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PAPER

Polyphenol-grafted collagen fiber as reductant and stabilizer for one-step synthesis of size-controlled gold nanoparticles and their catalytic application to 4-nitrophenol reduction†

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A facile method for one-step synthesis of size-controlled gold nanoparticles (AuNPs) supported on collagen fiber (CF) at room temperature was proposed. Epigallocatechin-3-gallate (EGCG), a typical plant polyphenol, was grafted onto CF surface to serve as reducing/stabilizing agent, so that the AuNPs were generated on CF surface without introduction of extra chemical reagents or physical treatments. The prepared AuNPs were fully characterized, and the results showed that the dispersed AuNPs were successfully produced and the mean particle size of AuNPs could be effectively controlled in range of 18 to 5 nm simply by varying the grafting degree of EGCG on CF surface. These stabilized AuNPs were found to be active heterogeneous catalysts for the reduction of 4-nitrophenol to 4-aminophenol in aqueous phase. The catalytic behaviors of AuNPs depended on the particle size and the grafting degree of EGCG. A distinct advantage of these catalysts is that they can be easily recovered and reused at least twenty times, because of the high stability of the AuNPs supported by EGCG-grafted CF.

Introduction

Gold was long regarded as chemically inert and catalytically inactive due to its completely filled *d*-band. However, since Haruta's discovery that supported gold nanoparticles (AuNPs) are exceptionally active for low temperature oxidation of CO,[1] the interest of exploring gold-based catalysts has been substantially booming. Highly dispersed AuNPs have been found to be very active for a number of chemical reactions such as oxidation[2] and reduction.[3] Although a high dispersion of AuNPs is basically important to present high catalytic activity, the associated tendency of AuNPs to aggregate would lower their catalytic activity and reuse life-time. Therefore, how to design and prepare AuNPs with long-term dispersion stability and high catalytic efficiency is a primary challenge. During the past few decades, solid inorganic materials like activated carbon (AC)[4] and metal oxides[5] have been widely used as supports to protect AuNPs against aggregating and facilitate their recovery. However, use of these supports often suffers from failure in obtaining highly dispersed and stable gold nanoparticle catalysts. For example, agglomeration of AuNPs supported by SiO_2 occurs easily because the interaction between gold and SiO_2 is inherently weak.[5c] Conventional deposition-precipitation method is unlikely to produce highly dispersed AuNPs supported by AC, due to the acidic nature of AC.[4b]

Organic polymers have been recently recognized as a new class of supports for stabilizing AuNPs because in addition to stabilizing and protecting these particles, polymers can offer unique possibilities for modifying both the environment around AuNPs and access to the catalytic sites.[6] Polyvinyl alcohol (PVA),[6b,6c] polyvinyl pyrrolidone (PVP)[2a,6d] and polystyrene (PS)[6e] are the commonly used polymeric supports in the synthesis of size-controlled AuNPs, in which the stabilized AuNPs displayed distinct catalytic activities. Ishida's work showed that the catalytic activity for oxidation of glucose over AuNPs deposited on ion-exchange resins was more influenced by the nature of the polymer supports than the size of the AuNPs.[2c] However, the synthesis steps of these polymer supported AuNPs often involve addition of chemical reducing agents such as sodium borohydride, sodium citrate, hydrazine, or organic solvents like *N*,*N*-dimethylformamide (DMF) and glycol. All these chemicals are highly reactive and have potential environmental and biological risks, which could be a problem for large-scale production of AuNPs and their subsequent applications.

As an alternative to these hazardous chemicals, a variety of naturally-occurring macromolecules have attracted growing

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interest in the synthesis of gold and silver nanoparticles over the last decade.[7] Huang *et al.* first reported the use of chitosan for reduction of Au^{3+} ions and stabilization of AuNPs without any additional reducing and stabilizing agents.[7a] Cellulose has been utilized as both reducing agent and stabilizer for the controlled "green" synthesis of gold and silver nanoparticles.[7d–7f] Singh *et al.* reported one-step *in situ* generation of AuNPs on spider-silk fiber which is accomplished by a simple reaction of the silk with aqueous chloroauric acid.[7g] These hydroxyl- and amine-containing biomacromolecules have shown potential as support with dual roles of reductant and stabilizer for AuNP synthesis, in which additional reducing agent is not needed. Among the biomacromolecules, collagen that has been widely studied in biomedical fields and tissue engineering is recognized to be an ideal candidate for synthesis of AuNPs.

Collagen, coming from skin, tendon, and other tissues of animals, is one of the most abundant renewable biomass in nature. A wealth of merits of collagen such as biological origin, specific molecular structure, excellent biocompatibility and biodegradability make the fabrication of collagen-stabilized AuNPs more fascinating. In recent years, there has been several literatures referring to collagen-mediated AuNPs assembly and synthesis,[8] but use of collagen for the reduction of Au^{3+} to Au^0 is often time-consuming, and almost all the present research still required an introduction of extra chemical reductants (sodium borohydride and citrate) or physical approaches (heat treatment and UV irradiation) in order to achieve rapid and efficient reduction. In addition, although collagen itself contains some stabilizing groups for AuNPs, our previous research showed that collagen-stabilized AuNPs as heterogeneous catalysts lacked sufficient stability for reuse in practical catalysis applications.[8c] The same problem is sometimes faced by other biomacromolecule-stabilized AuNPs used in catalytic reactions, *e.g.* chitosan.[7b]

Quite recently, we found that grafting of epigallocatechin-3-gallate (EGCG) onto the surface of collagen fiber (CF) could significantly improve the dispersion and stabilization of Pd^0 nanoparticles on CF support,[9] which prompted us to investigate the capability of the modified collagen serving as an efficient support for preparing stable AuNPs. We describe herein a one-step, reductant-free, and size-controlled synthesis of stable AuNPs by using EGCG-grafted CF (EGCG-CF) as the support that possesses efficient reducing/stabilizing ability for the formation AuNPs. EGCG, a natural plant polyphenol extracted from tea, contains a large number of phenolic hydroxyls which endows it with the capability of reducing Au^{3+} to Au^0.[10] Moreover, due to the high affinity of phenolic hydroxyls with AuNPs, EGCG may provide AuNPs robust shielding to prevent them from aggregation. The synthesis processes were carried out in aqueous solution at room temperature without any extra reagents or treatments, which is compatible with green chemistry principles. The main physical and chemical properties of the prepared AuNPs were fully characterized. The reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) by $NaBH_4$ in aqueous phase was chosen as a model reaction to investigate the catalytic behaviors of the AuNPs. The effects of EGCG-grafting degree on the particle size and catalytic activity of AuNPs were studied, and the reuse stability of AuNPs was also investigated.

Experimental

Chemicals

Collagen fiber was prepared from cattle skin according to our previous work.[11] Epigallocatechin-3-gallate (99%) was provided by the Department of Tea Science, Sichuan Agricultural University. $HAuCl_4$ (99%) was purchased from Sigma-Aldrich. Sodium borohydride ($NaBH_4$), glutaraldehyde (50%), 4-nitrophenol (99%) and other chemicals were all analytical reagents.

Preparation of EGCG-grafted CF (EGCG-CF)

The procedure of preparing EGCG-grafted CF was the same as that in our previous work.[9] In brief, 0.5 g of EGCG was dissolved in 100 mL of distilled water and then mixed with 5 g of CF. The mixture was stirred at 298 K for 2 h. Then, 50 mL of glutaraldehyde solution (2.0 wt%) at pH 6.5 was added into the mixture and stirred at 318 K for 6 h. Subsequently, the product was filtrated, fully washed with distilled water and dried in vacuum at 308 K, and then EGCG-grafted CF (EGCG-CF) was obtained. The concentrations of EGCG in the reaction solution before and after grafting reaction were determined by High-performance Liquid Chromatography (HPLC, Agilent 1100),[12] and the actual amount of EGCG grafted on CF was determined by the mass balance calculation.

Preparation of AuNPs supported by EGCG-CF (Au-EGCG-CF)

In a typical preparation of Au-EGCG-CF, 1 g of EGCG-CF was suspended in 100 mL of 0.5×10^{-3} M $HAuCl_4$ aqueous solution. The mixture was stirred at room temperature for 6 h, and then filtrated, fully washed with distilled water and dried in vacuum at 308 K. Finally, a wine red product of Au-EGCG-CF was obtained. As control, Au-CF was also prepared in the same procedure as described above, where Au was directly loaded onto CF. To ascertain the actual loading amount of Au, the catalyst was digested to obtain Au solution. Then, the Au amount on catalyst was determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, Perkin-Elmer Optima 2100DV, Germany).

Characterization

Ultraviolet-visible diffuse reflectance spectra (UV-vis DRS) were recorded by means of UV-vis-NIR spectrophotometer (UV-3600, Shimadzu, Japan) equipped with an integrating sphere and using $BaSO_4$ as reference. X-Ray diffraction (XRD, Philips X' Pert Pro-MPD) studies were performed by using Cu-Kα X-radiation (λ = 0.154 nm). The morphology of Au-EGCG-CF was observed by Scanning Electron Microscopy (SEM, JEOL LTD JSM-5900LV). The size and distribution of AuNPs on EGCG-CF were determined using Field Emission Transmission Electron Microscopy (FE-TEM, 200kV, Tecnai G^2 F20, FEI, Netherlands) equipped with an energy-dispersive X-ray analysis (EDAX) attachment. Fourier Transform-infrared Spectroscopy (FT-IR, Perkin-Elmer, USA) analyses were carried out by using compressed films of KBr pellets and sample powders. X-Ray Photoelectron Spectroscopy (XPS, Kratos XSAM-800, UK) analyses were conducted by employing Mg-Kα X-radiation ($h\nu$ = 1253.6 eV) and a pass energy of 31.5 eV. All of the binding energy

peaks of XPS spectra were calibrated by placing the principal C 1s binding energy peak at 284.8 eV. Peaks from all the high-resolution core spectra were fitted with XPSPEAK 4.1 software, using mixed Gaussian-Lorentzian functions.

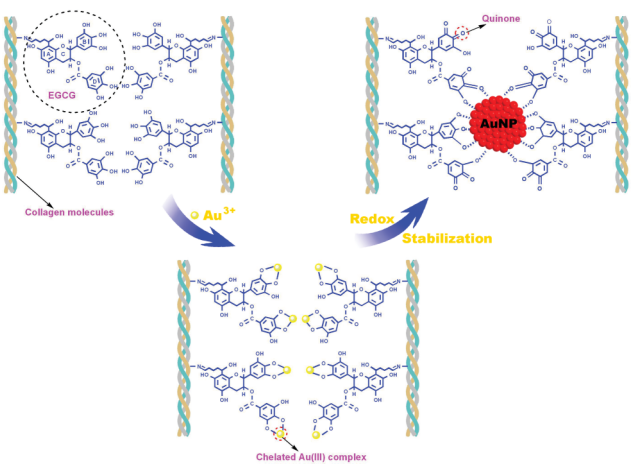
Catalytic reduction of 4-nitrophenol

In a typical run, 1 mL of 4-NP aqueous solution (1 mM) was mixed with 25 mL of distilled water containing a certain amount of catalyst (Au: 1.0×10^{-3} mmol). The suspension was then purged with N_2 for 30 min to remove the dissolved O_2. Freshly prepared 4 mL of $NaBH_4$ aqueous solution (0.33 M) was then added to start the reduction reaction. The solution mixture was stirred during the reaction. A sample of 1.5 mL was withdrawn at a regular interval and measured by UV-vis spectrophotometer in the range of 200–600 nm. The reduction process was monitored through measuring the change of absorbance at 400 nm as a function of time.

Results and discussion

Preparation of AuNPs supported by EGCG-CF

In the present study, EGCG can be easily grafted onto CF surface *via* a Mannich reaction.[9,13b] As shown in Scheme 1, EGCG is covalently bonded with amino groups of collagen molecules by the crosslinking of glutaraldehyde, which results in an increase of anchoring sites on CF surface for gold precursors. As a typical plant polyphenol, EGCG consists of multiple orthophenolic hydroxyls, and it has been proven to be excellent bidentate ligand to bond with Au^{3+} ions by forming a stable five-member chelating ring.[13] Due to the high redox potential of Au^{3+}, the chelated Au^{3+} ions could be reduced into Au^0 atoms *in situ*, while a part of phenolic hydroxyls of EGCG are simultaneously oxidized to corresponding carbonyl groups, quinone.[10c,13a,14] According to Tripathy's research,[14a] both the formed carbonyls and free hydroxyls are able to stabilize AuNPs by the interaction between the surface Au atoms of AuNPs and oxygen atoms of EGCG molecules. More importantly, high density of hydroxyl groups in EGCG could lead to extensive inter- and intramolecular hydrogen bonding, which favors formation of unique supramolecular assemblies.[15b] These supramolecular cages provide steric hindrance to the AuNPs formed, and subsequently prevent individual particles from coagulating.[13a,15]



Scheme 1 Schematic plot illustrating the formation and stabilization of AuNPs with EGCG-grafted CF as support.

The typical preparation procedure of Au-$EGCG_{0.1}$-CF (subscript number stands for initial mass ratio of EGCG to CF) is illustrated in Fig. 1. When the synthesized $EGCG_{0.1}$-CF was added into the aqueous solution of $HAuCl_4$, Au^{3+} ions were chelated by the orthophenolic hydroxyls of EGCG and then were spontaneously reduced to Au^0 *in situ* at room temperature without any other chemical reducing agents. The generation of AuNPs on $EGCG_{0.1}$-CF matrix can be visually witnessed by the color change of $EGCG_{0.1}$-CF from pale brown to wine red.

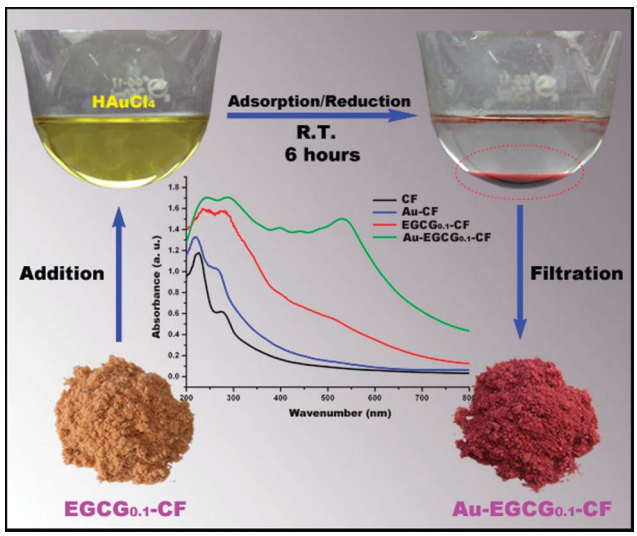


Fig. 1 Preparation of gold nanoparticles supported on EGCG-grafted CF. The inset shows the UV-vis DRS spectra of CF, Au-CF, $EGCG_{0.1}$-CF and Au-$EGCG_{0.1}$-CF.

UV-vis DRS measurement was performed to further confirm the formation of AuNPs. The inset in Fig. 1 presents the UV-vis DRS spectra of CF, Au-CF, $EGCG_{0.1}$-CF and Au-$EGCG_{0.1}$-CF. Compared with the spectrum of $EGCG_{0.1}$-CF, the UV-vis DRS spectrum of Au-$EGCG_{0.1}$-CF displays an intense absorption band with the maximum at 530 nm, which is attributed to the characteristic surface plasmon resonance (SPR) of metallic gold nanoparticles.[2d] The SPR in metal nanoparticles arises from the collective oscillation of the free conduction band electrons induced by incident electromagnetic radiation, and it is sensitive to particle size, shape, distribution, surrounding medium, *etc.* Moreover, the time-dependent variation of the UV-vis DRS spectrum for the reduction of Au^{3+} is presented in Fig. S1 (see the ESI† for details), in which the intensity of SPR band at 530 nm increases systematically with the increase of reaction time. About 6 h later, the intensity reaches a maximum and almost keeps constant. This observation indicates the formation of AuNPs on EGCG-CF. Furthermore, as can be seen in Fig. 1, the unmodified CF only has two relatively weak absorption peaks, located at 210 nm and 280 nm, mainly ascribed to the polypeptide chains and the benzene rings in side chains, respectively. However, after grafting reaction, EGCG-CF exhibits enhanced absorbance in the range of 200–800 nm, which is consistent with the color change of CF from white to pale brown. For comparison, CF was directly used

as support for preparing gold catalyst (Au-CF) without adding any reductant, and its UV-vis DRS spectrum is also presented in Fig. 1. Clearly, Au-CF does not show any adsorption peak around 530 nm, indicating that almost no metallic Au particles are generated in Au-CF. The color of CF after reaction with $AuCl_4^-$ changed from white to faint yellow (image not shown), implying that the gold loaded on CF are mainly in the form of ionic state. This observation demonstrates that CF itself has no ability to reduce Au^{3+} to form Au^0 nanoparticles under present preparation conditions, even though it can combine with Au^{3+} through its plenty of functional groups, such as –COOH and $–NH_2$, in side chains. Additionally, as reported in our previous work,[9] the grafting degree of EGCG on CF can be easily tunable depending on the initial mass ratio of EGCG to CF. As summarized in Table S1 (see ESI†), a series of Au-EGCG$_x$-CF (x = 0.01, 0.05, 0.1, 0.3, 0.5, and 1.0) with varying grafting degree of EGCG on CF were prepared in this study. It is found that the actual grafting quantity of EGCG on CF increases with the increase of initial amount of EGCG, which simultaneously enhances the interaction of CF with Au ions.

Characterization of Au-EGCG-CF

Fig. S2 shows the typical X-ray diffraction patterns of EGCG$_{0.1}$-CF, Au-EGCG$_{0.1}$-CF, and Au-CF (see ESI†). The XRD patterns of all these samples exhibit a broad signal around 23°, which is attributed to the amorphous polymer phase of collagen fiber. As expected, we can note the absence of crystal diffraction peaks in the EGCG$_{0.1}$-CF. In contrast, the XRD pattern of Au-EGCG$_{0.1}$-CF shows four diffraction peaks at 2θ = 38.1, 44.2, 64.6, and 77.5° corresponding to the (111), (200), (220), and (311) crystal planes, respectively, which are the characteristic of face-centered cubic (*fcc*) gold (JCPDS-4748). As for Au-CF, however, no obvious crystal diffraction peaks are observed in its XRD pattern, implying no gold nanocrystals were formed in Au-CF, which is consistent with the result of UV-vis DRS characterization. Fig. S3 presents the SEM images of Au-EGCG$_{0.1}$-CF (see ESI†), in which the sample is in a highly ordered fibrous state, indicating that the inherent fibrous morphology of natural collagen fiber is still preserved well.

The morphology and size of AuNPs in Au-EGCG$_x$-CF (x = 0.01, 0.05, 0.1, 0.3, 0.5 and 1.0) were further determined by TEM observation, and the results of size distribution are summarized in Table S1 (see ESI†). It is noted that the particle size distribution of AuNPs supported on EGCG-CF shows a remarkable dependency on the grafting degree of EGCG on CF. The representative TEM images of Au-EGCG$_x$-CF (x = 0.01, 0.1, 0.3, and 1.0) and their corresponding histogram of particle size distribution are presented in Fig. 2. As shown in Fig. 2a, the AuNPs with nearly spherical shape are presented in Au-EGCG$_{0.01}$-CF, and their mean diameter is estimated to be 18.6 ± 7.4 nm. The high-resolution TEM micrograph (HRTEM) of an individual gold nanoparticle in Au-EGCG$_{0.01}$-CF and its fast Fourier transform (FFT) image (inset) are presented in Fig. 3a. The multiple lattice fringes with an interplanar spacing of 0.238 nm were observed, which is consistent with the interplanar distance of gold (111) planes.[16] Evidently, the size distribution of AuNPs on EGCG$_{0.01}$-CF is in a wide range, which suggests that a relatively low grafting degree of EGCG on CF is not

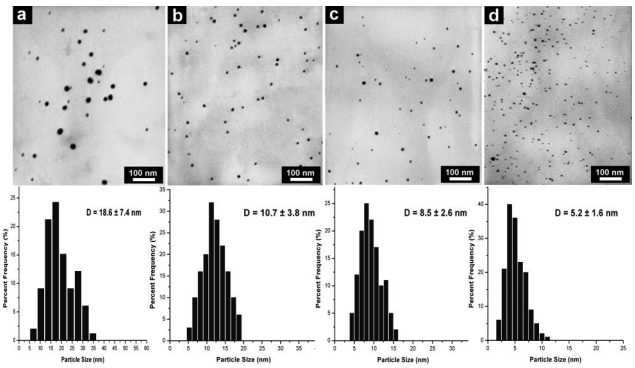


Fig. 2 TEM images and the corresponding particle size distribution of Au-EGCG$_x$-CF with various EGCG/CF initial mass ratio: x = (a) 0.01, (b) 0.1, (c) 0.3 and (d) 1.0. The bar 100 nm.

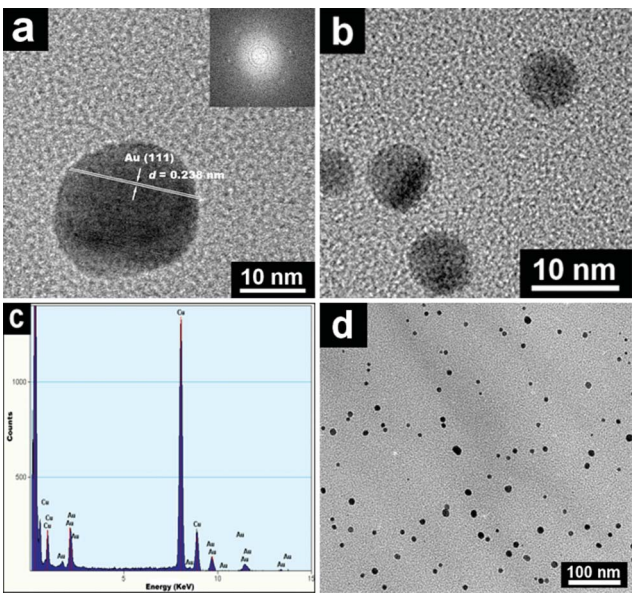


Fig. 3 HRTEM images of (a) Au-EGCG$_{0.01}$-CF, (b) Au-EGCG$_{0.1}$-CF and (d) Au-EGCG$_{0.01}$-CF after twenty cycles in reduction reaction. (c) EDAX pattern of Au-EGCG$_{0.1}$-CF. The inset in Fig. 3a shows the FFT image of an individual Au nanoparticle.

enough to efficiently control the dispersion of AuNPs. The AuNPs with small size and narrow distribution can be obtained if the initial mass ratio of EGCG to CF was increased from 0.01 to 1.0, as shown in Fig. 2b–2d. The HRTEM observation of Au-EGCG$_{0.1}$-CF in Fig. 3b also reveals that the small and well-crystallized AuNPs were produced. The EDAX analysis (Fig. 3c) confirms that the nanoparticles observed in the TEM images consist of pure gold atoms. In addition, no AuNPs were observed in Au-CF by TEM (image not shown), which is in agreement with the results of UV-vis and XRD analyses. All these observations suggest that the grafting degree of EGCG on CF plays an important role in the formation of AuNPs as well as their distribution.

Accordingly, we propose a kinetic-controlled process for the formation of AuNPs stabilized by EGCG-CF. At a low dosage of EGCG, as in Au-EGCG$_{0.01}$-CF, the auto-reduction rate of Au^{3+} is relatively faster than the coating speed of AuNPs with EGCG molecules, which easily leads to the assembly and growth of the reduced Au^0 species into large-sized AuNPs. However,

as increasing dosage of EGCG from 0.01 to 1.0, the hydrogen bond interactions among EGCG molecules are strengthened, which consequently construct dense supermolecular cages. In these cages, the coordination affinities of EGCG to Au ions are improved and therefore, the coating speed is increased to that being comparable to the reduction of Au^{3+}. The strong coating effect could sterically prevent or slow individual particles from coalescing with each other, and thus give a small size and narrow distribution of AuNPs.[15]

The chemical state of gold in Au-EGCG-CF was determined by XPS analysis, and the typical XPS spectra are presented in Fig. 4. As seen in Fig. 4a, the survey scan spectrum of Au-$EGCG_{0.1}$-CF shows the presence of C 1s, N 1s, O 1s, Au 4d, and Au 4f core-levels with no evidence of impurities. It is worthwhile to mention that almost no chlorine ions were detected in the Au-EGCG-CF, because of the absence of Cl 2p peak at the 197 eV binding energy region, as shown in the inset of Fig. 4a. This fact suggests that the Cl^- ions were completely washed out and no $AuCl_4^-$ precursor was reserved on the surface of AuNPs.[17] Fig. 4b shows the Au 4f core-level spectra of Au-$EGCG_{0.1}$-CF. The Au 4f core-level is curve-fitted with two pairs of doublets from spin-orbital splitting of $4f_{7/2}$ and $4f_{5/2}$. According to Tripathy's research,[14a] the most intense doublets observed at 84.0 and 87.8 eV arise from the inner Au atoms of AuNPs, being consistent with zero valent Au^0, while the second set of doublets located at 85.3 and 89.1 eV originate from the outer surface Au atoms bonded with EGCG molecules. These surface Au atoms of AuNPs serve as the main interaction sites with EGCG-CF to stabilize AuNPs. Negishi and Tanaka have also pointed out that the Au 4f peaks located at relatively higher binding energy should be attributed to the surface Au atoms of AuNPs bonded to surface surrounding stabilizer or passive molecules, suggesting that a substantial electron donation from AuNPs to stabilizer molecules is present.[17a,17b]

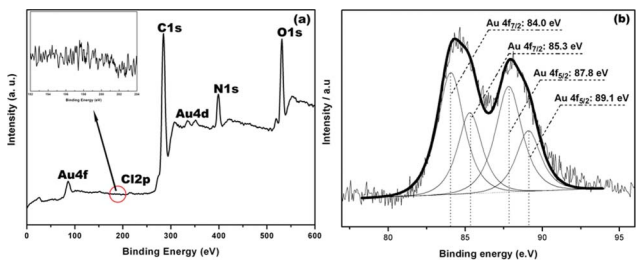


Fig. 4 XPS spectra of Au-$EGCG_{0.1}$-CF. (a) Survey scan spectrum. (b) Au 4f core-level spectrum. The inset in Fig. 4a shows Cl 2p core-level spectrum.

Moreover, the O 1s XPS spectra of CF, $EGCG_{0.1}$-CF and Au-$EGCG_{0.1}$-CF are also presented in Fig. S4 (see ESI†). There is only one peak at 531.6 eV in the O 1s signal of CF, which is mainly attributed to O═C–N in peptidic carbonly groups (Fig. S4a†).[17c] However, as for $EGCG_{0.1}$-CF, the O 1s signal peak changes, where a new peak with a higher intensity clearly appears at 532.8 eV (Fig. S4b†), which is assigned to the oxygen atoms in phenolic hydroxyl (HO–C).[17d] This fact indicates that EGCG was successfully grafted onto CF. After reaction with Au^{3+}, the O 1s peak corresponding to the HO–C group dramatically decreases in intensity, while the O 1s signal around 531.8 eV is greatly strengthened (Fig. S4c†). This observation should be attributed to the oxidation of phenolic hydroxyls and the formation of quinones.[17e] It should be noted that a new peak arising at a relatively lower binding energy of 530.6 eV implies the presence of the Au→O electron charge transfer, which indicates the nature of the interaction between the AuNPs and EGCG to stabilize AuNPs on CF.[14a,19e]

Fig. 5 presents the FT-IR spectra of CF, $EGCG_{0.1}$-CF and Au-$EGCG_{0.1}$-CF. In the IR spectrum of CF (Fig. 5a), the amide I adsorption band around 1660 cm^{-1} arises predominantly from protein amide C═O stretching vibration, while amide II adsorption band at 1548 cm^{-1} arises from amide N–H bending vibration and C–N stretching vibration. The amide III adsorption band at 1238 cm^{-1} consists of the components from C–N stretching vibration and N–H in-plane bending vibration from amide linkages.[18a] For $EGCG_{0.1}$-CF (Fig. 5b), the adsorption band around 3385 cm^{-1} appears to be broadened, mainly due to the strong hydrogen bond interaction between the phenolic hydroxyls of EGCG and the amino/amide groups of CF.[18b] The appearance of a new adsorption peak at 1120 cm^{-1} is ascribed to the C–O–H stretching vibration of phenolic hydroxyls in EGCG, while the two strengthened adsorption peaks at 1045 and 1337 cm^{-1} belong to the C–O–C stretching vibration and O–H in-plane bending vibration, respectively.[18c] As for Au-$EGCG_{0.1}$-CF (Fig. 5c), however, the adsorption band around 3385 cm^{-1} appears to be narrowed and shifted to long wavelength probably due to the partial destruction of hydrogen bonds between EGCG and CF, which implies the involvement of the O–H groups in the reduction of gold.[9,14d] In addition, the damping of the peak at 1120 cm^{-1} further indicates that the

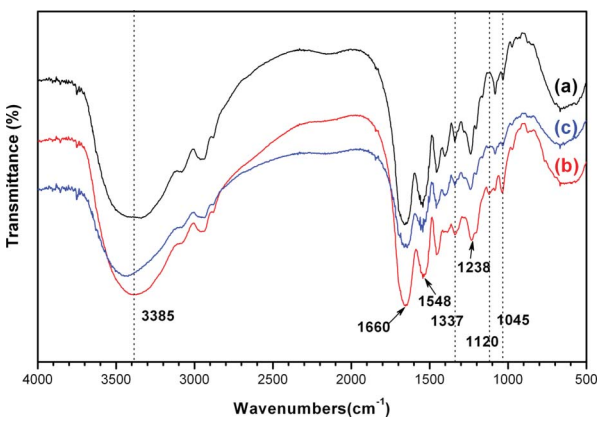


Fig. 5 FT-IR spectra of (a) CF, (b) $EGCG_{0.1}$-CF and (c) Au-$EGCG_{0.1}$-CF.

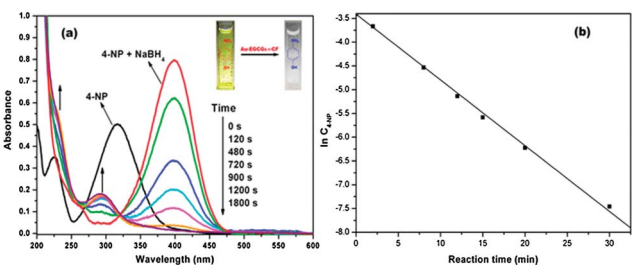


Fig. 6 (a) Successive UV-vis adsorption spectra taken after adding $NaBH_4$ into 4-NP solution in the presence of Au-$EGCG_{0.1}$-CF as catalyst. (b) Plot of $\ln(C_{4\text{-NP}})$ *versus* time corresponding to the reduction of 4-NP catalyzed by Au-$EGCG_{0.1}$-CF at 298 K.

reduction of gold ions could be coupled to the oxidation of phenolic hydroxyls of EGCG.[14d]

Catalytic properties of Au-EGCG-CF

To study the catalytic characteristics of Au-EGCG-CF, the reduction of 4-nitrophenol to 4-aminophenol by sodium borohydride in aqueous phase was chosen as a model reaction. This reaction has been extensively used as a benchmark system to evaluate the catalytic activity of noble metal nanoparticles.[3d,19] Metal nanoparticles can effectively catalyze the reduction of nitro compounds by acting as an electronic relay system, wherein the electron transfer takes place from donor BH_4^- to acceptor nitro groups. As shown in Fig. 6a, the adsorption peak of 4-NP was red-shifted from 317 to 400 nm immediately upon the addition of $NaBH_4$ solution, which corresponds to a color change of 4-NP solution from light yellow to yellow-green due to the formation of 4-nitrophenolate ion in alkaline condition. In absence of catalyst, the adsorption peak at 400 nm remained unaltered even for a couple of days. In contrast, addition and mixture of a proper amount of Au-EGCG$_{0.1}$-CF into the solution caused a decolorization of the yellow-green 4-nitrophenolate solution (inset of Fig. 6a), while the adsorption peak height at 400 nm successively decreased with a concomitant appearance of two new adsorption peaks at 235 and 295 nm due to the formation of 4-AP. Moreover, two points are visible in the UV-vis spectra, where all the spectra intersect each other, indicating that the catalytic reduction of 4-NP to 4-AP proceeded without formation of by-products.[19a] The blank experiment using EGCG$_{0.1}$-CF did not show any change in color or adsorption peak of 4-nitrophenoloate for more than 24 h, clearly demonstrating that the reduction of 4-NP by $NaBH_4$ is solely activated by AuNPs stabilized on EGCG-CF.

As the initial concentration of $NaBH_4$ largely exceeds that of 4-NP, the reduction rate can be assumed to be independent of $NaBH_4$. Therefore, the pseudo-first-order rate kinetics with respect to the 4-NP concentration could be used to evaluate the catalytic rate.[19] The reaction kinetics can be described as $-\ln(C_t/C_0) = kt$, where k is the rate constant at a given temperature and t is the reaction time. C_0 and C_t are the concentration of 4-NP at beginning and at time t, respectively. As expected, a good liner correlation of $\ln C_t$ *versus* reaction time t was obtained (shown in Fig. 6b), whereby a kinetic rate constant k was estimated to be 14.16×10^{-2} min^{-1}. Furthermore, Fig. 7a shows that the rate of the reduction reaction is increased when reaction temperature rises from 298 to 328 K. According to the principle of the Arrhenius equation, the activation energy (E_a) of the reduction of 4-NP catalyzed by Au-EGCG$_{0.1}$-CF is determined by plotting $\ln k$ *vs* $1/T$ (Fig. 7b). The E_a obtained is 37.3 ± 1.8 kJ mol^{-1}. This value is relatively small, and is comparable to those of other nanoparticle catalysts in the reduction of 4-NP such as Au/poly(methyl methacrylate) (38 kJ mol^{-1}),[19b] Au/ion-exchange resin (31 kJ mol^{-1}),[19a] Au/polyelectrolyte brushes (43 kJ mol^{-1}).[19c]

Fig. 8 presents the dependency of reduction rate on the grafting degree of EGCG on CF. It was found that Au-EGCG$_{0.01}$-CF exhibited a relatively lower catalytic activity, whereas the rate of the reduction reaction was greatly increased by using Au-EGCG$_{0.1}$-CF. This increased reduction rate should be mainly

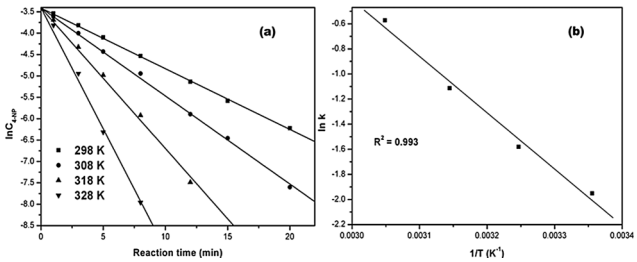


Fig. 7 (a) Effect of reaction temperature on reduction rate. (b) Arrhenius plots of ln(k) *versus* $1/T$ in the temperature range of 298–329 K.

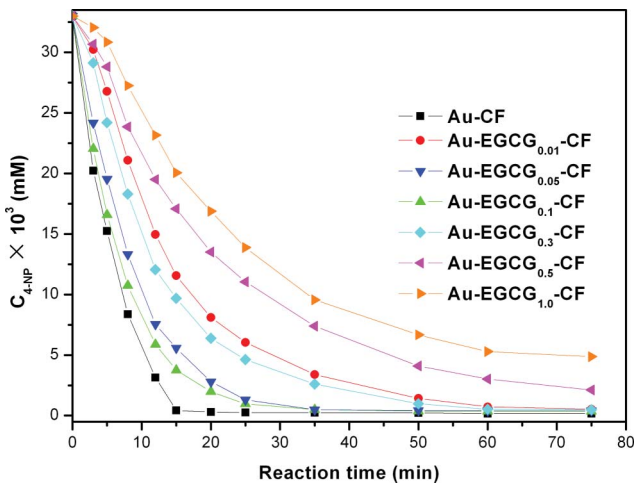


Fig. 8 Reduction of 4-NP catalyzed by Au-CF and Au-EGCG-CF with varying grafting degree of EGCG.

attributed to the size effect of AuNPs in catalysts. As confirmed by TEM above, the gold particle size in Au-EGCG$_{0.1}$-CF is much smaller than that in Au-EGCG$_{0.01}$-CF, which would significantly promote the accessibility of reactants to catalyst active centers and thus enhance the catalytic reduction rate. However, when the particle size of gold further decreased from 18.6 ± 7.4 nm in Au-EGCG$_{0.1}$-CF to 5.2 ± 1.6 nm in Au-EGCG$_{1.0}$-CF, a tendency of decreasing reduction rate was observed, as shown in Fig. 8. In general, a smaller size of metal particle implies that a larger fraction of metal atoms are exposed at surfaces and available for catalysis. The unusual phenomenon in our experiment should be ascribed to the fact that a high grafting degree of EGCG may introduce a high density of phenolic hydroxyls around Au nanoparticle, which can sterically hinder the diffusion of reactant toward the active sites of catalyst and eventually the catalytic activity is depressed.[9] Accordingly, it is remarkable that the grafting degree of EGCG on CF surface is an important factor in controlling the formation of AuNPs and affecting their catalytic activity.

An attention should be paid to the fact that a delay time at the beginning of the catalytic reduction was found in the cases of Au-EGCG$_{0.01}$-CF, Au-EGCG$_{0.3}$-CF, Au-EGCG$_{0.5}$-CF, and Au-EGCG$_{1.0}$-CF. This is in accordance with other studies of this reduction reaction catalyzed by metal nanoparticles.[19] According to the literature, two factors including gold particle size and support (EGCG-CF) structure should be responsible for the appearance of the induction period in this study. In the case of Au-EGCG$_{0.01}$-CF, since the size of AuNPs is

relatively larger, and they are mainly composed of low-index and high-coordinative saturated surface atoms related with lower surface roughness,[19a] which depresses the chemisorption of 4-nitrophenolate ions and thereby hampers the reaction. Panigrahi *et al.* has also reported that the induction period decreased and subsequently vanished with decreasing particle size of AuNPs.[19a] As for Au-EGCG$_{0.3}$-CF, Au-EGCG$_{0.5}$-CF and Au-EGCG$_{1.0}$-CF, however, the induction period may be resulted from the diffusion process of reactant on the catalysts. When the grafting degree of EGCG on CF was increased to some extent, the interactions among EGCG molecules were largely strengthened, which constructs dense supermolecular cages around Au^0 nanoparticles. As a result, the reactants and reduced products will take time to diffuse into and out of these cages, once they reach and leave the catalyst active centers. Kuroda *et al.* has concluded in detail the dependency of the induction period in 4-NP reduction on the type and structure of various polymer supports.[19b]

Additionally, one phenomenon beyond our expectation should be noted in Fig. 8, in which a maximum catalytic reduction rate was displayed by Au-CF as compared with that of other six catalysts despite the absence of gold nanocrystals in Au-CF proved by TEM above. To uncover the underlying reason accountable for this unexpected result, an additional experiment was employed. We performed a filtration to remove the Au-CF catalyst from the 4-NP solution when the reduction reaction proceeded for 180 s. Following that, the filtrated solution was continuatively stirred and monitored by UV-vis spectrophotometer at a regular interval, and the spectrum is presented in Fig. S5a (see ESI†). It is clearly seen that although the solid Au-CF catalyst was removed from the reaction system, the reduction of 4-NP was still proceeded and completed within 1500 s, as indicated by the decrease of adsorption peak height at 400 nm with time. This phenomenon implies that Au-CF suffered a leaching of active Au species during the initial 180 s, which was further confirmed by the fact that a certain amount of Au species remained in the filtrated solution detected by element analysis (ICP-AES). Due to the relatively weak stabilizing action of CF, some unstable Au ions loaded on CF could be leached into the reaction solution and further reduced into Au^0 in the presence of $NaBH_4$. Accordingly, catalytic reduction of the 4-NP solution containing these naked active Au^0 species can be considered as a quasi-homogeneous catalysis system, in which the reduction of nitrophenol could be extremely promoted. For comparison, a similar filtration experiment in the case of Au-EGCG$_{0.1}$-CF was also carried out. As shown in Fig. S5b†, the reaction almost stopped as soon as the Au-EGCG$_{0.1}$-CF was removed, indicating the heterogeneous nature of the reduction of 4-NP catalyzed by AuNPs supported by EGCG-CF. Such a difference in catalytic behaviors between naked Au^0 and supported Au^0 species was also reported by Xiong and co-workers in their research.[19g]

It is known that reusability is the main advantage of using heterogeneous catalyst rather than homogeneous catalyst for industrial application. Although several catalytic studies of 4-NP reduction using AuNP catalysts have been reported in the literature, there exist only a few reports where the AuNP catalysts were successfully recovered for consecutive reuses.[3d,3e,19b,19e,19h] To check the reusability of Au-EGCG-CF catalyst, the solid catalyst Au-EGCG$_{0.1}$-CF was recovered from the reaction mixture simply by filtration and washing with distilled water, and reused under the same conditions as for the initial cycle. As shown in Fig. 9, Au-EGCG$_{0.1}$-CF can be successfully recycled and reused in twenty successive reactions with a conversion of >98%. The catalyst was digested and analyzed by ICP-AES after twenty cycles. Compared to the Au amount on the initial catalyst (1.0×10^{-3} mmol), no significant Au loss on the used catalyst (0.98×10^{-3} mmol) was determined. Further TEM observation confirms that almost no aggregation of AuNPs occured in the used catalyst (Fig. 3d), indicating the high stability of AuNPs supported by EGCG-CF. For comparison, the results of recycling uses of Au-CF are also presented in Fig. 9. Au-CF suffered a sharp decrease in catalytic activity after twenty cycles, due to the leaching of Au species from Au-CF, proved by the fact that a Au loss of 69% was detected by ICP-AES analysis. It should be noted that the catalytic activity of Au-CF decreased step by step after its first cycle, which could be attributed to the fact that collagen itself possesses certain ability of stabilizing Au because of its functional groups. Such recycling behavior of Au-CF was quite similar to that of Pd^0 nanoparticles supported by CF in our earlier report.[9]

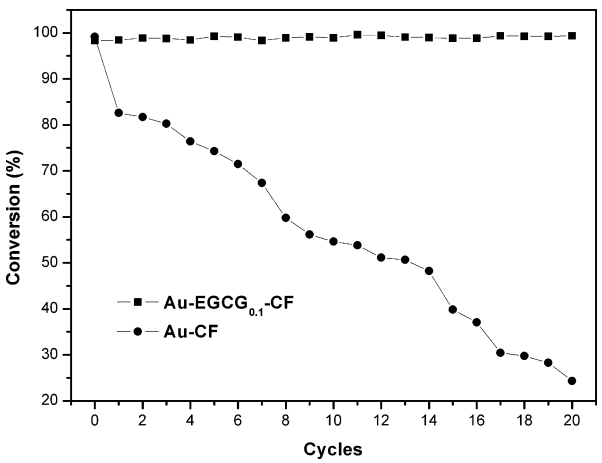


Fig. 9 Conversion of 4-nitrophenol in twenty successive uses of Au-EGCG$_{0.1}$-CF and Au-CF as catalysts.

Conclusions

In conclusion, EGCG-grafted CF can be used as a support for preparing stable AuNPs. The EGCG acts as both reductant and stabilizer in the process of preparation, so that no additional reducing agents or treatments are needed. This process is performed in aqueous solution and is a green approach. The particle size and distribution of AuNPs can be easily controlled by tuning the grafting degree of EGCG on CF surface. These supported AuNPs exhibited a good catalytic activity and high reusability for the reduction of 4-nitrophenol in aqueous phase. This preparation strategy of AuNPs appears very simple, green and cost-effective, and exhibits great potential for practical applications. Further works to explore the application of the AuNPs system in other fields are currently ongoing in our laboratory.

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