

Using Chondroitin Sulfate to Synthesize Nanoparticles

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ABSTRACT

We provide evidence that chondroitin sulfate (CS), a member of the glycosaminoglycan (GAG) family of polysaccharides found in the extracellular matrix of all higher order species, can be used as a matrix to generate nanoparticles. Aqueous mixtures containing the select cation and CS are kept at RT and metallic nanoparticles are synthesized due to reducing power of the CS polysaccharide or by adding NaBH_4 as a reducing agent. The generation of nanoparticles was confirmed by transmission electron microscopy or was strongly hinted from UV/VIS spectroscopic scans. In addition, UV/VIS spectroscopic studies hint that sulfide, hydroxide or carbonate nanoparticles could be generated in the presence of CS.

Keywords: chondroitin sulfate, nanoparticle synthesis

1 INTRODUCTION

The various types of chondroitin sulfate (CS) belong to a family of anionic polysaccharides, ubiquitously found in the extracellular matrix (ECM) of virtually all mammalian cells, called the glycosaminoglycans (GAG).[1] The CS polysaccharides are usually covalently linked to extracellular or membrane proteins, the proteoglycans (PG).[1] Like all GAGs, CS polysaccharides serve passive biological functions in the ECM, although in recent years many important, active cell-biological functions have been attributed to these polysaccharides.[2]

Using biosynthetic approaches, i.e., employing microorganisms or plant extracts, so-called biogenic Au or Ag nanoparticles have been produced. Extracts from Neem leaf, geranium leaf, lemongrass plant or Aloe vera plant have been shown to reduce aqueous Au(III) or Ag(I) solutions and generate metallic nanoparticles with varying shapes and morphology under mild experimental conditions.[3-7]

Here we provide evidence that CS, a naturally occurring polysaccharide, can serve as a matrix and stabilizing agent to generated nanoparticles. The capacity of CS to chelate cations like Ca(II) or Cu(II) has long been recognized.[8, 9] In fact, Rhee et al. have shown that CS serves as a template to generate hydroxyapatite nanocrystals during bone formation.[10]

2. EXPERIMENTAL

2.1 Materials

Chondroitin sulfate (type A, sodium salt from bovine trachea, approx 70% and balanced with CS type C) was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were purchased from Fisher Scientific (Suwanee, GA).

2.2 Synthesis of nanoparticles

Aqueous solutions (1mL) containing a mixture of CS (final concentration varying between 6 and 8 mg/mL) and the select cation (final concentration varying between 1 and 2 mM) were prepared at room temperature (RT). To such mixtures, 5 μ L of either NaBH_4 (final concentration varying between 8 and 10mM), saturated Na_2S solution, NaOH (final concentration 3.5mM) or saturated Na_2CO_3 solution was added. The occurrence of precipitations or changes in color were noted and compared to cation solutions prepared in the absence of CS. Samples were filtered through a 0.2 μ m filter prior to UV/VIS spectroscopic measurements or transmission electron microscopy.

2.3 UV/VIS spectroscopy

UV/VIS spectroscopic scans were made between 200 and 1,000 nm using the Perkin Elmer Lambda 25 UV/VIS spectrophotometer (Shelton, CT).

2.4 Transmission electron microscopy

Transmission electron microscopy (TEM) pictures of select samples were obtained in the laboratories of Dr. Charles Lukehart of the Chemistry Department at Vanderbilt University using a Philips CM20T TEM operating at 200 kV (Hillsboro, OR).

3. RESULTS

3.1 Metallic nanoparticles

When mixtures containing CS and Ag(I) or Au(III) cations were kept at RT for three days, the color of the solutions changed gradually from colorless to yellow (Ag) or from yellow to purple-red (Au). These color

changes were reflected in UV/VIS absorption features present in the spectra of the filtered samples that were not observed in the UV/VIS spectra of solutions of CS or the cations alone (results not shown). Such color changes are indicative of the formation of Ag or Au nanoparticles and TEM pictures taken of the filtered samples confirmed the presence of such particles (figures 1 and 2).

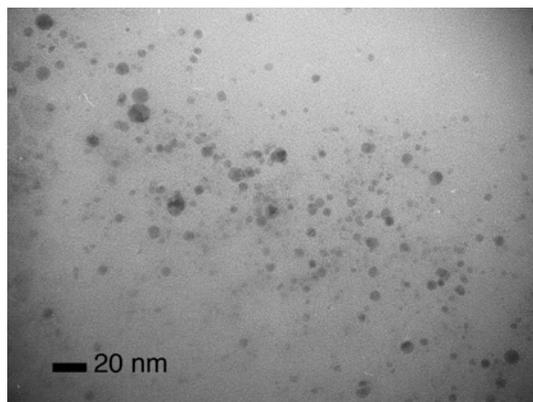


Figure 1: TEM picture of Ag nanoparticles prepared as outlined in the main text.

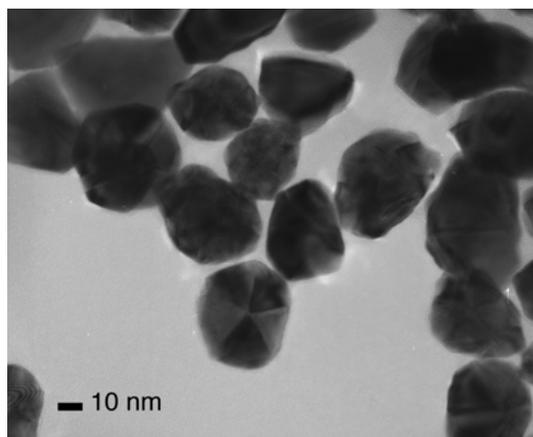


Figure 2: TEM picture of Au nanoparticles prepared as outlined in the main text.

When other cations like Rh(III), Pd(II), Cu(II), Hg(II) or Mn(II) were mixed with CS, no changes in color were observed when such mixtures were kept at RT for multiple days. However, when NaBH₄ was added to these CS/cation mixtures, instant changes in color were observed without any obvious signs of precipitation. When these mixtures were filtered through a 0.2 μm filter, the filtrate was colored like the original solution. When similar cation solutions were mixed with NaBH₄ in the absence of CS, clear precipitations were immediately observed in the mixtures and the filtered solutions were colorless. UV/VIS spectra of the filtered samples of these CS/cation mixtures reduced with

NaBH₄ were recorded (figure 3) and TEM pictures of select samples were taken (figures 4 and 5).

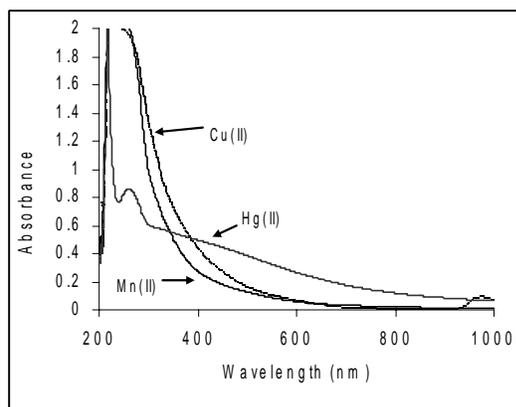


Figure 3: UV/VIS spectra of mixtures of CS and Cu(II), Mn(II) or Hg(II) cations reduced with NaBH₄ at RT.

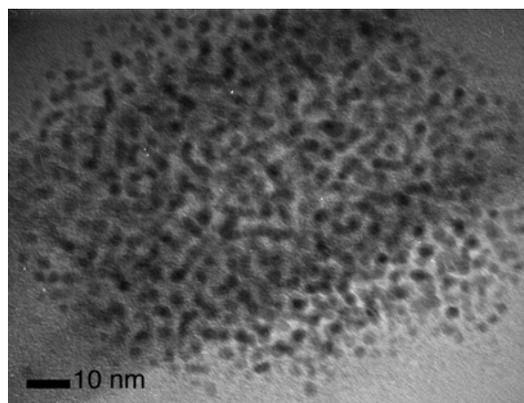


Figure 4: TEM picture of Pd nanoparticles made by NaBH₄ reduction at RT of a mixture containing CS and Pd(II) cations.

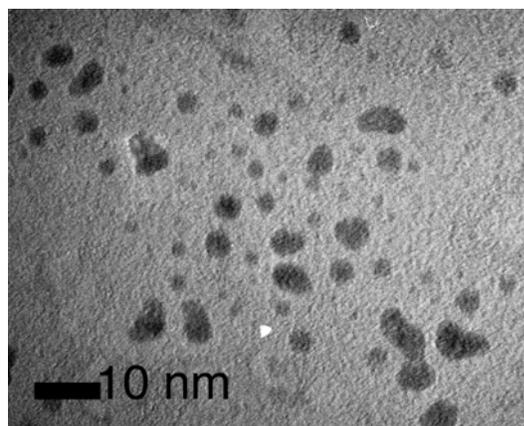


Figure 5: TEM picture of Rh nanoparticles made by NaBH₄ reduction at RT of a mixture containing CS and Rh(III) cations.

3.2 Sulfides, hydroxides and carbonates

To mixtures containing CS and select cations, Na_2S , NaOH or Na_2CO_3 was added. The cations were selected on the basis of the fact that they commonly form water-insoluble salts with sulfide, hydroxide or carbonate at RT. However, in the presence of CS no precipitate could be observed and often the solution exhibited immediate changes in color. The color of these solutions were also present in the filtrate of the mixtures when filtered through a $0.2\mu\text{M}$ filter. UV/VIS spectra were taken of such filtered samples are shown in figures 6 (sulfides), 7 (hydroxides) and 8 (carbonates).

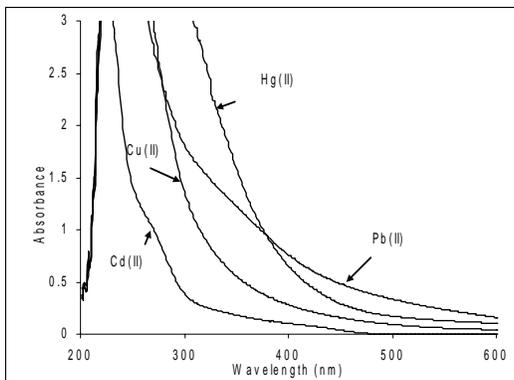


Figure 6: UV/VIS spectra of select “insoluble” sulfides prepared in the presence of CS as outlined in the main text.

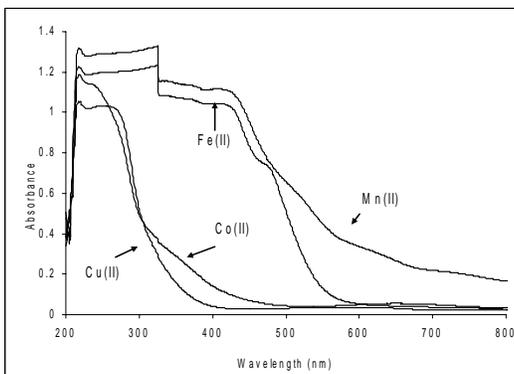


Figure 7: UV/VIS spectra of select “insoluble” hydroxides prepared in the presence of CS as outlined in the main text.

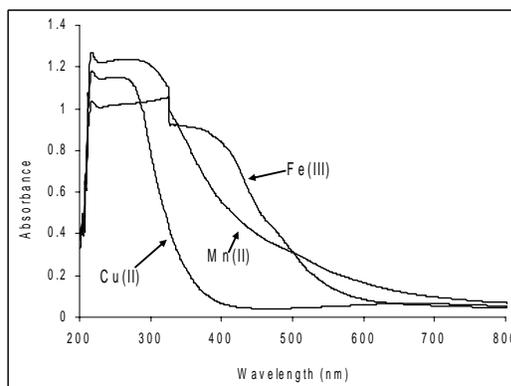


Figure 8: UV/VIS spectra of select “insoluble” carbonates prepared in the presence of CS as outlined in the main text.

4. DISCUSSION

Our experiments indicate that CS can serve as a matrix and stabilizing agent to generate nanoparticles and that these particles can be generated very fast, under mild conditions and in an aqueous environment.

In the cases of Ag and Au, metallic nanoparticles can be generated from the corresponding Ag(I) and Au(III) cations using the reducing properties of the CS polysaccharide alone.

Other metallic nanoparticles can be generated in the presence of CS using NaBH_4 as the reducing agent. Thus far, TEM pictures confirming the nano scale of the particles generated, have only been obtained from select samples (Rh and Pd). However, the fact that no obvious precipitations are observed in any of the other samples (Cu, Mn, Hg) reduced with NaBH_4 in the presence of CS and that the colors of these solutions remain even when filtered through a $0.2\mu\text{M}$ filter, provide strong hints that nanoparticles might be generated.

Similarly, in the synthesis of the sulfides, hydroxides or the carbonates in the presence of CS, the fact that no precipitations are observed and the fact that the color of the mixtures remains after filtration through a $0.2\mu\text{M}$ filter, provide strong indications that nanoparticles were generated.

Preliminary experiments (results not shown) indicate that this synthesis method can be expanded to generate potential phosphate, iodate or any other salt nanoparticle and that other polyanionic polysaccharides (hyaluronan, heparin, pectin) or polyanionic polymers (carboxymethylcellulose) could be used to provide a similar nanoparticle-synthesizing environment.

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