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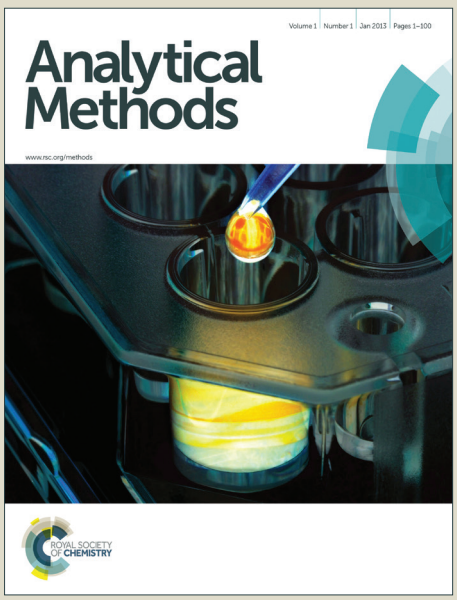

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Fast detection of paracetamol on gold nanoparticles-chitosan substrate by SERS

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Abstract

A fast method of detecting pharmaceutical drugs, such as paracetamol, by surface-enhanced Raman spectroscopy (SERS) using gold nanoparticles substrate was studied. Gold nanoparticles were synthesized using chitosan (AuNPs-chitosan) as reductant and capping agent and subsequently deposited on glass slides as a thin film. The SERS performance of AuNPs-chitosan films were evaluated using methylene blue (MB, 10^{-6} mol L^{-1}) as SERS probe molecule. The method is based in drop-drying an analyte solution (paracetamol, 10^{-3} mol L^{-1}) onto substrate surface and subsequently analyzed by Raman spectroscopy. The spectra were obtained in 10 seconds with two accumulations and exhibit an high signal-to-noise ratio. This preliminary study supports AuNPs-chitosan substrate as a SERS sensor, convenient analytical method for paracetamol detection and other pharmaceutical drugs molecules.

***Keywords*:** gold nanoparticles, chitosan, SERS sensor, molecules detection.

Introduction

The occurrence and fate of pharmaceutical compounds in aquatic environment has been recognized as one of the emerging issues in environmental chemsitry.[1,2] Depending on the area many of these compounds are found in concentrations of around μg/kg, ng/kg and so on.[1,2] For example, 4-Hidroxyacetanilide, also named paracetamol and acetaminophen, is one of the most frequently used analgesic and antipyretic drugs.[3] As other analgesic drugs, paracetamol rapidly becomes absorbed and distributed after oral administration and it is easily excreted in urine.[4] The presence of pharmaceuticals from human medical care in aquatic environment may, however, also be caused by others sources such as landfill leachates,[5] disposal of unused medication via the toilet,[6] and manufacturing residues.[7] Several works have shown some evidences that substances of pharmaceutical origin are often not eliminated during the waste water treatment and also not biodegraded in the environment.[8,9] In this context, fast methods for determination of this kind of compounds can be an efficient strategy to investigate the quality of the water after the waste water treatment. For this purpose, recently, several investigations have shown some methodologies, using SERS/Raman spectroscopy as analytical tool, to detect molecules that are found in samples collected in aquatic environment.[10-12]

Raman spectroscopy is a fast and non-destructive technique and it can be carried out directly on the samples without any extensive sample preparation.[13,14] However, the low strength of the Raman signal is a limitation to detect molecules in low concentrations, since the Raman line is directly proportional to the concentration of the scattering component of a sample in a laser beam.[15,16] Moreover, the intensity of Raman scattering (characterized by the Raman cross-section) can vary by many orders of magnitude depending on the molecules under study and the incident laser. It is

important to clarify here that the condition for a molecule to be a "good Raman scattering" is not enough to make it a good SERS probe.[15,16] Then, a study of the individual Raman spectrum of the target molecule in SERS condition is an important step before proceeding with the application.

The challenge of detecting chemicals in low concentrations has been overcame by using surface enhanced Raman spectroscopy.[17,18] SERS relies on electronic and chemical interactions among the excitation laser, molecule of interest, and the SERS substrate.[14] The nature of the SERS enhancement of the Raman signal is caused by two contributing mechanisms.[14,15] First, is the electromagnetic mechanism (long-range), that is a consequence of the interaction of the electric field (from the incident radiation) with the electrons in the metal surface, leading the excitation of surface plasmon. The second mechanism (short-range) is due to charge-transfer from the metal to the molecules adsorbed on the metallic substrate surface. The maximum SERS enhancement, up to 10^{14}-fold the normal Raman signal, typically is observed at specific positions in the substrate surface (hot spots) and only those molecule adsorbed there can gain from it. Substrates that sustain high magnitude of the SERS enhancement have been applied in the detection of biological and chemical species in several kinds of samples with high sensitivity.[16-18]

The stabilization of Ag and Au nanoparticles with reductant and capping agents have been reported as a promise strategy to prepare stable and efficient SERS substrates.[10,11,19-21] Chen *et al.* demonstrated a simple method to detect sulfide in environmental and biological media at the nM level by SERS using silver nanoparticles substrate.[10] Péron *at al.* described a quantitative SERS sensor based on gold nanoparticles for environmental analysis of naphthalene in the range of 1-20 ppm.[11] In this work, is reported a simple and fast SERS sensing method for the detection of

pharmaceuticals drugs, such as paracetamol with high sensitivity. For this purpose, gold nanoparticles was synthesized using chitosan as reductant and capping agent, and subsequently deposited as a thin film on glass slides. The SERS properties of the material were probed using methylene blue as Raman probe molecule. Thus, to evaluate the possibility of employing the AuNPs-chitosan substrate in future applications, as a platform in the detection and analysis of pharmaceutical drugs, it was tested to detect paracetamol in a simple and fast method.

Experimental

Chitosan powder sample (high molecular weight, 78% deacetylated) was acquired for free from C.E. Roeper, Hamburg - Germany. Tetrachloroauric(III) acid, 3-mercaptopropyl-trimetoxisilane (MPTMS), methylene blue and 4-hydroxyacetanilide (paracetamol) were purchased from Sigma-Aldrich. All chemicals were used without further purification. All glassware were cleaned with piranha solution (4:1 sulphuric acid:hydrogen peroxide) before using and then rinsed thoroughly with deionized water.

AuNPs were prepared following the procedure reported elsewhere with some modifications.[22] Briefly, a solution of chitosan 1 mg mL^{-1} was prepared by dissolving the polymer in acid acetic solution (pH = 2.5). Due to the low solubility of chitosan, the mixture was kept under stirring for 8 h to obtain a clear solution. A mixture of 6 mL solution of 10^{-3} mol L^{-1} tetrachloroauric(III) acid and 36 mL solution of 1 mg mL^{-1} chitosan was prepared. The AuNPs-chitosan synthesis was carried out under stirring at 100 ^{o}C for 10 min, resulting in a red color solution.

Microscope regular glass slides were cut in 1.2 cm^2 pieces, cleaned, and their surfaces were modified following the procedure reported elsewhere, but in the present work using MPTMS.[23] The AuNPs-chitosan films were prepared by dropping 100 μL of

AuNPs solution onto thiol groups modified glass pieces. To solvent evaporation the pieces were placed into an oven at 50 ^{o}C for 15 min. To complete the films preparation this procedure was repeated more two times, drop-drying a total volume of 300 μL for each AuNPs-chitosan film prepared. Before the second and third AuNPs-chitosan deposition the films were washed with deionized water to displace any residual material of the synthesis and dried with N_2 flow.

UV-visible absorption spectra of the AuNPs-chitosan solution and of the AuNPs-chitosan films were collected on an Agilent Cary probe 50 UV-vis spectrometer. High resolution transmission electron microscopy (HRTEM) images were obtained using a JEOL JEM-3010 microscope (300 kV, 1.7 Å point resolution). The sample was prepared by drop-drying the AuNPs-chitosan solution on a holey carbon coated Cu grid. SERS spectra were acquired using a confocal Jobin-Yvon T64000 Raman spectrometer system, equipped with a liquid-N_2-cooled CCD. The excitation source was a laser at 633 nm. The laser power at the sample surface was about 7.2 mW. The laser was focused with a 100x focal-lens objective to a spot of about 1 μm. For all measurements, the laser exposure time was 10 s with two accumulation. An aliquot of 50 μL of MB (10^{-6} mol L^{-1}) aqueous solution was dropped onto the 1.2 cm^2 AuNPs-chitosan film surface. The film was dried in air atmosphere. After that, the sample was ready to be analyzed. The same procedure was used to detect paracetamol (10^{-3} mol L^{-1}).

Results and discussion

Figure 1(a) shows the UV-vis absorption spectrum of the AuNPs-chitosan solution with surface plasmon band at 525 nm. This is the standard optical signature for the formation of AuNPs spheres in solution. A representative AuNPs-chitosan film

shows the surface plasmon band at 537 nm, indicating a red shift of 12 nm in the absorption maximum (Fig. 1(b)). This red shift is caused due to increase the refractive index of the media surrounding metallic nanoparticles.[24,25] The UV-vis spectral profile of the chitosan solution (Fig. 1(c)) does not shows any absorption band at UV-vis range. As observed in the HRTEM image in Fig. 2(a) the AuNPs are immersed into the chitosan structure forming a composite. Measuring the AuNPs diameter, in different HRTEM images, is obtained an average size distribution of 5.2 nm (Fig. 2(b)). The narrow size distribution (1.5 - 12.5 nm) indicates that chitosan plays an important role of controlling the AuNPs size as well as provides a high density of gold nanoparticles on its structure. That condition is related with the short distance among the AuNPs immersed into the chitosan structure, being an appropriate condition to plasmon coupling, which promoted Raman signal enhancement.

Insert Figure 1

Insert Figure 2

As shown in the literature, stable gold nanoparticles can be easily synthesized with excellent size control using chitosan as reductant and capping agent.[26,27] Its dual role is a great advantage, once it is not necessary add other compound to promote the reaction. Despite the widespread use of chitosan in the gold nanoparticles synthesis, the reaction mechanism has not been explained. In the present work, the gold nanoparticles synthesis using chitosan was conducted in acetic acid solution. In that condition, the chitosan is soluble and the amine groups can be protonated ($-NH_3^+$), consequently, this

biopolymer reach a positive residual charge. Then, these groups can to attract the $AuCl_4^-$ ions from the solution, suggesting that they are the sites where the reduction step takes place to size-controllable AuNPs. Finally, the chitosan chains, loaded of AuNPs, self-assemble into larger structures as shown in Fig. 2(a). The overall reaction could be represented as illustrated in Fig. 3 according to our results and to some related researches.[26,27]

Insert Figure 3

To evaluate the SERS efficacy of the AuNPs-chitosan film, Raman measurements were conducted employing MB as the model molecule. As shown in Fig 4(a), it is clear that the characteristic Raman bands of MB are very weak to be observed and the spectrum was multiplied twenty times. The bands of MB at 1622 and 446 cm^{-1}, which have been assigned to C-C stretching and C-N-C skeletal bending, respectively, are the most intense bands in the SERS spectrum (Fig. 4(b)). This result indicates that the MB molecules were adsorbed on the AuNPs-chitosan substrate.[28,29] The bands at 236 and 306 cm^{-1}, that were not observed in the powder MB spectrum can be ascribed to Au-N and Au-S stretching of the Au-MB complex, respectively.[29,30] The bands at 360, 670 , 1031, and 1230 cm^{-1} are observed only in the MB SERS spectrum (Fig. 4(b)). At the same time, the intensities of the bands at 478 and 888 cm^{-1} are very prominent in the SERS condition, that is a strong evidence of this enhancement effect.

Insert Figure 4

To check the repeatability of the SERS spectra, measurements were made in five different points, randomly chosen, in the same AuNPs-chitosan film. The MB Raman signature is observed in all collected SERS spectra (Fig. 5). The difference was only between the relative intensities of the spectra collected from point-to-point, while the spectral position and the full width of Raman bands show no noticeable difference (Fig. 5). The band at 1622 cm^{-1} exhibit the highest intensity, indicating that this band can be used as MB reference in future analyses.

Insert Figure 5

The Raman signal scattered from the chitosan is very weak and it does not interfere in SERS spectra of MB model molecule (Fig. 5). This is an interesting result, indicating that this kind of substrate can be employed to detect molecules without any interference from the substrate background. As a demonstration of a practical application of the above AuNPs-chitosan substrate, it was tested as SERS sensor to detect paracetamol. Fig. 6 shows the measured SERS spectra for paracetamol powder and for paracetamol after drop-drying 50 μL of 10^{-3} mol L^{-1} solution on the AuNPs-chitosan substrate. The SERS spectra differ mainly in intensity and positions when comparing with the Raman spectrum of the paracetamol powder. Also, the SERS

spectra differ each other (Fig. 6 (b) and (c)). This can be explained based on paracetamol chemical structure,[31] that can undergo deprotonation and achieve a stable equilibrium in water (inset Fig. 6). Both structures, paracetamol and its conjugated base (oxyanion), can interact with the AuNPs-chitosan substrate in different ways due to their different charges. In that situation molecules can be differently orientated on the sensor surface, resulting in different intensities and positions of some Raman bands as observed in Fig. 6.

Insert Figure 6

The band at 1168 cm^{-1} for σ^{ip}(HCC, ip: in-plane) and for □(CC) is shifted to 1145 and 1154 cm^{-1} and also is observed in the SERS spectrum in Fig. 6(C), indicating that there are molecules attached on the AuNPs-chitosan substrate surface with different orientations.[32,33] The band at 1237 cm^{-1} for □(CC) and σ^{ip}(HOC) is observed in both SERS spectra, appearing as shoulder in the spectrum in Fig. 6(C). The band at 1258 cm^{-1} for □(C-O), σ^{ip}(HCC) and □(CC) appear only in the spectrum in Fig. 6(C) as the highest one, indicating that the SERS effect is operating on the system. The band at 1325 cm^{-1}, attributed to Amide III band (C-N stretch/C-N-phenyl stretch/C-N-H band), is split and becomes more intense in Fig. 6(C). The band at 1371 cm^{-1} for σ^{s}(CH_3, s: symmetric) appear in different positions in the SERS spectra; unlike the band at 1445 cm^{-1} for σ^{as}(CH_3, as: asymmetric) is observed in the same position in the SERS spectra, but it is much more intense in Fig. 6(C). The band at 1565 cm^{-1} for σ^{ip}(HNC) and □(CC) is observed only in Fig. 6(b), while the band at 1585 cm^{-1} for □(CC) and

σ^{ip}(HNC) is observed only in Fig. 6(C).[33] The band at 907 cm^{-1} observed only in Fig. 6(b) could not be assigned, since any band was observed in this position in the paracetamol powder Raman spectrum. These changes observed in the SERS spectra bands are correlated with orientation differences in the paracetamol molecules adsorbed on the AuNPs-chitosan SERS sensor. The high intensity observed for some bands, in the SERS condition, indicates that the substrate is a promise sensitive sensor to detect paracetamol even in concentrations below10^{-3} mol L^{-1}. This kind of SERS sensor has potential applications in the analytical field and the straightforward procedure used has several advantages as a fast and efficient method.

Conclusions

AuNPs were synthesized in a simple and convenient method using chitosan as reductant and capping agent. The AuNPs-chitosan composite is easily deposited on glass slide forming a film which exhibited an excellent performance as SERS substrate. AuNPs-chitosan film showed sensitivity to detect MB in low concentration by SERS. Such AuNPs-chitosan film was employed as a SERS sensor to detect and identify paracetamol by drop-drying 50 μL onto the substrate surface. This methodology is simple, fast and cost-effective. Also, the originality and advantage of this SERS-active substrate reside in the fact that the chitosan Raman signal does not interfere on the Raman spectra of the analytes. In the near future, AuNPs-chitosan can be employed as a sensor of other pharmaceutical drugs using the same methodology, even in low concentrations.

Acknowledgments

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References

1 B. Halling-Sorensen, N. Nielsen, P. F. Lansky, F. Ingersley, L. Hansen, H. C. Lutzhoft, S. E. Jorgensen, *Chemosphere*, 1998, 36, 357.

2 C. G. Daughton, T. A. Ternes, *Environ. Health Perspect.*, 1999, 107, 907.

3 K. Klimova, J. Leitner, *Thermochimica Acta*, 2012, 550, 59.

4 J. Parojcic, K. Karljikovic-Rajic, Z. Duric, M. Jovanovic, S. Ibric, *Biopharm. Drug Dispos.*, 2003, 24, 309.

5 J. V. Holm, K. Rugge, P. L. Bjerg, T. H. Christensen, *Environ. Sci. Technol.*, 1995, 29, 1415.

6 T. A. Ternes, *Water Res.*, 1998, 32, 3245.

7 K. Reddersen, T. Heberer, U. Dunnbier, *Chemosphere*, 2002, 49, 539.

8 C. Zwiener, T. Glauner, F. H. Frimmel, *High Res. Chromatogr.*, 2000, 23, 474.

9 H. R. Buser, T. Poiger, M. D. Muller, *Environ. Sci. Technol.*, 1999, 33, 2529.

10 L-X. Xen, Da-W. Li, L-L. Qu, Y-T. Li, Y-T. Long, *Anal. Methods*, 2013, 5, 6579.

11 O. Peron, E. Rinnert, T. Toury, M. L. Chapelle, C. Compere, *Analyst*, 2011, 136, 1018.

12 R. A. Alvarez-Puebla, L. M. Liz-Marzan, *Angew. Chem. Int. Ed.*, 2012, 51, 11214.

13 M. Fleischmann, P. J. Hendra, A. J. McQuillan, *Chem. Phys. Lett.*, 1974, 26, 63.

14 E. C. Le Ru, P. G. Etchegoin, Principles of Surface-Enhanced Raman Spectroscopy. Amsterdam: Elsevier; 2009.

15 R. Aroca, Surface-enhanced Vibrational Spectroscopy. Southern Gate: John Wiley & Sons; 2006.

16 Y. Zhang, S. Liu, L. Wang, X. Qin, J. Tian, W. Lu, G. Chang, X. Sun, *RSC Advances*, 2012, 2, 538.

17 G. Moula, R. F. Aroca, *AnaL. Chem.*, 2011, 83, 284.

18 W. W. Yu, I. M. White, *Analyst*, 2012, 137, 1168.

19 W. L. Zhai, D. W. Li, L. L. Qu, J. S. Fossey, Y. T. Long, *Nanoscale*, 2012, 4, 37.

20 M. Alsawafta, S. Badilescu, M. Packirisamy, V. V. Truong, *Reac. Kinet. Mech. Cat.*, 2011, 104, 437.

21 K. Esumi, N. Takei, T. Yoshimura, *Colloids Surf. B*, 2003, 32, 117.

22 M. Potara, D. Maniu, S. Astilean, *Nanotechnology*, 2009, 20, 315602.

23 E. B. Santos, F. A. Sigoli, I. O. Mazali, *Vib. Spectrosc.*, 2013, 68, 246.

24 Y. Q. Wang, W. S. Liang, C. Y. Geng, *Nanoscale Res. Lett.*, 2009, 4, 684.

25 E. F. S. Vieira, A. R. Cestari, E. B. Santos, F. S. Dias, *J. Colloid Interf. Sci.*, 2005, 289, 42.

26 Y-C. Cheng, C-C. Yu, T-Y. Lo, Y-C. Liu, *Mater. Res. Bull.*, 2012, 47, 1107.

27 T. T. Nhung, Y. Bu, S-W. Lee, *J. Cryst. Growth*, 2013, 373, 132.

28 G. N. Xiao, S. Q. Man, *Chem. Phys. Lett.*, 2007, 447, 305.

29 C. M. Ruan, W. Wang, B. H. Gu, *J. Raman Spectrosc.*, 2007, 38, 568.

30 Y. Peng, Z. Niu, W. Huang, S. Chen, Z. Li, *J. Phys. Chem. B*, 2005, 109, 10880.

31 M. A. Elbagerma, G. Azimi, H. G. M. Edwards, A. I. Alajtal, I. J. Scowen, *Spectrochim Acta Mol. Biomol. Spectros.*, 2010, 75, 1403.

32 B. Chazallon, Y. Celik, C. Focsa, Y. Guinet, *Vib. Spectrosc.*, 2006, 42, 206.

33 J. B. Nanubolu, J. C. Burley, *Mol. Pharmaceutics*, 2012, 9, 1544.

FIGURE CAPTIONS

Figure 1. UV-visible absorption spectra of (a) AuNPs-chitosan solution, (b) a representative film prepared with AuNPs-chitosan, and (c) chitosan solution.

Figure 2. (a) HRTEM image showing the AuNPs into the chitosan structure and (b) Histogram of the AuNPs average size distribution into the chistosan structure.

Figure 3. Simplified schematic representation of the formation of AuNPs-chitosan composite.

Figure 4. (a) Raman spectrum of solid methylene blue powder and (b) SERS spectrum of 10^{-6} mol L^{-1} methylene blue droped onto a AuNPs-chitosan film.

Figure 5. SERS spectrum of the AuNPs-chitosan substrate and SERS spectra of 10^{-6} mol L^{-1} methylene blue onto a AuNPs-chitosan film recorded in five different points.

Figure 6. (a) Raman spectrum of paracetamol powder, (b) and (c) SERS spectra of 10^{-3} mol L^{-1} paracetamol recorded onto a AuNPs-chitosan film. Inset shows the deprotonation equilibrium of paracetamol and its conugated base in water.

342 **Figure 1**

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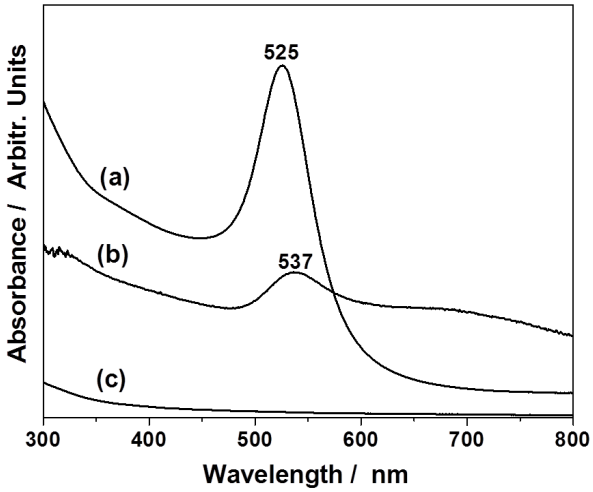

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346 **Figure 2**

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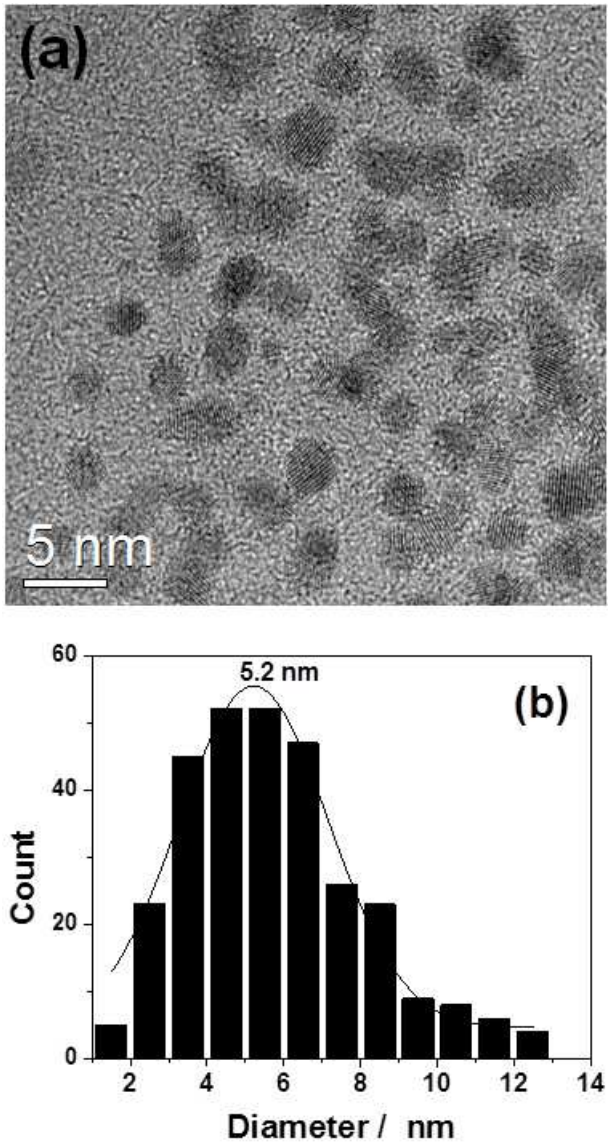

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Figure 3

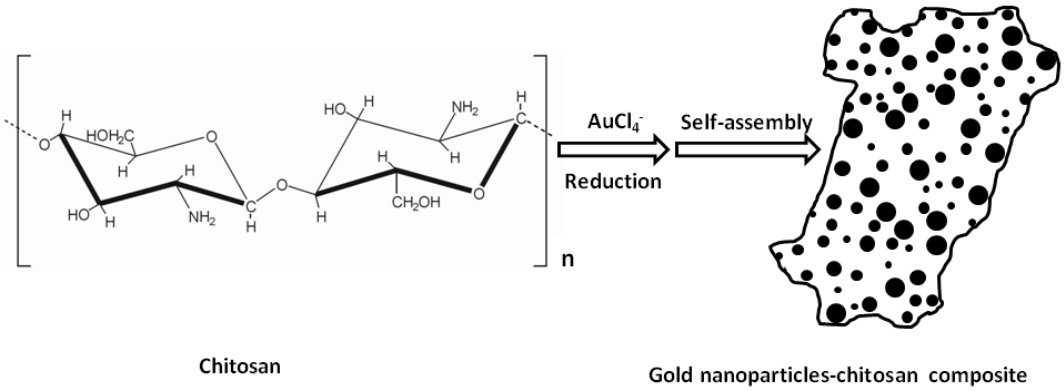
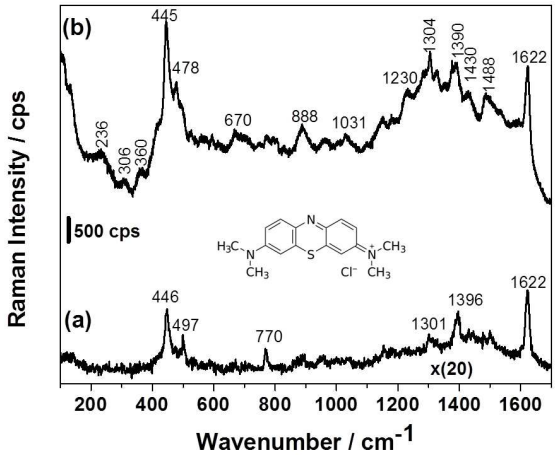


Figure 4



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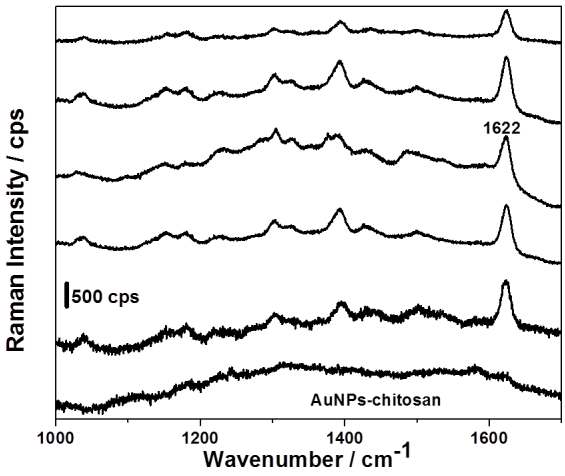

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Figure 6.