Gold-Conductive Polymer Nanoparticles: A Hybrid Material with Enhanced Photonic Reactivity to Environmental Stimuli

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Abstract

We have designed a simple synthetic procedure to encapsulate colloidal gold nanoparticles by electrostatic adsorption with water-soluble poly(aniline-2-carboxylic acid). The composite nanoparticles are stable in aqueous buffer and retain the respective optical reactivity of the gold colloid to refractive index increases, and of the conductive polymer to pH changes and oxido-reduction. The new composite displays however significant enhancements in photonic performance when compared to the individual components, which seem to result from electronic interplay between the two materials in the hybrid structure. The enhanced photonic reactivity of the composite structure offers new opportunities for biosensing application.

KEYWORDS

Gold colloid; conductive polymer; composite; encapsulation; photonic reactivity; surface plasmon resonance; pH; oxido-reduction.
1. Introduction

Both noble metal colloids and conductive polymers independently present interesting optical properties that make them suitable for various applications, particularly in homogeneous sensors for biomolecular recognition [1, 2]. For instance, the exquisite sensitivity of the localized surface plasmon resonance (LSPR) absorbance of gold and silver nanocolloids to increases in refractive index at the particle surface has been advantageously applied to the real-time monitoring of ligand-receptor interactions in both liquid and solid phase [3-6]. Similarly, the chromic transitions experienced by watersoluble conductive polymers linked to antibodies or receptors upon biomolecular recognition of their cognate ligand, resulting respectively from the responsiveness of their electronic structure to localized redox changes or departure from coplanarity [1], has been successfully applied in homogeneous immuno- and receptor assays [7, 8]. Therefore, the idea to combine both materials into a composite nanocolloid has been tempting. The underlying hypothesis is that the combination of such materials into composite nanoparticles should lead, provided that their electronic absorptions characteristics overlap, to photo-induced electronic interplay or charge and energy transfers between the components, resulting in an enhancement of their original optical reactivity [9, 10]. Similar molecular plasmonic constructs have already been shown to carry out active energy transfer through the surface plasmon of the metal [10, 11]. Therefore, their possible applications are numerous and in biotechnology alone, range from nanoscale optical spectroscopy to surface plasmon resonance sensing and nanolithography [12]. However, the practical realization of hybrid nanostructures containing conductive polymers is far from straightforward, primarily because of their poor solubility. Several strategies were however developed to combine a noble metal and a conductive polymer in a composite nanostructure. A first such attempt used gold and silver nanocolloids as templates for poly(acetylene) photopolymerization in ethanol-water solutions [13, 14]. Other attempts were subsequently made to simultaneously oxidize the monomer and reduce the metal salt in order to create nanoparticles of mixed composition [15-17]. The resulting materials obtained were unfortunately unsuitable for optical biosensing and biological applications because either the optical properties of the components were not matched and their absorption peaks did not overlap [13, 14], the nanoparticles were not miscible in aqueous solvents [15], or the nanocolloids obtained were ill-defined and unstable [16, 17].

In order to circumvent these drawbacks, we have attempted the encapsulation by electrostatic adsorption of preformed gold nanoparticles with a poly(aniline-2-carboxylic acid) (PANI-COOH) aqueous solution synthesized separately. We report here the facile synthesis and characterization of such a stable composite nanomaterial, and detail its enhanced photonic reactivity in solution as compared to that of the original components toward environmental stimuli such as changes in refractive index, oxido-reduction and pH.
2. Materials and Methods

2.1. Reagents and equipment.

Chemicals of the highest available grade were obtained from SAF (Bornem, Belgium) and used without further purification. Distilled water was used throughout. UV-vis spectra, difference spectra and absorbance measurements were recorded in an Ultrospec 2000 spectrophotometer (Amersham Biosciences) using disposable UV-transparent cuvettes of 1 cm optical path length. For the spectra, the spectrophotometer was zeroed on water. For the difference spectra, the spectrophotometer was zeroed on the mixture containing the material (PANI-COOH, gold colloid or composite nanoparticles) in absence of the additive studied. After synthesis, the polymer solution (20 µl) was chromatographed on a 7.5x600 mm TSK2000SW size-exclusion HPLC column eluted with 50 mM phosphate pH 7.4 using a 2150 HPLC pump (LKB), and the elution was monitored at 254 nm using a Uvicord SII detector (LKB). The column was calibrated with globular proteins. After synthesis, the composite nanomaterial (0.5 ml) was chromatographed on a 25x300 mm Biogel P10 column (Biorad; exclusion limit 20 kDa for globular proteins) eluted with 50 mM borate pH 9 containing 5mM sodium dodecylsulfate using an Alltech 301 HPLC pump, and the elution was monitored simultaneously at 520 and 540 nm in a 18µl quartz cell placed in the Ultrospec spectrophotometer.

2.2. In silico experiments

The 45-mer chain of PANI-COOH was build and optimized in terms of geometry using a convergence root mean square limit of 0.01 Kcal/Å.mol (Alchemy 32, Tripos Inc.). The geometric characteristics of the molecule (length, depth and height) were evaluated using the DS ViewerPro 5.0 from Accelrys Inc.

2.3 Syntheses of the gold colloid and of water-soluble PANI-COOH.

The gold nanoparticles were produced by reduction under boiling of an hydrogen tetrachloroaurate aqueous solution with sodium citrate, according to previously reported procedures [3, 4]. The PANI-COOH solution was synthesized by oxidizing a solution of 2-aminobenzoic acid in 1M HCl with ammonium persulfate and ferric chloride as previously described [7].

2.4. Synthesis of the gold-PANI-COOH composite nanoparticles.

We modelled the encapsulation procedure with the polymer on a technique we use routinely with proteins, as extensively detailed elsewhere [18]. Briefly, the optimization involved first the determination of the suitable pH for adsorption of the polymer on the gold colloid. The gold colloid in water at various pH values was mixed and incubated (10 min. RT) with a fixed saturating amount of aqueous PANI-COOH solution. After incubation, the nanocolloid solution was challenged with an equal volume of NaCl 1M so as to agglutinate the gold particles that were not stabilized by a polymer layer. The next optimization step consisted in incubating the gold colloid solution set at the optimal coating pH with increasing volumes of water-soluble polymer solution, so as to determine the minimal amount of polymer required to fully stabilize the gold colloid.
against agglutination induced by the eventual salt addition. The stabilization of the gold nanoparticles by adsorption of the polymer was monitored by calculating the percentage shift in $\lambda_{\text{max}}$ of the composite solution versus the $\lambda_{\text{max}}$ of the gold colloid ($\Delta\lambda_{\text{max}}/\lambda_{\text{max}} \times 100$). Any increase in this parameter is indicative of gold nanoparticle agglutination by the salt. The gold colloid nanoparticles were considered fully stabilized by a polymeric layer against salt agglutination when the $\lambda_{\text{max}}$ shift was minimal. The optimal conditions being determined, the encapsulation process was scaled-up to a larger volume (100 ml) and the resulting composite nanoparticle solution was buffered at pH 9 (50mM borate).

2.5. Particle size characterization.

The apparent diameter and dispersity of gold and composite nanoparticles were deduced from published relationships between the vis. spectrum characteristics and actual size measurements by transmission electron microscopy [18, 19] and small angle X-ray scattering [19] of sets of colloidal gold preparations with various sizes. The training sets of nanoparticles used to derive the relationships were homeodisperse preparations respectively characterized by the following actual sizes and dispersities: 12.0 ± 2.7; 25 ± 4.6; 40 ± 7.2; 50 ± 10.0; 63 ± 11.3; 77 ± 14.3; 92.0 ± 19.0 nm [18] and 5.0 ± 0.7; 14 ± 8.7; 22 ± 2.2; 32 ± 3.5; 40 ± 3.4; 57 ± 6.7; 95 ± 6.3; 102.0 ± 8.0 nm [19]. The average sizes of the nanoparticles ($d_{\text{av}}$) were estimated from the relationship between the $\lambda_{\text{max}}$ and actual size ($r = 0.94$) as follows:

$$d_{\text{av}}(\text{nm}) = \frac{\lambda_{\text{max}}(\text{nm}) - 511.5}{0.399}$$

and the dispersity ($\sigma$) was estimated from the relationship between the ratio $A_{600\text{nm}}/A_{\lambda_{\text{max}}}$ and actual dispersity ($r = 0.99$) as follows:

$$\sigma(\text{nm}) = \frac{A_{600\text{nm}}}{A_{\lambda_{\text{max}}}} - 0.0664 \times \frac{\Delta \lambda_{\text{max}}}{0.0259}$$

2.6. Effects of pH.

The polymer and composite nanoparticles solutions were adjusted at the same concentration in terms of PANI-COOH and buffered with 50mM borate. The pH was raised to 11, which was set as the reference. The pH of both solutions was then progressively decreased by addition of 300 mM HCl and a difference spectrum was recorded at each pH value.

2.7. Quantitative assay of ascorbic acid.

The technique was based on a previously described assay using poly(aniline) as the reagent in microtiter plates [20]. Solutions containing increasing concentrations of ascorbic acid (0.5 to 100 mg/l) were prepared in 3% (v:v) acetic acid. These solutions (0.5 ml) were mixed in triplicate with an equal volume of either composite nanoparticle or PANI-COOH solutions, containing the same polymer concentration respectively, both reagents having been adjusted to pH 5 with acetic acid immediately before use. After incubation during 40 minutes at room temperature, the absorbance of the mixtures was recorded at 600 nm.
2.8. Effects of increases in refractive index (RI) of the surrounding medium.

Aqueous solutions of either gold or composite nanocolloids, each respectively containing the same nanoparticle concentration, were mixed with twice their volume of water containing increasing concentrations of glycerol (0, 7.5, 15, 25, 50, 75 and 100 %, v:v) so as to increase progressively the RI of the surrounding medium from 1.33 (water) up to 1.45 (66.7% glycerol final). In order to avoid any influence of a pH-buffering effect in the composite material, a new synthesis was made for this experiment and the resulting composite nanoparticle aqueous solution was used as such.

3. Results and Discussion.

3.1. Syntheses and product characterization.

The gold nanoparticles obtained by citrate reduction and used as templates for the composite synthesis had an apparent diameter and dispersity of 27.0 ± 3.3 nm, as deduced from the optical spectrum [18, 19]. From the optical density at the $\lambda_{\text{max}}$, the solution was estimated to contain $6 \times 10^{11}$ particles per ml [21]. After oxidative polymerization and extraction [7], the PANI-COOH aqueous solution used for encapsulation was chromatographed by HPLC on a size exclusion column. As shown in Fig. 1, the chromatogram displayed a 92% polymer homogeneity with an apparent average molecular weight of 6-kDa (45-mer). The remaining 8% were constituted by an homogeneous polymer population with an apparent average molecular size of 15-kDa.

The encapsulation process was optimized by incubating the gold nanocolloid solution with a saturating amount of PANI-COOH at various pH values, and once the optimal pH selected, with increasing amounts of polymer at that optimal pH. In each case, the mixture was challenged with an equal volume of 1M NaCl so as to agglutinate the particles that were not stabilized by a polymer layer. Since the agglutination of the gold colloid by salt induces a red-shift in the plasmon resonance absorption band, the stabilization was evaluated by measuring the percentage shift of the mixture absorption band versus the $\lambda_{\text{max}}$ of the original gold nanocolloid solution ($\Delta \lambda_{\text{max}}/\lambda_{\text{max}} \%$). The gold nanocolloid was considered stabilized with PANI-COOH when that shift was minimal. As shown in Fig. 2, the optimization of the encapsulation process allowed to select pH 2.5-3 and a minimal volume of 10 µl of PANI-COOH per ml of gold nanocolloid as the best conditions for stabilizing the gold nanoparticles with a layer of conductive polymer. Based on these conditions, the encapsulation process was scaled-up to a larger volume (100 ml) and the resulting composite nanoparticle solution was buffered at pH 9 (50 mM borate). After buffering, no agglutination of colloidal gold could be observed, indicating that the colloid was stabilized by polymeric encapsulation. In order to further verify that PANI-COOH was adsorbed on the gold nanoparticles, we chromatographed the nanocomposite solution on a gel permeation column and as shown on Fig. 3, most of the material eluted in the void volume as an homogeneous peak, a small fraction of free PANI-COOH eluting before the total volume of the column. In order to avoid any interaction between the particles and the gel, we included 5mM of the anionic detergent sodium dodecylsulfate in the eluting buffer [22]. The comparative UV-vis. spectra displayed in the insert of Fig. 3 show that the composite nanomaterial was
characterized by a single absorption maximum (532 nm) closer to that of the original gold colloid (522 nm, data not shown) than of the free PANI-COOH solution (573 nm), further indicating a successful encapsulation. The spectrum of the composite nanomaterial solution taken before chromatography displayed an identical profile. The stability of the nanocomposite was verified by recording a spectrum four months after synthesis, which was superimposable to the original record.

From the optical absorption characteristics [18, 19], the size of the composite nanoparticles was estimated to be of 62.6 ± 10.2 nm (apparent diameter and dispersity). With the aim to gain an idea of how the polymer might orient on the nanoparticles, we constructed and optimized in silico a 45-mer chain of PANI-COOH. From its geometric characteristics, we inferred that the average gold nanoparticle (27 nm) surrounded by a single layer of head-attached linear polymer chains would generate a composite nanoparticle with a diameter of 53.7 nm, close to the size of the smallest particles apparently present in the solution (52.6 nm). The same average gold nanoparticle surrounded by two such polymeric layers would generate a composite nanoparticle with a diameter of 80.4 nm, close to the size of the largest particles apparently present in the composite solution (73.1 nm). Consequently, these data suggest that the composite nanoparticles of average size are covered by a single layer of head-attached linear PANI-COOH chains in which a second layer of such chains is possibly semi-embedded.

3.2. Comparative photonic sensitivity to pH and oxido-reduction of the polymer and nanocomposite solutions.

Like poly(aniline) films [23, 24], the optical spectrum of water-soluble PANI-COOH changes dramatically as a function of pH [1, 7, 18]. A progressive protonation of the water-soluble polymer induces a progressive decrease in intensity of the 310 nm absorption band corresponding to the π-π* transition, and of the 560 nm band corresponding to the emeraldine base. Concurrently, new absorption bands appear at 350, 670 and 800 nm of which the intensity increases with a decreasing pH, corresponding respectively to the formation by progressive protonation of the charged polaronic and bipolaronic states of the doped emeraldine salts and pernigraniline.

In the first instance, we compared the optical reactivity to pH of the composite with that of a PANI-COOH solution, in the same buffer and at the same concentration as that present in the composite. The UV-vis spectra of both polymer and composite solutions displayed identical transitions upon titration from pH 11 to 1.5. However, except for the progressive decrease in absorbance at 310 nm that occurs with protonation, the extent of which was superimposable for both materials, significant quantitative differences were observed at the other typical wavelength transitions. These are displayed in Fig. 4. Firstly, the absorbances corresponding to the doped polarons that appear in acidic conditions (350 and 670 nm; Fig. 4 A and C) were more intense in the composite than in the polymer alone by 1.5 and 2 fold, respectively. Secondly, and most importantly, although the absorbance at 800 nm, which is indicative of the appearance of the conducting charged bipolaron at acid pH, is nearly inexistent in the polymer solution, it was close to 8 fold more intense in the composite colloid material (Fig. 4D). The presence of more intense intraband absorptions in the composite should be expected to be associated with a similarly more important decrease in the absorbance intensity of the
emeraldine base species at 560 nm. Whilst this seems to be the case from pH 11 to 5, the trend is seriously dampened and reversed at lower pH values (Fig. 4B). Such data are likely to indicate the occurrence of charge transfer mechanisms between the surface plasmons of gold and the π electrons of emeraldine base [25], which seem to facilitate its further oxidation in the polaronic emeraldine salts and pernigraniline species [26].

Another interesting feature of water-soluble PANI-COOH, which is also shared with insoluble poly(aniline), is the photonic sensitivity of the material to oxido-reduction. Both protonation and changes in oxidation states of emeraldine salts give rise to marked transitions in the optical spectrum [27]. That property was recently applied in a highly sensitive assay for ascorbic acid, using a poly(aniline) film deposited in microtiter plates [20]. When compared to currently available analytical techniques, this new assay presents several marked advantages because it uses smaller sample volumes, displays a lower detection limit and proves reproducible in an extended range of analyte concentrations. In order to compare the redox sensitivity of the composite nanomaterial to that of PANI-COOH, we incubated at different pH values both materials with increasing concentrations of ascorbic acid (0.9 to 500 mg/L) and recorded the difference spectra versus the mixture devoid of reductant. Whatever the pH, both materials reported their reduction by a progressive decrease in absorbance at 600 nm. In agreement with previous results [20], we observed the strongest changes in the 600 nm absorbance intensity for both materials at pH values below 4. The composite nanomaterial displayed however a stronger optical reactivity to reduction than water-soluble PANI-COOH. With an aim to further document the difference, we designed a simple assay for ascorbic acid in solution, based on the previously described procedure, using the reagents in aqueous solution rather than as a solid film. In presence of ascorbic acid, the reproducibility of the optical measurements was rather poor with PANI-COOH solution (Fig. 5), most probably because of its progressive loss of solubility at such low pH values. In contrast, the optical response of the composite nanomaterial to reduction with ascorbic acid was highly reproducible and the dose-response curve was much steeper (Fig. 5). With PANI-COOH, the absorbance change for a 20 mg/l ascorbic acid dose (0.076 AU) was comparable to that observed in the microtiter plate assay (0.118 AU) [20]. For the same analyte dose, the response was however much higher (0.22 AU). We further compared the least detectable doses (LDD, i.e. dose measured at 3 S.D. of the zero response) of each system. The calculated LDD was 3.39 mg/l with PANI-COOH, close to the 1 mg/l detection limit of the microtiter plate assay [20]. Comparatively, the composite was 60 times more sensitive with a LDD calculated at 0.057 mg/l. Such high redox sensitivity with good reproducibility and linearity in solution makes therefore the composite nanomaterial an excellent candidate for the further development of biosensing reagents requiring a level of redox sensitivity at the lower micromolar range. Examples of such possible applications are the measurement of the plasmatic antioxidant capacity and of superoxide radicals in cells [28, 29].

3.3 Comparative photonic reactivity toward refractive index changes of colloidal gold and composite nanoparticles in solution.

We then turned our interest to the LSPR reactivity as a function of increases in refractive index (RI) of the surrounding medium, which is the mechanism involved in biomolecular interaction sensing using colloidal gold [3-5]. The question remained to know as to whether the composite material would keep this reactivity, given the fact that
the colloidal gold nanoparticles are encapsulated by conductive polymer layers. Indeed, the optical spectra of poly(aniline) do display neither transitions nor absorbance changes when the RI of the surrounding medium is increased by the addition of various polyols [30]. Although gold nanoparticles capped with proteins retain this reactivity [3-5], it was not obvious that nanoparticles encapsulated by PANI-COOH would. In order to answer this key question, we mixed solutions of either colloidal gold or composite material nanoparticles, each respectively containing the same nanoparticle concentration, with twice their volume of water containing increasing concentrations of glycerol so as to increase progressively the RI of the surrounding medium by over 0.1 unit, from 1.33 (water) to 1.45 (66.7% glycerol final). Since gold nanoparticles are unstable in presence of salts and in order to avoid any influence of a pH-buffering effect in the composite material, a new composite synthesis was made for this experiment and we used the aqueous solution of composite nanoparticles in absence of any buffer. After mixing, the difference spectra of these solutions were recorded versus the aqueous mixture devoid of glycerol. Fig. 6 compares the difference spectra recorded from 340 to 900 nm with the nanogold sol (part A) and with the composite nanoparticles solution (part B), in presence of increasing glycerol concentrations. For the sake of clarity, the same absorbance scales have been kept in both graphs so as to enable the comparison.

As expected [2], increasing the RI of the gold nanoparticle environment with glycerol (Fig. 6A) induced a progressive red-shift of the original LSPR absorption band (525 nm), reflected by progressive increases in absorbance intensity at longer wavelengths (530-550 nm). At the highest glycerol concentration however, the 700 nm peak was likely to result from particle aggregation. The progressive LSPR peak shift was linearly related to RI increases (Fig. 6A insert) by 1.7 nm/0.01 RI units. Transitions in the same wavelength range were observed with the composite nanomaterial (Fig. 6B), but no systematic red-shift in the LSPR absorption band could be observed with increasing RI (Fig. 6B insert). Notwithstanding the absence of LSPR peak shift in the composite spectrum, a progressive linear increase in absorbance intensity could be observed at the $\lambda_{\text{max}}$ (532 nm) with increasing RI. The increase in LSPR peak absorbance intensity with RI changes was fourfold greater with the composite when compared to gold nanoparticles. This observation could suggest a progressive desorption of the polymer from gold nanoparticles as induced by increasing glycerol concentrations. However, such interpretation can be refuted by the following arguments. Firstly, a desorption of the polymer from the nanoparticles would induce the gold particles to aggregate at highest glycerol concentration and hence the difference spectrum to produce the intense 700 nm band observed with the naked gold (Fig. 6A). This is not observed with the composite (Fig. 6B). Secondly, adsorbing the polymer to the gold nanoparticles results in a 1.5 fold increase in absorbance intensity at the LSPR wavelength (532 nm, data not shown). Hence, polymer desorption would accordingly result in a decrease, not in an increase in absorbance intensity at this wavelength. These results further support the occurrence of charge transfer mechanisms from gold surface plasmons to the $\pi$ polymer electrons, which are likely to compensate for the changes in the dielectric constant of the surrounding medium that occur with increasing glycerol concentrations. These effects probably explain the much greater increases in photonic absorption at the LSPR band of the composite when compared to gold, possibly masking the wavelength red-shifts of gold in the composite spectrum. Most remarkable however, was the appearance of a
tremendous absorbance at 350nm, the intensity of which increased linearly with RI changes. As shown in Table 1., the absorbance changes per increase in RI unit were several fold greater for the composite when compared to the gold colloid. The gain in optical sensitivity of the composite versus gold toward RI increases ranged from 11 to 1.6 fold in the 500-700nm wavelength range, i.e. where normal LSPR transitions occur with gold, but rose up close to 200 fold at 350nm, a wavelength where gold nanocolloids hardly respond to RI changes.

The fact that this increasing new absorption band occurs at 350nm is particularly interesting. This is indeed the wavelength corresponding to the polaronic transition of PANI-COOH that develops with protonation (see Fig. 4A). However, during the pH titration, this transition occurred at pH values lower than 6, with a maximal optical difference due to the resonance behavior of the polarons occurring near pH 4. In our RI experiments, the composite-glycerol mixtures were characterized by pH values of 7-8, a pH range where the protonation of the polymer is too low for such a transition to occur. In order to check if the polaronic transition of PANI-COOH present at the gold nanoparticle surface in the composite were actually occurring with RI increases, despite an unfavorable pH and hence absence of charge transfers due to protonation, we subtracted the respective spectra of the neat gold nanocolloid recorded in presence of increasing glycerol concentrations from the spectra of the composite nanoparticles recorded in the same conditions. We reasoned that such subtraction would display directly the spectral transitions of PANI-COOH that were supposed to occur on the gold surface with RI increases. Accordingly, if the 350 nm polaronic transition were occurring with RI changes, it should be matched by a decrease in the 560 nm absorbance of the emeraldine base, such as during pH titration (see Fig. 4 A and B). In contrast to the 350 nm transition, the 560 nm transition could not be observed directly in the composite spectra because of the increasing absorbance at this wavelength of the gold nanocolloid LSPR peak tail induced by RI increases. As shown in Fig. 7, the increase in absorbance intensity at 350 nm that occurs with RI increases and calculated by the spectral subtraction operations (Fig. 7, triangles) matched quite fairly the increases in absorbance intensity at the same wavelength (Fig. 7, circles) measured from the experimental difference spectra of the composite nanocolloid shown in Fig. 6B. The spectral subtractions further permitted to identify the 560 nm emeraldine base peak, the absorbance intensity of which decreased progressively (Fig. 7, squares) with increasing RI, in parallel to the increase in absorbance intensity at 350 nm and in a way similar to what occurred during pH titration (compare with Fig. 4B). Although the changes in absorbance measured with RI increases were much greater than what was observed during pH titration, the absolute ratio between the highest increases at 350 nm and the corresponding decreases at 560 nm were of the same order of magnitude, respectively 2.55 for RI experiments and 2.67 for pH. Thus, the 350 nm transition that occurs in the composite nanoparticles with increasing the RI of the surrounding medium results most probably as suggested earlier, from the polaronic transition of the PANI-COOH emeraldine base to emeraldine salt as the result of charge transfers at the gold-polymer interface. The phenomenon is thus likely to result specifically from a resonant absorption enhancement by the particles induced by RI changes, due to the polaronic transition of PANI-COOH at this wavelength. Interestingly, a similar phenomenon has been observed with colloidal gold or silver nanoparticles coated with human serum albumin and
deposited on a quartz substrate upon binding to an anti-albumin antibody [31]. Beside the expected red-shifts of the respective LSPR absorption bands, the authors observed also the appearance of a new strong absorption band at 280nm that they attributed to a transition in the aromatic aminoacids of the proteins. They concluded that the increase in RI occurring at the particle surface upon binding of the anti-albumin was resonant of the aminoacids and increased the absorption probability of the nanoparticles at this excitonic light frequency. We can argue similarly in the present case, that the increases in RI dielectrics at the nanocomposite surface are resonant of the polarons of PANI-COOH emeraldine salt, which confers this new resonance frequency to the composite material.

The enhancement in photonic reactivity to pH changes and redox processes of the composite over the plain conductive polymer, already constitutes an interesting advantage for the development of sensing platforms to be used with biological samples and requiring high sensitivity, which currently requires the use of sophisticated instrumentation [28, 29]. However, the new and tremendously enhanced photonic reactivity of the composite material to RI increases when compared to a plain gold nanocolloid offers a lot of prospects for its application in surface plasmon resonance sensing. The current level of sensitivity with gold or silver colloids reaches in the best cases the pM level with proteins and the nM with small ligands [2-6]. A 200 fold increase in the sensor optical reactivity would therefore allow to increase the sensitivity accordingly and hence achieve, with a common spectrophotometer, sensitivity levels which currently require costly reagents and more sophisticated instruments [32]. Moreover, matching the optical transitions of the noble metal with those of the conductive polymer paves the way to other possible combinations. As an example, a polythiophene derivative absorbing around 350-380 nm could be used to encapsulate a colloidal silver nanoparticle core with a LSPR band around 400nm. Such combinatorial sensing could allow to multiplex various reactions in a single microtiter plate or microarray, increasing significantly assay throughput, a current need in both diagnostics and pharmaceutical laboratories. Moreover, because of optical sensitivities to different changes in the environment gathered in a single nanoparticle, this kind of reagent offers in our opinion, a huge potential for in vitro biopharmaceutical (ADME-Tox) studies in a high throughput format, a currently nearly desperate need in the pharmaceutical industry [33].

Summary

We report the simple and successful synthesis of a composite nanoparticulate colloid made of a gold core encapsulated by PANI-COOH. Matching the optical properties of the individual materials included in the composite permits the charge transfers that occur between them upon light absorption to increase tremendously the photonic reactivity of the new nanomaterial toward various environmental stimuli such as pH, redox or RI changes. The fact that the composite nanocolloid remains stable in aqueous solution further allows for the development of applications in biological sciences.
References

Table 1. Comparison of the extent of absorbance changes per increase in refractive index (RI) unit at typical wavelengths between gold and composite nanocolloid solutions. The absolute gain in signal change between the composite nanomaterial and gold nanoparticles at each wavelength is given in the right-hand side column.

<table>
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<th>Wavelength, nm</th>
<th>Absorbance Change per RI unit increase</th>
<th>Absolute fold increase in signal change for the composite vs gold</th>
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<td>Gold nanocolloid</td>
<td>Composite nanocolloid</td>
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Fig. 1. Size-exclusion HPLC profile of the PANI-COOH solution used for the composite nanomaterial synthesis. The major peak corresponds to a 6-kDa polymer (second peak, 92%; 45-mer) whilst the minor peak corresponds to a 15-kDa polymer (first peak, 8%; 112-mer). The arrows indicate respectively the elution time of bovine serum albumin (BSA) and the column total volume (VT).
Fig. 2. Optimization of the encapsulating conditions. This was evaluated by measuring the $\lambda_{\text{max}}$ shift ($\Delta \lambda_{\text{max}}/\lambda_{\text{max}} \%$) of the gold nanocolloid spectrum after incubation in presence of either a saturating amount of PANI-COOH at various pH values (closed triangles, upper abscissa), or at the optimal pH value with increasing PANI-COOH volumes (closed triangles, lower abscissa) respectively, after dilution with NaCl 1M so as to agglutinate the unstabilized nanoparticles.
Fig. 3. Gel permeation chromatography of the composite nanomaterial on a Biogel P10 column. Elution was monitored at 520 nm (solid line) and 540 nm (dotted line). Most of the material eluted in the void volume fraction as an homogeneous peak. Insert: UV-vis. spectra of the collected peak fractions.
Fig. 4. Differences in absorbance changes at typical wavelengths (A: 350 nm; B: 560 nm; C: 670 nm and D: 800 nm) observed with solutions of PANI-COOH (closed triangles) and of the nanocomposite colloid (closed circles) upon pH titration from pH 11 to 1.5.
**Fig. 5.** Dose-response curves observed respectively with PANI-COOH (open circles) or colloidal nanocomposite (closed circles) solutions after reaction with increasing doses of ascorbic acid. Data are averages ± S.D. of triplicate measurements.
Fig. 6. Difference UV-vis. spectra of gold (part A) and composite (part B) nanocolloid solutions as observed when the refractive index (RI) was increased from 1.33 (water, reference cell) to 1.45 by progressive addition of glycerol. Inserts: relationships between the respective LSPR peak wavelength shifts (nm) and RI of the surrounding medium.
Fig. 7. Evolution of absorbance changes at 350 nm and 560 nm of the composite nanocolloid spectra as a function of the refractive index. The absorbance changes were respectively inferred either from the experimental difference spectra shown in Fig. 6B (350 nm, closed circles) or calculated from the composite nanocolloid spectra after subtraction of the gold nanocolloid spectra recorded in the same conditions (350 nm, closed circles; 560 nm; closed squares).