Digest Journal of Nanomaterials and Biostructures Vol. 12, No. 4, October-December 2017, p. 1089-1095

ONE POT MICROWAVE ASSISTED SYNTHESIS OF CYCLODEXTRINS CAPPED SPHERICAL GOLD NANOPARTICLES

G. STIUFIUC[a], V. TOMA[b], A. I. MOLDOVAN[b], R. STIUFIUC[b,c*], C. M. LUCACIU[c]
[a]*Faculty of Physics, ''Babes-Bolyai'' University, Kogalniceanu 1, 400084, Cluj - Napoca, Romania*
[b]*Department of Bionanoscopy, MedFuture Research Center for Advance Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Pasteur 4-6, 400337 Cluj-Napoca, Romania*
[c]*Department of Pharmaceutical Physics-Biophysics, "Iuliu Hatieganu" University of Medicine and Pharmacy, L. Pasteur 6, 400349 Cluj-Napoca, Romania*

In this letter we report a new, simple and very rapid method for the synthesis of monodispersed spherical gold nanoparticles (AuNPs) using as reducing and capping agents the three classes of natural cyclodextrins (alfa *α*-, beta *β*- and gamma *γ*-CDs). The as-synthesized nanoparticles are remarkably stable in aqueous solutions and possess very interesting plasmonic properties allowing the detection of low concentration of analyte molecules, using Surface Enhanced Raman Spectroscopy (SERS). The spherical cyclodextrin capped gold nanoparticles (*α*-, *β*- or *γ*CD@AuNps) were prepared in a very short period of time (less than 10 minutes) by mixing the Au^{3+} solutions with aqueous solution of each class of the natural cyclodextrins, followed by a very short heat treatment (2 minutes) inside of a microwave oven. The UV-Vis absorption spectra, measured right after the synthesis, revealed the existence of a strong and narrow plasmonic peak in the visible region, suggesting the successful synthesis of monodispersed spherical gold nanoparticles. The TEM and DLS measurements confirmed the presence of spherical gold nanoparticles having a mean diameter of 20 nm (*α*CD@AuNPs) and 30 nm (*β*CD@AuNPs and *γ*CD@AuNPs) respectively. The plasmonic properties of AuNPs have been evaluated by means of SERS measurements performed on aqueous solution and on dry samples, using a standard test molecule (methylene blue) as well as a pharmaceutical compound having a very low Raman cross section (atenolol).

(Received August 11, 2017; Accepted November 11, 2017)

Keywords: Microwave assisted synthesis, Gold nanoparticles, TEM, DLS, SERS

1. Introduction

The development of new synthesis methods of different classes of nanoparticles, having controllable sizes and shapes and possessing uniform and reproducible properties, represents today one of the hottest scientific topic in the field of nanotechnology [1]. The increased scientific interest for these tinny objects is fueled by their fascinating, size and shape dependent, optical, chemical, mechanical and electronic properties that could lead to promising applications in nanoelectronics, nanomedicine and pharmaceutical sciences [2],[3],[4].

Among the different classes of nanoparticles synthesized and tested so far for different applications, gold nanoparticles hold a great promise due to their intrinsic properties. One of the most interesting properties of gold nanoparticles is represented by the occurrence of localized surface plasmons as a result of their interaction with an external electromagnetic field. In the same time, gold's chemical properties allow the attachment of different molecules on their surface by means of covalent and/or electrostatic interactions [5]. These two properties have transformed gold

---

[*] Corresponding author: rares.stiufiuc@umfcluj.ro

nanoparticles into ideal candidates for the detection of very small amounts of analytes using the Surface Enhanced Raman Spectroscopy (SERS) technique.

The scientific literature is abundant in synthesis methods reporting the creation of pure gold nanostructures of different shapes such as rods [6], wires [7], cubes [8] and spheres [9] or multifunctional nanostructures containing gold nanoparticles such as magnetoplasmonic nanoparticles [10] or plasmonic liposomes [11]. The vast majority of this methods use very toxic chemical products in the synthesis process.

From the point of view of biomedical applications, the need for the development of new synthesis methods using only biocompatible materials is an important obstacle that must be overcome in the next period. As a consequence, several research groups have proposed different classes of nontoxic agents for nanoparticles synthesis such as polyethylene glycol [12], or even *α*- or *β*- cyclodextrins (CDs) [13], [14].

CDs represent a class of water soluble and non-toxic oligosaccharide possessing a hydrophobic inner cavity, that have been extensively studied in the macrocyclic host-guest chemistry. They have been used for the functionalization of metallic nanoparticles for the heavy metal detection applications [14]. Based on their ability to specifically interact with the molecular species Stiufiuc *et al.* have shown that CDs can be used for chiral discrimination of propranolol enantiomers by means of SER spectroscopy [15].

In this paper, we present a very rapid, green and one stage synthesis method of biocompatible spherical gold nanoparticles capped with *α*, *β* and *γ* CDs. The only chemicals used in the synthesis are the gold salt and the three classes of natural CDs. The very homogenous conditions, created by placing the reaction vials in the center of a microwave oven, allowed the synthesis of stable monodispersed gold nanospheres covered with either *α*-, *β*- or *γ*- CDs in a very simple and reproducible manner. The successful synthesis has been proven by means of UV-Vis absorption spectroscopy, Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) while the plasmonic properties have been evaluated by means of SER spectroscopy performed directly on solutions and/or dry samples. The synthesis method proposed in this study allows the rapid synthesis of monodispersed spherical gold nanoparticles of controllable sizes and possessing very interesting plasmonic properties in a very simple, rapid and reproducible manner.

2. Materials and methods

2.1 Materials

All reagents used in this study were of analytical grade. Tetrachloroauric (III) acid trihydrate ($HAuCl_4$) was purchased from Roth and *α*, *β*, and *γ* CDs were purchased from Alfa-Aestar. The sodium hydroxide was purchased from Merck. Ultrapure water (18.2 MΩ·cm, Barnstead EASYPure ROdi) was used for the preparation of all aqueous solutions used in this study.

2.2 Preparation of Gold Nanoparticles

The synthesis procedure started with the preparation of aqueous stock solution (1 mM) of tetrachloroauric acid and 10mM stock solutions of *α*-CDs, *β*-CDs and *γ*-CDs. In a typical synthesis procedure 8 ml of ultrapure water were added in a glass bottle followed by the addition of 2.5 ml of $HAuCl_4$ (1mM), 2 ml of *α* CDs (10 mM) and 20 µl of NaOH (1M). This procedure has been repeated for *β* CDs and *γ* CDs solutions using the same volumes and the same concentrations. Each of the three resulting glass bottles have been sealed and gently mixed for 30 s at room temperature. In a final step, the sealed glass bottles have been placed in the center of a microwave oven (1050 W) operating at 2450 MHz and heated for 2 minutes. After 1 minute, the solutions started to boil and a sudden color change from colorless to ruby red has been observed for each bottle, indicating the successful synthesis of gold nanoparticles. The three bottles have been let to cool down at room temperature for another few minutes and the colloids were ready for characterization. The whole synthesis procedure completed in less than 10 minutes for each colloid.

2.3. Methods and instrumentation

The UV–VIS absorption spectra were recorded using a T92+ UV–Vis Spectrophotometer from PG INSTRUMENTS. The absorption curves have been acquired on standard quartz cells at room temperature, over a spectral range between 350 nm and 900 nm. The spectral resolution has been set at 2 nm.

Electron microscopy measurements were performed on a Hitachi HT7700 (Hitachi, Japan) Transmission Electron Microscope (TEM) operating at 120kV, using the high-resolution operation mode.

The Dynamic Light Scattering (DLS) analysis has been performed on a Vasco$^{\gamma}$ nanoparticle analyzer (Cordouan, France) using a monochromatic laser beam having a wavelength of 658 nm and 65 mW power.

The SERS measurements performed on solutions have been recorded in 1 ml glass vials filled with 540 µl of colloid and 60 µl of analyte in the 200–2000 cm^{-1} range, using a DeltaNu Advantage spectrometer (DeltaNu, Laramie, WY) equipped with a laser diode emitting at 785 nm. The laser power was 100 mW and the spectral resolution was 5 cm^{-1}. Each SERS spectrum is the average of 5 recordings taken with an acquisition time of 20 seconds.

For a further evaluation of nanoparticles plasmonic properties, SERS measurements have been performed on dry samples by employing a multilaser confocal Renishaw *inVia* Reflex Raman spectrometer (Renishaw, UK) using a 785 nm excitation laser and a 20x objective. The laser power was set to 12.5 mW and the spectral resolution was 0.5 cm^{-1}. In this case 50 µl of gold colloids and 100 µl of analyte solution have been mixed together and the resulting solution has been poured on a microscope glass slide covered with Al foil and let to dry. Each spectrum has been recorded using an acquisition time of 10 seconds.

3. Results and discussions

The UV-Vis absorption spectra of the colloids synthesized using α, β, and γ CDs together with the optical images of the three classes of colloids are shown in figure 1. Each colloid exhibits a well-defined surface plasmon resonance (SPR) band in the visible region located at 521 nm (αCD@AuNPs) and 529 nm respectively (βCD@AuNPs and γCD@AuNPs). According to Mie theory, these values would correspond to spherical gold nanoparticles having a theoretical mean diameter between 15-20 nm [16]. By comparing the SPR bands of the three colloids one can expect that the AuNPs synthesized using αCDs have a smaller diameter compared with those synthesized using β and γ CDs. Very recently Scarabelli *et al.* have shown that the absorbance value at 400 nm (A_{400}) can be quantitively correlated with the number of Au^{3+} ions that have been reduced to Au^0, regardless the nanoparticles shape [17]. By comparing the A_{400} value of the three classes of colloids, which is higher in the case of αCD@AuNPs, and by considering that in all cases the amount of gold precursor was the same, one can conclude that αCDs are the most efficient reducing agents among the three classes of CDs used in this study.

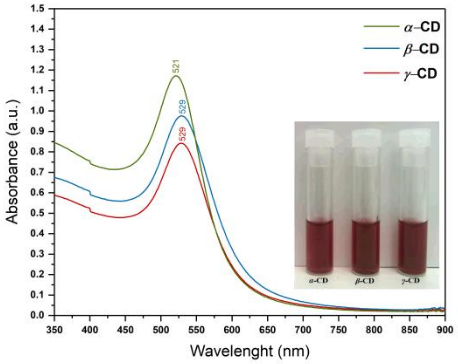

Fig. 1. UV-Vis absorption spectra of the colloidal solutions synthesized using αCDs (green curve), βCDs (blue curve) and γCDs (red curve). The inset show an optical image of the three colloidal solutions.

To confirm this hypothesis, we have performed TEM measurements on the three types of colloids (figure 2). As it can be seen in the upper panel of figure 2 (a, b, c) all three classes of gold nanoparticles have a spherical shape. The statistical analysis performed on a large number of nanoparticles (more than 100 nanoparticles for each class) indicated a mean diameter of 20 nm for *α*CD@AuNPs respectively 30 nm in the case of *β*CD@AuNPs and *γ*CD@AuNPs. The small difference between the predicted theoretical values obtained using the Mie theory and UV-Vis absorbance curves and the experimental ones obtained from TEM measurements can be explained by the fact that, the theoretical values of the mean diameter have been obtained by considering the refractive index of pure water (1.333). This value does not take into account the presence of a molecular layer covering the outer surface of the nanoparticles, thus yielding smaller value of the plasmon resonance band and hence smaller values of nanoparticles diameters. However, one has to observe the small differences between the theoretically predicted values and those obtained from the statistical analysis of TEM images.

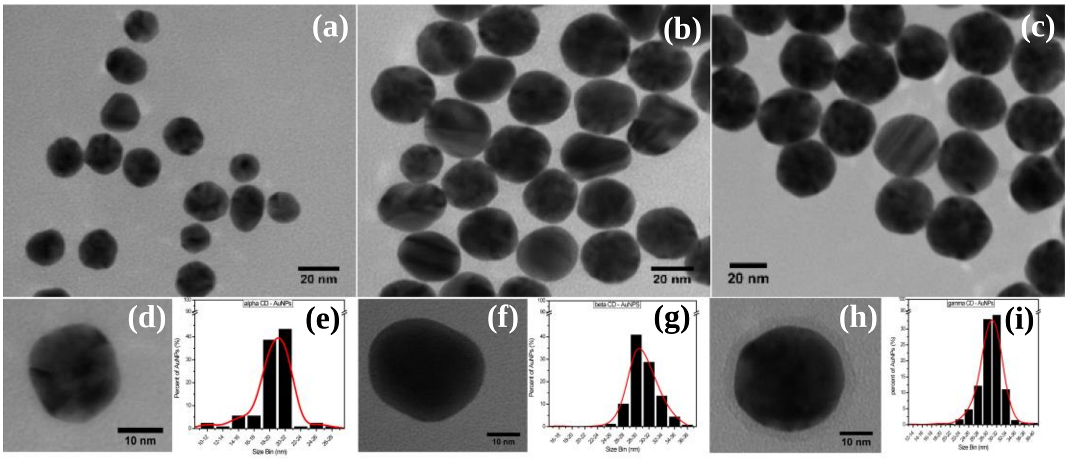

Fig. 2. TEM images of the monodispersed αCD@AuNPs (a), βCD@AuNPs (b) and γCD@AuNPs (c). High resolution TEM images of an individual αCD@AuNP (d), βCD@AuNP (f) and γCD@AuNP (h). Size distribution of αCD@AuNPs (e), βCD@AuNPs (g) and γCD@AuNPs (i) obtained from TEM images.

The high resolution TEM images acquired on each class of single nanoparticles evidenced the presence of CDs molecules covering the outer surface of *α*CD@AuNPs (figure 2d), *β*CD@AuNPs (figure 2f) and *γ*CD@AuNPs (figure 2h). The molecular layer is the most visible in the case of *γ*CD@AuNPs, probably because the geometrical dimensions of *γ*-CDs are slightly larger than those of *α*- and *β*-CDs.

The DLS measurements presented in figure 3 confirmed the observations presented above. The higher values of the mean diameters measured using the DLS technique (26 nm for *α*CD@AuNPs and 31 nm for *β*CD@AuNPs and *γ*CD@AuNPs), as compared with those obtained from TEM image analysis, can be explained if one considers that, in the case of DLS experiments, one measures the hydrodynamic diameter of the nanoparticles. This hydrodynamic diameter includes the nanoparticles diameter as well as the molecules attached to nanoparticles outer surface when travelling in solution.

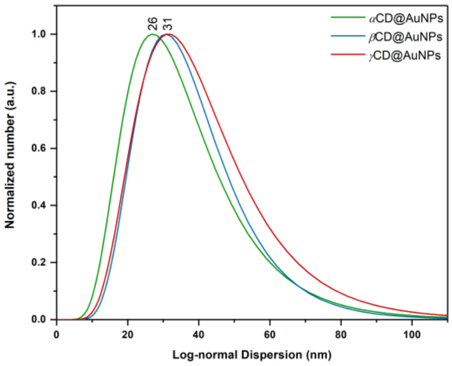

Fig. 3. Normalized hydrodynamic size of gold colloids

For a proper assessment of nanoparticles plasmonic properties we have performed SERS measurements on the three types of colloids using as analyte methylene blue (MB) and an excitation wavelength of 785 nm. The measurements have been performed on solutions, by mixing the colloids with aqueous solutions containing the analytes. As it can be seen in figure 4 the SER spectra of MB are almost identical, independent of the colloidal type used as SERS substrate. This could represent a strong evidence of the fact that MB molecules interact identically with the three types of colloids. Moreover, the shapes of the spectra are very similar with those obtained on "classical" citrate capped gold nanoparticles (data not shown).

The major vibrational peaks of MB, as described in the literature, are clearly visible even for an analyte concentration of 10^{-6} M. The main vibrational bands of MB are located at 447, 500, 771, 1181, 1397 and 1622 cm^{-1}. The most visible ones can be assigned to C-N-C skeletal deformations (447 and 500 cm^{-1}), asymmetric C-N stretching (1397 cm^{-1}) and C-C ring stretching (1622 cm^{-1}).

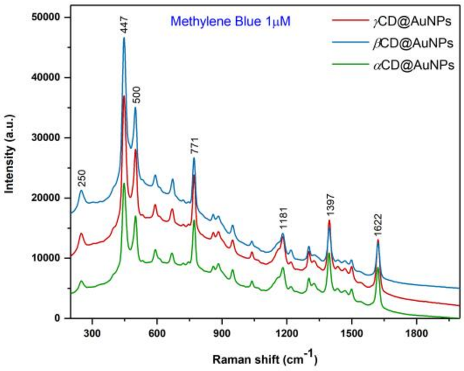

Fig. 4. SER spectra of MB acquired in liquid solutions containing αCD@AuNPs (green curve), βCD@AuNPs (blue curve) and γCD@AuNPs (red curve) using a 785 nm excitation wavelength.

The clarity of the spectra acquired in aqueous solution (pH=7.5) at an analyte concentration of 1 µM can be explained only by taking into account the plasmonic properties of the nanoparticles, making them good candidates for SERS applications. All the acquired spectra are highly reproducible under the same measurement conditions.

The nanoparticles capacity to enhance the electromagnetic field in the close vicinity of their surface and to amplify the molecular specific Raman signal of an analyte molecule has been also tested on dry samples on a pharmaceutical compound having a very low Raman cross section: atenolol. In this case 50 µl of colloids (*α*CD@AuNPs) have been mixed with 100 µl of 10 mM atenolol. The resulted solution has been poured on a microscope slide and let to dry. The recorded

SER spectrum of atenolol is presented in figure 5. The spectrum has been recorded using a 785 nm excitation wavelength. In the literature there are very few papers reporting the reproducible acquisition as well as a proper assignment of the vibrational bands because this pharmaceutical compound has a very low Raman cross-section.

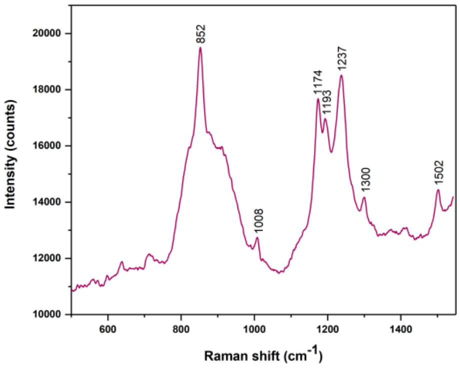

Fig. 5. SER spectra of atenolol (3 mM) acquired on dry samples using as SERS substrates αCD@AuNPs

The spectrum presented in figure 5 is dominated by several vibrational bands located at 852, 1174, 1193, 1237 and 1502 cm^{-1}. The 852 cm^{-1} band is the most visible one. Based on previous Raman and DFT results performed in our group [18] this band can be attributed to a ring breathing mode and could be used for the quantitative evaluation of atenolol in biological fluids using the SERS technique.

4. Conclusions

A very rapid, non-toxic and highly reproducible method for the synthesis of spherical and monodispersed gold nanoparticles, using CDs as both reducing and capping agents, has been proposed in this study. The nanoparticles have been synthesized in covered bottom flasks via a microwave assisted procedure allowing the process completion in less than 10 minutes. The UV-Vis, DLS and TEM measurements confirmed the very low polydispersity index of the as-synthesized spherical gold nanoparticles. The precise control of experimental conditions allowed the synthesis of nanoparticles possessing very interesting plasmonic properties, allowing the rapid detection of different analyte molecules (MB and atenolol) in solution and/or dry samples by means of Surface Enhanced Raman Spectroscopy.

Acknowledgement

This research was supported by the Romanian National Authority for Scientific Research, CNCSIS-UEFISCDI, project no. PN-II-RU-TE-2014-4-1770.

References

[1] S.S. Agasti, S Rana, M.H. Park, C.K. Kim, C.C. You, and V.M. Rotello, Adv. Drug Deliv. Rev. **62**(3), 316 (2010).
[2] E. Boisselier, D. Astruc, C.A. Mirkin, A.P. Alivisatos, R.B. Bhonde, M. Satry, G. Schmid, V. Brandau, C. Li, S.H. Cho, J.W. Tunnell, J.D. Hazle and S. Krishnan, Chem. Soc. Rev., **38**(6), 1759 (2009).
[3] P. Ghosh, G. Han, M. De, C. Kim, V. Rotello, Adv. Drug Deliv. Rev., **60**(11) 1307 (2008).

[4] L. Mikoliunaite, R. Rodriguez, E. Sheremet, V. Kolchuzhin, J. Mehner, A. Ramanavicius, D. R.T. Zahn, Sci. Rep. **5**, 13150 (2015).
[5] S. Eustis, M. El-Sayed, M.A. El-Sayed, S. Meltzer, A. Requicha, H. Atwater, A. Willkinson, E. Matveeva, J. Zhann, R. Badugu, J. Huang, Chem. Soc. Rev. **35**(3), 209 (2006).
[6] N. R. Jana, L. Gearheart and C.J. Murphy, J. Phys. Chem. B, **105**(19), 4065 (2001).
[7] G. M. Kim, A. Wutzier, H. Radusch, G. Michler, P. Simon, R. Sperling, W.J. Parak, Chem. Mater., **17**(20), 4949 (2005).
[8] T. K. Sau, C.J. Murphy, J. Am. Chem. Soc., **126**(28), 8648 (2004).
[9] G. Marek, P.J. Jorge, M. Paul, L.M. Liz-Marzan, Chem. Soc. Rev. **37**(9), 1783 (2008).
[10] C. Iacovita, G. Stiufiuc, A. Florea, R. Stiufiuc, and C.M. Lucaciu, Dig. J. Nanomat. Biostruct., **10**(4), 1209 (2015).
[11] R. Stiufiuc, C. Iacovita, G. Stiufiuc, A. Florea, M. Achim, and C.M. Lucaciu, J. Colloid Interface Sci., **437**, 17 (2015).
[12] R. Stiufiuc, C. Iacovita, R. Nicoara, G. Stiufiuc, A. Florea, M. Achim, C.M. Lucaciu, J. Nanomater., **2013**, 1 (2013).
[13] T. Huang, F. Meng, and L. Qi, J. Phys. Chem. C, **113**(31), 13636 (2009).
[14] B. Aswathy, G.S. Avadhani, S. Suji, G. Sony, Front. Mater. Sci., **6**(2), 168 (2012).
[15] R. Stiufiuc, C. Iacovita, G. Stiufiuc, E. Bodoki, V. Chis, C.M. Lucaciu, Phys. Chem. Chem. Phys., **17**(2), 1281 (2015).
[16] K.L. Kelly, E. Coronado, L.L. Zhao and G.C. Schatz, J. Phys. Chem. B, **107**(3), 668 (2003).
[17] L. Scarabelli, A. Sánchez-Iglesias, J. Pérez-Juste and L.M. Liz-Marzán, J. Phys. Chem. Lett., **6**(21), 4270 (2015).
[18] A. Farcas, C. Iacovita, E. Vinteler, V. Chis, R. Stiufiuc and C.M. Lucaciu, J. Spectroscopy, **2016**, 1 (2016).