and covalent bond interactions. Based on these works, GNPs with different dimensional, morphology, and crystal lattice are obtained, which can be further apply to a variety of applications in sensing, imaging, therapy, and catalysis. Copyright © 2019 VBRI Press.

Keywords: Gold nanoparticles, chitosan derivatives, surface modification, self-assembly, bio-related application.

Introduction

Soluble gold, known as gold colloids, shows desirable curative power for several diseases since Middle Ages, including dysentery, tumors, and syphilis [**1, 2**]. Gold colloids are composed of many gold nanoparticles (GNPs), which disperse well in the solvent with the protection of stabilizers, such as small molecules, polymers, and biomolecules [**3**]. With the rapid development of biomedicine in the nanoscale, the shape, size, and surface modification of GNPs attracted more attentions because the possible biological effects mainly depend on these parameters. Keeping other parameters constant, the surface chemistry of GNPs shows critical influence in the performance regulation of GNP-based diagnostic and therapeutic systems [**4**].

Chitosan (CS), a kind of naturally produced polysaccharide, show good biocompatibility and biodegradation, especially in biomedicine applications. Since no obvious genetic toxicity and acute toxicity are found [**5**], it has attracted much attention in terms of ligand-functionalized modification to stabilize GNPs [**6**]. Recently, several GNPs with different morphologies have been synthesized using CS as both reducing and stabilizing agent [**7, 8**]. However, CS is difficult to be dissolved in neutral aqueous solution, which limits its applications in the field of biomedicine. Therefore, the structure modification by functional groups to break the intramolecular hydrogen bonds and increase the hydrophilicity of the polymer was carried out [**9**]. Based on its chemical structure, CS is easy to be modified because of the presence of reactive groups such as amino group (C2-NH_2), secondary hydroxyl group (C3-OH), and primary hydroxyl group (C6-OH). The chemical modification on these active sites produces novel derivatives with satisfied solubility, suitable electric charge (positive, negative, and zwitterionic), and favorable physicochemical properties of CS materials. Subsequently, preparing GNPs in the presence of these CS derivatives will offer improved performance of GNPs in the practical applications.

In this review, the preparation and characterization of CS derivatives and their stabilized GNPs as well as their self-assembly in the solution are introduced. The rational design and synthesis of CS derivatives make it a platform for controlling the properties and biological functions of GNPs and their multi-dimensional assemblies.

Chitosan derivative-modified GNPs via electrostatic interaction

Positively charged CS derivative-stabilized GNPs

A two-phase synthesis for the preparation of GNPs is a classic method, in which a quaternary ammonium bromide salt ($R_4N^+Br^-$) was used as the phase-transfer reagent [**10**]. The phase-transfer reagent is believed to be adsorbed on the surface of GNPs through the surface ion pairs between the reagent and gold surface. Thus, the introduction of $R_4N^+Br^-$ into the chitosan structure is expected to be an effective ligand to stabilize the GNPs. N, N, N-trimethyl chitosan chloride (TMC) is a positively charged polysaccharide. Due to its cationic structure, it was usually used to compact DNA or/and proteins [**11-14**]. High-degree-substituted TMC (~ 40%) has been proved to be an effective enhancer for the

absorption of drugs [**15-17**]. To prepare the TMC-modified GNPs and investigate their interactions between this positive polymer and particles, TMC was synthesized by modifying methyl groups on the C2-NH_2 via a two-step method [**9, 18**]. After derivatization, TMC can be well dissolved in water. In the presence of TMC, chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was reduced by sodium borohydride.

Comparing with the citrate protected-GNPs, the freshly prepared TMC-coated GNPs had an average size of 2.9 nm detected by TEM technology [**9**]. These GNPs showed high stability in the daylight and at room temperature. No aggregates were observed after the storage for 2 months, and the particle diameter and dispersity were unchanged. Using the same approach, TMC-protected silver colloid in light yellow and platinum colloid in grey were also prepared. A narrow size distribution in the nanoscale for the silver and platinum nanoparticles were observed as well. Due to the protection effect of TMC on the surface of GNPs, the freeze-dried TMC-protected nanoparticles can be well-dissolved and dispersed in aqueous solutions for the further use [**9**].

To investigate the mechanism of the relatively low affinity between TMC and GNPs, a variety of spectral methods have been employed. It was found that signals of methyl groups in TMC became broad in FTIR, ^{1}HNMR, and ^{13}CNMR spectra, which demonstrated the 'surface ion pairs' interactions between TMC and GNPs via N, N, N-trimethyl groups as shown in **Fig. 1**.

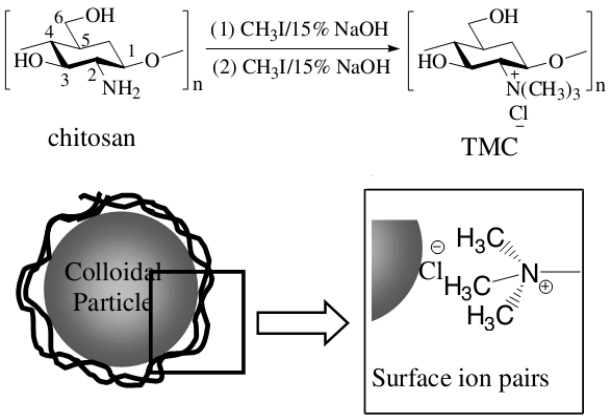

Fig. 1. Illustration of the synthesis method for TMC and the surface ion pairs between TMC and gold surface. Reproduced from Ref. 9 with permission from IOP Publishing.

Zwitterionic CS derivative-stabilized GNPs

Smart materials in response to the external stimuli, especially the disease microenvironments, cause widespread concern [**19-21**]. The rational design of the nanoparticle protectors in the chemical structure, the shape, size, and assembly of nanoparticles can be finely controlled [**22**]. By controlling these structure-regulated properties, it is expected to develop new materials with desirable functions [**23**].

Up to now, a variety of ligands were designed and modified on polymeric skeleton that were employed as templates for assembling nanostructures [**24-27**]. Nanostructures that arrange in solutions and on substrate surfaces from one-dimensional to three-dimension have been reported [**1**]. The polymers having natural repeating units are desirable templates to assemble materials in a controlled way. CS provides a perfect periodic structure that can be designed for the artificial stimuli-responsive templates via the chemical modification of homogeneous D-glucosamine units [**28**].

Amino acids, possessing both α-amino and carboxyl groups, are the simplest zwitterionic templates. GNPs became water soluble after being capped with lysine and aspartic acid. The interspaces between nanoparticles could be regulated by the protonation degree of amino stabilizer at different pH values [**29, 30**]. In the case of basic amino acids (pI_{lysine} is ca. 9.4), the aggregation of gold colloids only occurs either in acidic solutions [**31**]. On the opposite, those acidic amino acids (e.g. $pI_{aspartic\ acid}$ is ca. 2.77) induced gold aggregates in basic solutions [**32**]. However, the increase of assembling dimension for GNPs did not occur in the aggregation system of these small molecule templates.

For the aim of assembling GNPs in a multi-dimensional manner, a water-soluble and zwitterionic CS derivative, 6-O-carboxylmethyl chitosan (CMC), was synthesized by Ding *et al.* [**33**] in which the substituting degree of the carboxymethyl group was about 1.0 [**34, 35**]. It means almost equal number of cationic (-NH_4^+) and anionic (-COO^-) groups was in each D-glucosamine unit of CS. GNPs was prepared by sodium borohydride reduction using CMC as the stabilizer [**32, 36**]. The zwitterionic structure of CMC was a pH-sensitive template that played an important role in the reversible assembly and disassembly of GNPs through the conformation changes of the polymer and the change of ligands as the stabilizer **(Fig. 2)**.

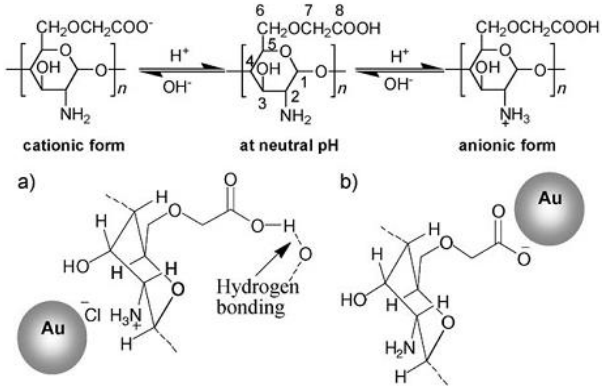

Fig. 2. Zwitterionic structure and charge change of CMC (top), and the relative chelation mode to GNPs as a function of pH: (a) acidic and (b) basic conditions. Reproduced from Ref. 33 with permission from Wiley Online Library.

Different from the previous literature using the amino acid templates, the aggregation of GNPs occurred at two pH values, e.g. 2.0 and 12.0, resulting in different

shapes formed by GNPs (**Fig. 3**). At pH 9.6, the GNPs had a spherical morphology and well-dispersed in an aqueous solution. An average diameter was detected to be about 5 nm by TEM technology. After the pH was adjusted to a high value (pH 12.0), dispersed GNPs were clustered into needle-like aggregates. On the contrary, in acidic solution (e.g. pH 2.0), GNPs aggregates become much larger and denser than those observed at pH 12.0. Gold aggregates were produced with a more regular morphology about 360 nm long and about 150 nm wide and with symmetrical angles on both ends of the clusters.

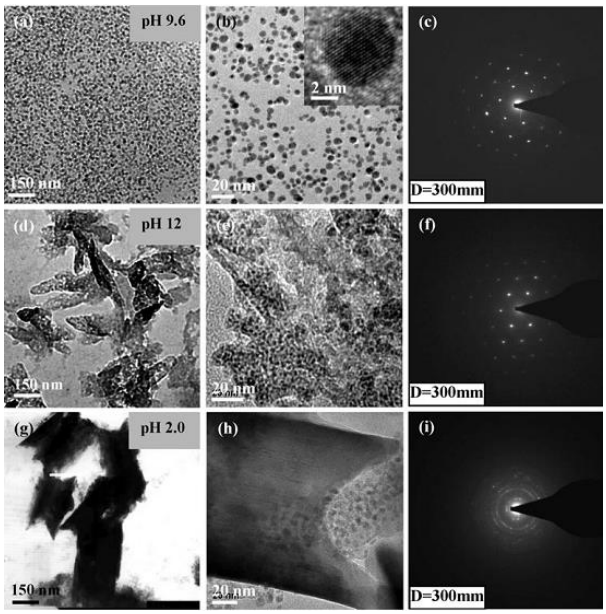

Fig. 3. TEM images and SAED patterns of CMC-protected GNPs at different pHs. Reproduced from Ref. 33 with permission from Wiley Online Library.

The color of gold colloid changed from purple (pH 2.0) to pink (pH 9.6) and to mauve (pH 12.0) [**33**]. This process is reversible to some extent and could be explained by the Mie scattering theory [**37, 38**]. The color changes associated with the aggregation of GNPs can be attributed to the changes of the particle-particle distance. However, the intensity of the ultraviolet absorption peak continuously decreased, particularly in the process adjusting pH from 12 to 2. This phenomenon could be partly due to the irreversibility when the pH value experienced the isoelectric point at pH 6. Therefore, based on this proton responsive changes of color and UV absorption, the CMC-modified GNPs can be used as a visible pH meter to the naked eye.

Chitosan derivatives-modified GNPs via the covalent bond

Other than the surface ion pair, the covalent bond of S-Au is an important surface interaction between ligand and GNPs [**39, 40**]. Modifying CS with thiol-containing molecules will anchor GNPs on or along the CS chain. Furthermore, adjusting the solution environments, the anchoring GNPs will realize multi-dimensional assembling and aggregation. Ding, *et al.* [**41**] designed and synthesized a Cysteine (Cys)-modified chitosan derivative with a neutral thiol ligand [**42, 43**]. Cys is a natural generating and mercapto group-containing amino acid. Its carboxylic acid group provides a reaction site to connect on the amine group of CS and the colloidal gold surface can bind to Cys molecule via thiolate linkages to obtain thiolated CS modified GNPs (TCS-GNPs, **Fig. 4**) [**44-46**]. In this work, cysteine molecules connected with the 2-amino group of chitosan via an amidation, and the grafted mercapto groups acted as anchoring sites for GNPs via S-Au bonds.

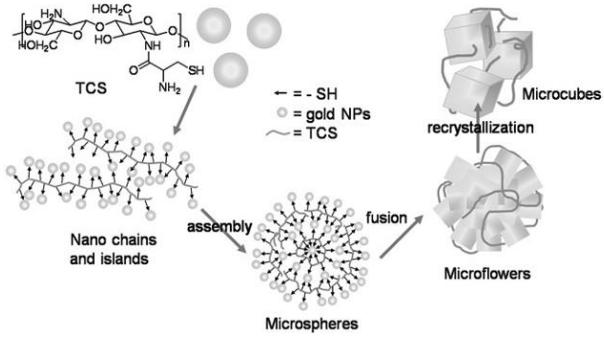

Fig. 4. Assembly of GNPs to be different nano- and micro- structures by adjusting the solution condition of thiolated chitosan (TCS). Reproduced from Ref. 41 with permission from The Royal Society of Chemistry.

Different from above methods [**47**], GNPs were not in situ prepared in the presence of TCS. The citrate-protected GNPs with the particle size of 12 ± 2 nm were prepared firstly using citrate as the reduction and protection regent. When the TCS was added in the gold colloids, the ligand exchange reaction occurred. The study revealed that by adjusting (1) the substitution degree of mercapto groups on CS structure and (2) GNPs: S ratio, various shaped nanoparticle assemblies from one dimension to three dimensions were obtained, including nanowires, needle-like crystals, microclusters, micro-flowers, and single crystal microcubes **(Fig. 5)**.

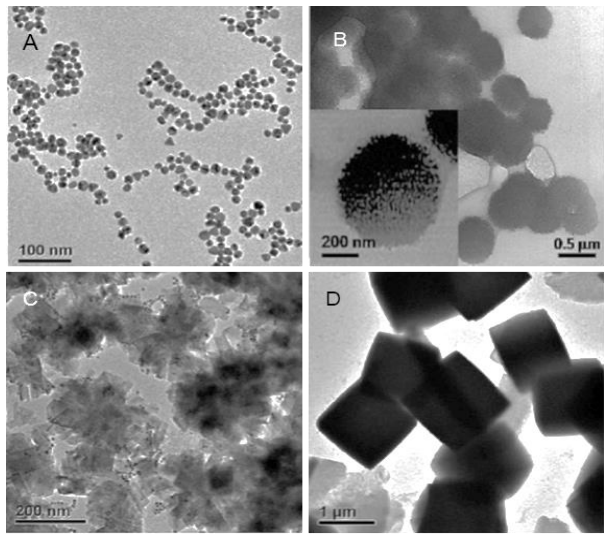

Fig. 5. TCS-mediated gold assemblies detected by TEM technology. Reproduced from Ref. 41 with permission from The Royal Society of Chemistry.

When the gold colloid prepared by citrate reduction method was mixed with TCS solutions, the molecular recognition process between mercapto groups and gold surface resulted in the formation of 1D nanowires **(Fig. 5A)**. After the aging process overnight, nanowires further self-assembled into spherical morphology around 0.5 μm **(Fig. 5B)**. When the above gold assembles were further dialyzed in a frequently refreshed HCl solution (pH 1.6), gold flowers (e.g. flower-shaped clusters) with a diameter of ca. 200 nm were obtained **(Fig. 5C)**. Further dialyzed in the HCl solution for 2 days, the removal of citrate protector from the gold surface caused the fusion of GNPs into large rectangular crystals, and then the formation of large gold cubes with a mean edge length of ca. 1.1 μm **(Fig. 5D)**. This phenomenon can be explained by the mechanism that halide ions etched and rearranged gold atoms in the presence of oxygen [**48-51**]. Based on this mechanism, GNPs were annealed into large crystals and induced the formation of nanoflowers and microcubes. Exchanging CH_3COO^- by Cl^-, no flowers and cubes were observed.

In the above assembly and shape change process of gold materials, many parameters played critical roles. They included the structure of CS derivatives, the ratio of gold nanoparticles to S atom (GNPs: S ratio), and the solution conditions. The number of GNPs can be calculated according to the molar amount of gold salt added in the feed solution, since a gold nanoparticle with the diameter of about 12 nm is composed of about 53,000 gold atoms [**1, 52, 53**]. And the S atom in the solution can be estimated by DS results obtained from element analysis. Thus, the ratio of gold nanoparticle to S atom can be well-controlled to modulate the morphology of gold assemblies. Based on this work, gold clusters with different dimensional, morphology, and crystal lattice are obtained, which can be further applied to a variety of applications in sensing, imaging, therapy, and catalysis.

Conclusion

In this review, we elucidate the preparation, characterization, and self-assembly of GNPs mediated by CS derivatives. Taking advantage of chemical reaction activity for CS, several representative derivatives of CS have been synthesized. Utilizing the long chain structure and their functional groups of CS, GNPs were protected and stabilized in the aqueous solution via electronic static and covalent bond interactions, which improve the biocompatibility of GNPs. By adjusting the ratio of CS derivative and GNPs, as well as their solution environment, the aggregation of GNPs can be well controlled to achieve the superior stability, pH sensing, and multi-dimension of GNPs. These may provide some inspirations for the applications of GNPs in the fields of nanomedicine, catalysis, and energy storage.

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