Synthesis, Purification, and Characterization of Negatively Charged Gold Nanoparticles for Cation Sensing

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S Supporting Information

ABSTRACT: In recent years, nanotechnology has been one of the major subjects of scientific and technological research. Currently, several applications of nanotechnologies are already available on the market. Particularly relevant are the fields of new materials and sensors, which have excellent potential future applications in the biomedical field. This paper describes a project in which the students were challenged to investigate the properties of gold nanoparticles they synthesized themselves. The activity, suitable for students with good chemical knowledge (last year of high school), is divided into three parts, each taking 2 h. In the first part, gold nanoparticles are synthesized and functionalized. In the second, students purify the sample and analyze its optical properties, focusing on the noncovalent interaction with metallic ions. Last (part three), the students realize a chemosensor for cations using the nanoparticles synthesized. At the end of the project, students use the sensing system they had set up to analyze an unknown sample containing bivalent or monovalent metal cations. The proposed activity turned out to be strongly motivating for the students involved and definitely improved their knowledge in the nanomaterials field. Different analytical techniques, such as UV−vis spectrometry, GPC, and TGA, were used, and consequently, both the understanding and the ability to use them were reinforced.

KEYWORDS: High School/Introductory Chemistry, Analytical Chemistry, Inquiry-Based/Discovery Learning, Nanotechnology, Qualitative Analysis, Surface Science, UV−Vis Spectroscopy
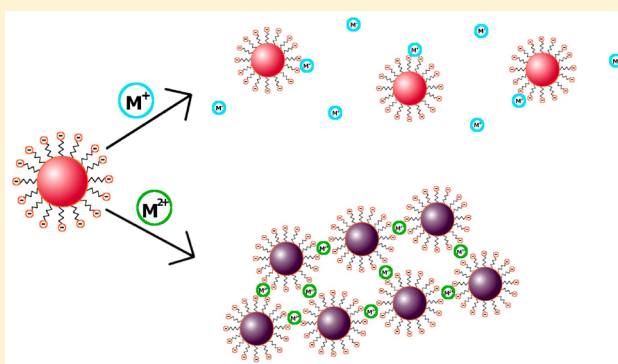
INTRODUCTION

Nanotechnologies are currently a leading research area. Yet, examples of teaching lab projects aimed to introduce the students to the basic concepts related to nanotechnology are quite scarce. This is particularly true when it comes to focusing on projects capable of dealing not only with the synthesis but also with the application of nanosystems. In this regard, passivated gold nanoparticles (PGNPs) are very interesting and versatile systems.[1] They are made up of a core made of metallic gold, with a diameter in the range between 1 and 100 nm, coated with a single layer of organic molecules, usually thiols. When larger than 3 nm, the gold core features strong light absorption at about 520 nm, called plasmonic absorption, whose intensity and wavelength depend on the size of the nanoparticles.[1] Consequently, the gold core is a scaffold to assemble the organic monolayer and also a chromophore.

Over the years, PGNPs covered with the most diverse functional groups have been synthesized and studied.[2] For example, crown ethers,[3] mono- and polysaccharides,[4] nucleic acids,[5,6] peptides,[7,8] chromophores,[9] catalysts,[10,11] and charged headgroups have been used[12−15] (Figure 1). One of the reasons that prompted the realization of such a diverse variety of structures is the observation that the coating layer has a

micelle-like structure (Figure 2). As a consequence, PGNPs can interact with chemical species in solution, as micelles do, by exploiting multiple noncovalent interactions. Moreover, PGNPs add to the self-organized nature, typical of both micelles and PNGPs, the advantages of better stability and versatility. The interactions that can be established between the organic monolayer and the guest cover all the most common ones such as electrostatic interactions, hydrogen bonds, hydrophobic interactions, and metal coordination. In particular, nanoparticles coated with charged molecules have shown different interesting properties and applications. Indeed, thanks to their polyionic nature, they can bind ions with the opposite charge with very high affinities.[16]

The interaction of the monolayer with chemical species in solution has made possible the development of several chemosensors.[12,17] The analytes can be detected by different working modes, which include the modification of the nanoparticle optical properties upon the binding with the analyte, the displacement of a fluorescent dye from the
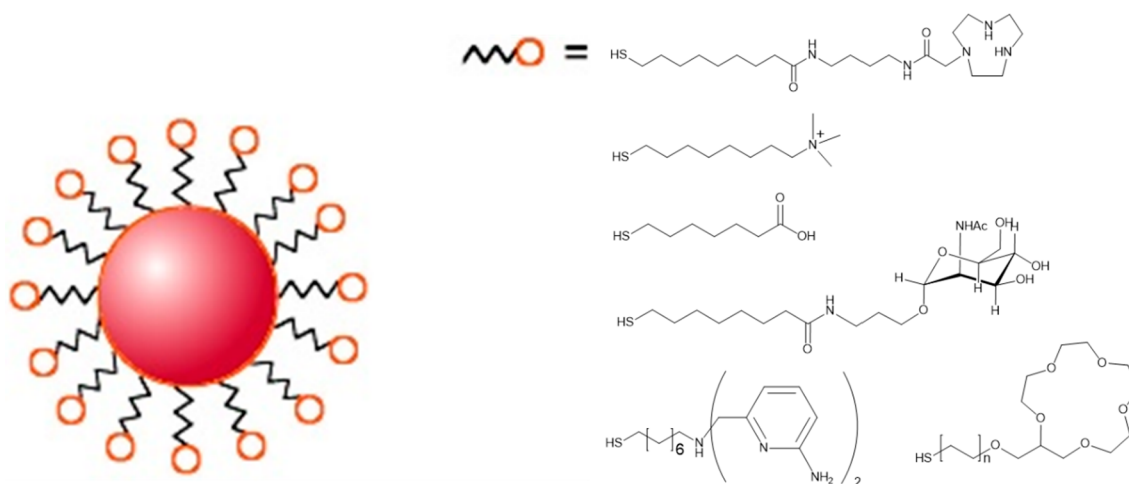
Figure 1. Some types of thiols with which it is possible to passivate the surface of a nanoparticle.
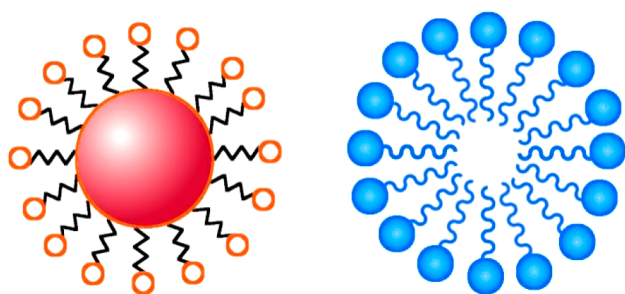

Figure 2. Structure of a passivated nanoparticle and a micelle.

monolayer, with the consequent restoration of its emission, or the color change caused by nanoparticle aggregation (Figure 3).[18]

This last method, in particular, is very sensitive, and it can be used for naked-eye detection. The phenomenon and its origin are well-described in the literature.[19,20] Nanoparticle aggregation can be caused by the decrease of the colloidal stabilization or by the presence of cross-linking species capable of interconnecting the particles either by covalent or noncovalent bonds. Two main morphologies of the aggregates are possible: necklace-like aggregates and globular close-packed aggregates (Figure 4).[21,22] Several parameters, including the solvent, the chemical nature of the passivating molecules, and the presence of salts, influence the aggregate morphology. When the distance between the metal cores of the nanoparticles is closer than their diameter, the plasmonic absorption changes. Such changes are influenced by the morphology of the super-

structures formed; in particular, the necklace-like structure shows a new plasmon resonance band at 650−750 nm. In contrast, the globular structure undergoes only a small shift to 550−570 nm.

The realization of colorimetric sensing systems based on the aggregation of PGNPs fits well the development of teaching laboratory projects for students. Nicely enough, in addition to the application of nanoparticles to a relevant chemical problem, such projects can also nicely include the synthesis of PGNPs.

The most diffused synthetic protocols involve the chemical reduction of Au(III) to metallic gold in solution and possibly the formation a stable coating layer made of thiols or other groups with a similar affinity for gold. The two processes can be simultaneous or separated, and the reaction can be performed in a single phase or in a two-phase solvent system. The structure of the coating thiols is usually linear, featuring a thiol functional group, an alkyl spacer, and an appropriate headgroup, which affects both the solubility of the nanoparticle and their recognition abilities. Many thiols usable for nanoparticle coating are currently commercially available, and consequently, the synthesis of MPGNs is suitable for a teaching laboratory. It has already been proposed over the years in different variants.[23−25]

In this paper, we describe how a group of fifth-year high school students synthesized, purified, and applied a simple PGNPs-based chemosensor to reveal the presence of bivalent ions. The chosen coating molecule was 12-mercaptododecyl-phosphonic acid (**I**), which is commercially available and
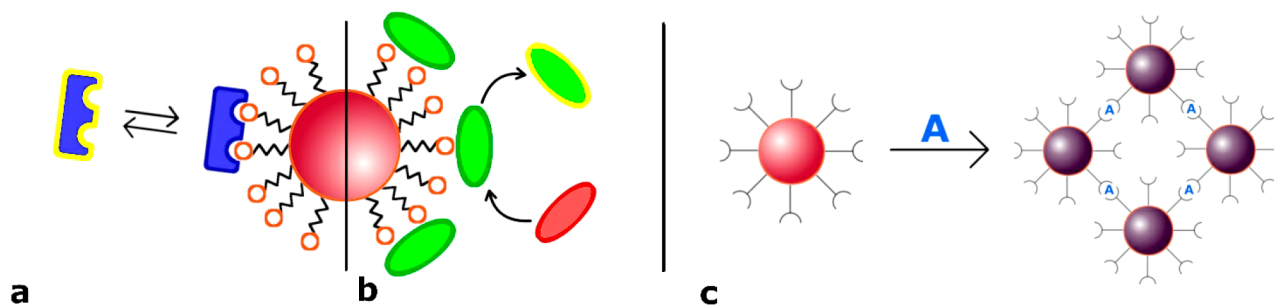

Figure 3. Representation of the process of interaction of chemical species with the monolayer (a, b) and the aggregation of nanoparticles by a generic analyte A (c)

Figure 4. Representation of the structures of the aggregates: necklace (a) and close-packed (b).

contains a phosphonate group that features two negative charges at neutral pH.

I

The students used the nanoparticles prepared for investigating the presence of divalent cations in unknown samples by the color change arising from target-induced nanoparticle aggregation. This allowed them to acquire familiarity with nanotechnologies and chemosensors.

At the same time, the "learning by doing" approach encouraged them to increase their interest and understanding of the research approach, both by satisfying their curiosity and by dealing autonomously with challenging problems such as the determination of surface, volume, concentration, and the average composition of PGNPs.

This activity can provide relevant benefits to the education of the students, both by enhancing their interest and by allowing them to come in contact with the relatively new field of nanotechnologies.[26] This can influence their career decisions and increase the attractiveness of university science programs.

■ EXPERIMENTAL OVERVIEW

The overall procedure has been tested with two classes of 22 students each. The experiment was proposed to students with a basic knowledge of chemistry and with good manual skills. In our case, the activity could be performed in a high school since this is a chemistry-oriented technical school where fifth-year students have already attended four years of chemistry courses including general chemistry, organic chemistry, industrial chemistry, and analytical chemistry.

The students were divided into groups of two, and they performed the experiment following accurate instructions (see detailed procedure in the Supporting Information). The presence of a laboratory assistant was also necessary. The material needed included basic glassware, a rotatory evaporator, and a UV–vis spectrometer. For additional analysis and characterizations on the product synthesized, instruments such as TEM and TGA (see the advice for teachers in the Supporting Information) can be used but are not indispensable. The activity was organized in three experimental sessions of 2 h each. Each session occurred on a different day, usually distributed through a week. A 1 h preparation session for cleaning and rinsing glassware and performing calculations is recommended. At the end of each session, the students gathered all the data and analyzed them in order to be able to answer the teacher questions (see the final questions in the Supporting Information).

■ EXPERIMENTAL PROCEDURES

Materials

The materials needed for the experiment are usually included in the standard equipment of any general chemistry laboratory (glassware, micropipettes, analytical balance, etc.). Tetrachloroauric acid ($HAuCl_4 \cdot 3H_2O$), trisodium citrate, sodium borohydride, Sephadex G-25, 12-mercaptododecylphosphonic acid, and the inorganic nitrate salts used were all obtained from

Scheme 1. Process of Synthesis and Layering of Gold Nanoparticles

Sigma-Aldrich. All the procedures were carried out with bidistilled or Milli-Q ultrapure water.

Synthesis of PGNPs

The synthesis of PGNPs was performed by chemical reduction in water, according to a modified Turkevich protocol,[27] using tetrachloroauric acid as source of Au(III) ions, $NaBH_4$ diluted solution as reducing agent, and trisodium citrate as surface stabilizer. Thiol monolayer formation simply occurred by the addition of the commercial thiol **I** to the nanoparticles solution. It is not necessary to work in an oxygen-free atmosphere since the nanoparticles are passivated immediately after the synthesis (Scheme 1).

Purification of the Sample

The second part of the experiment was the purification of PGNPs. Once passivated with **I**, the PGNPs are quite stable, and this procedure can be carried out on a day different from that of the synthesis. In our case, this was usually a week later. The nanoparticles' crude suspension obtained in the previous step was evaporated under vacuum, using a rotatory evaporator, up to a volume of about 5 mL. Then, column gel permeation chromatography (GPC), using a Sephadex resin, was performed. After conditioning the stationary phase in a beaker using water, the gel was poured into a column (diameter 2 cm) until it reached a height of approximately 20 cm. The nanoparticles' crude suspension was added and then eluted by gravity with a flow of about 5 mL/min. Fractions containing the purified nanoparticles were collected in a vial (see the purification of the sample in the Supporting Information). The nanoparticles could be easily detected during elution due to their intense reddish coloration. The obtained PNGP colloidal suspension is stable at room temperature for some months.

Characterization

The determination of the size of nanoparticles synthesized can be performed with UV−vis spectrometry, since the position and intensity of the plasmonic band are directly correlated to the diameter of PGNPs.[27−29] A calibration plot was provided to the students (see Supporting Information) to help them to estimate the size of the gold core. Further analysis (optional) was carried out through electron microscopy (TEM) and thermogravimetric analysis (TGA). The concentration of the solution was determined by its absorbance at the maximum absorption wavelength, using the molar absorption coefficient (ε)[27] provided. Using the measured diameter of the nanoparticles, the density of metallic gold, and the typical superficial density of the organic layer,[30] the students could estimate the average composition of nanoparticles (see the determination of dimension and concentration in the Supporting Information).

Development of the Sensor and Unknown Test

In order to test the aggregation of nanoparticles in the presence of metallic cations, two simple experiments were carried out. First, different amounts of different cation solutions were added to a small aliquot of the nanoparticle suspension, in order to determine the minimal analyte amount necessary to induce the nanoparticle aggregates. The aggregation could be easily detected since the color of the samples turned immediately from red to violet.

The second series of experiments consisted of the comparison of the color of different nanoparticle solutions after the addition of different cations (Na^+, K^+, Li^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Pb^{2+}, Al^{3+}) at the same concentration. Using the

information obtained by this experiment, students could determine the nature (i.e., whether it was singly or doubly charged) of the cation contained in an unknown sample.

■ HAZARDS

During all the phases of the experiment, students must wear lab coats, protective glasses, and gloves. The sodium borohydride solution is toxic by ingestion and corrosive. $HAuCl_4$ is corrosive and hygroscopic. Other diluted solutions used during the experiment are not dangerous; however, any contact with these substances must be avoided.

■ RESULTS

Sample Analysis

Solutions containing metallic nanoparticles have different colors from the bulk material. Particularly, solutions of gold nanoparticles with a diameter from 5 to 20 nm have a red color caused by a strong absorption around 520 nm, called plasmonic absorption. This effect is a consequence of the confinement of the valence electron in a reduced space, which allows them to oscillate in resonance with the electromagnetic radiation. The position and the intensity of the plasmonic band do not depend only on the dimension of the nanosystem, but also on the type of material and the shape and the presence of aggregation. In particular, the aggregation causes a broadening of the plasmonic band and its shift toward larger wavelengths. Figure 5 reports the UV−vis spectra of a nanoparticles sample
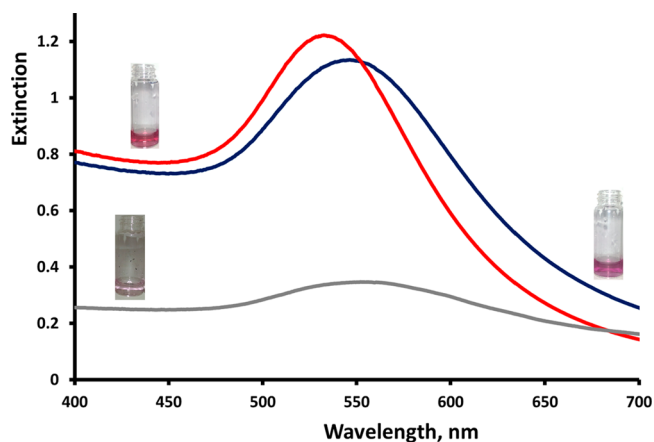
Figure 5. UV−vis spectra of a solution of PGNPs (red) and with the presence of Mg(II) ions (blue 0.50 mM, gray 5.0 mM). [PGNPs] = 75 nM.

in the absence and presence of Mg(II) ions. The position and shape of the nonaggregated nanoparticle plasmonic band (red) allowed estimation of the average size of PGNPs, which was found to be about 6 nm. After the addition of Mg(II), the shift of the plasmonic band from 535 to 550 nm was observed, with the consequent change of color of the samples from red to violet, detectable by the naked eye. This shift is compatible with the formation of globular aggregates. At large concentrations of metallic ions, we could observe the precipitation of PGNP ion aggregates in the form of a black powder in a few minutes. This behavior is reported as well as the investigation of its kinetics.[31] By the addition of ethylenediaminetetraacetic acid (EDTA), it was possible to break up the aggregates, restoring the initial nanoparticles' coloration.
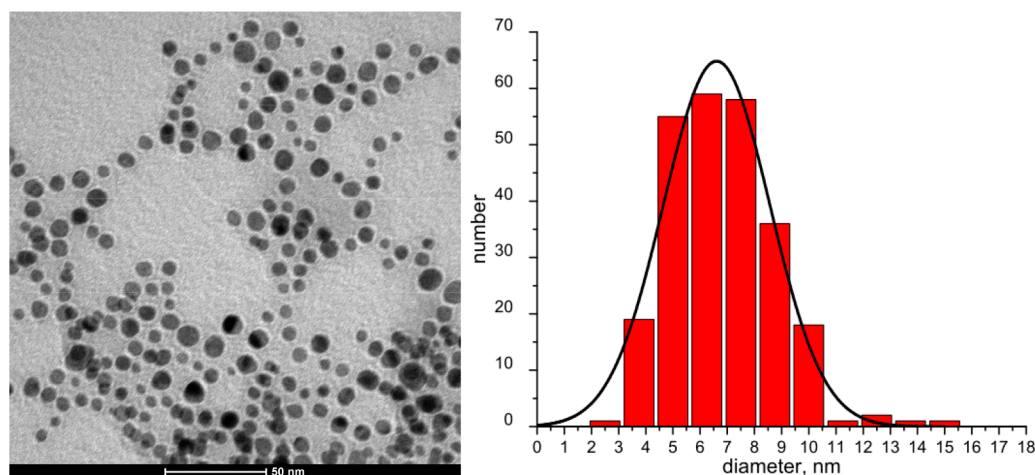
Figure 6. TEM image of the PGNPs synthesized by the students and size distribution.

The analysis of the sample with transmission electron microscopy (TEM) and thermogravimetric analysis (TGA) confirmed the dimensions estimated with UV−vis spectroscopy (Figures 6 and 7). Indeed, the average diameter obtained by TEM analysis was 6.6 nm with a standard deviation of 1.8 nm.
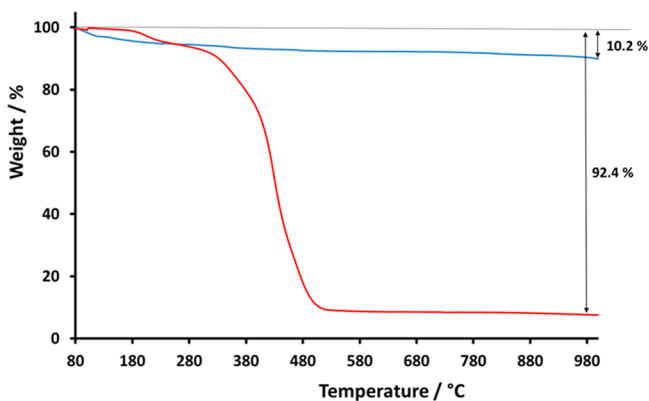

Figure 7. Thermogravimetric analysis of the obtained nanoparticles (blue) and thiol **I** (red).

The thermogravimetric analysis is a useful technique to confirm the dimension of the nanoparticles synthesized and to investigate their organic content. If the instrument is available, the laboratory experiment will be more complete and interesting for the students. If the sample is heated at 1000 °C, the analysis will provide the residual weight of the inorganic portion, allowing the calculation of the pondered ratio of the organic and inorganic parts. Considering the molar weight of gold (196.97 g mol^{-1}) and the molar weight of the coating thiol (282.38 g mol^{-1}), the theoretical molar ratio for nanoparticles with a diameter of 6.6 nm ($Au_{8880}I_{752}$) is 1.0:11.8.[30] The value calculated from the TGA results, with an organic content of 92.4% (Figure 6, red line), is 1.0:11.2 (Figure 7, blue line), in agreement with the theoretical data.

Aggregation of Nanoparticles

The first test performed was the study of the limiting concentrations of monovalent and bivalent cations inducing the aggregation of nanoparticles (Figures 8 and 9). Increasing amounts of metallic ions were added to different vials containing aliquots of a 75 nM nanoparticle solution, chosen
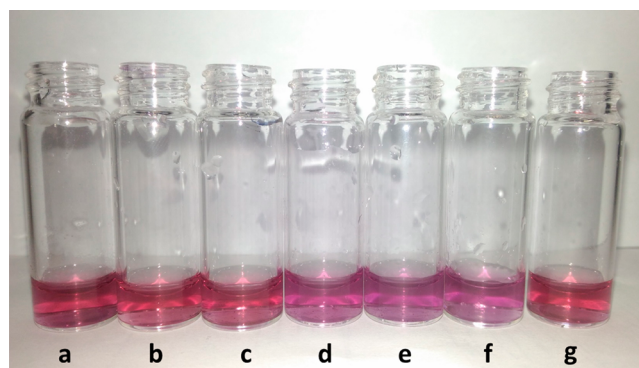

Figure 8. Nanoparticle solutions ([PGNPs] = 75 nM) in absence (g) and presence of Mg(II) ions: 2.0×10^{-6} M (a), 2.0×10^{-5} M (b), 2.0×10^{-4} M (c), 2.0×10^{-3} M (d), 2.0×10^{-2} M (e), 2.0×10^{-1} M (f).
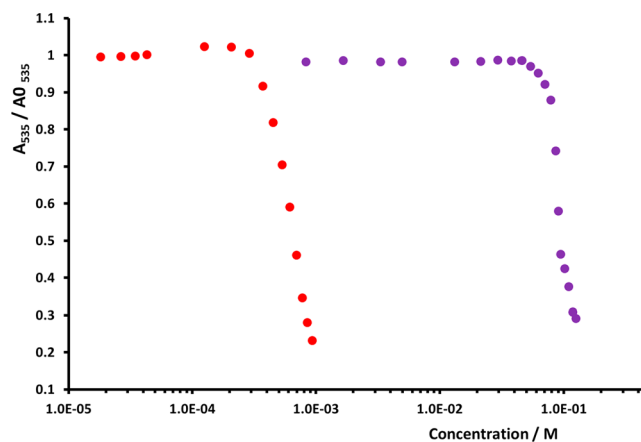

Figure 9. Normalized absorbance (with respect to the absorbance in the absence of analyte) at 535 nm of a PGNPs solution ([PGNPs] = 75 nM) as a function of the concentration of added $Mg(NO_3)_2$ (red) or $NaNO_3$ (violet).

as this corresponds to a highly visible red color. A change in color from red to violet was observed, as a consequence of the aggregation of PGNPs. After a few minutes, the solutions with higher concentrations of metallic ions precipitated forming a thin black powder (see the other considerations in the Supporting Information).

The substantial difference in behavior among the cations having different charges is highlighted in Figure 9, where the normalized absorbance at 535 nm is reported as a function of the concentration of magnesium and sodium ions. The difference of concentration necessary to induce the nanoparticles to aggregate increases by more than 2 orders of magnitude from Mg(II) to Na(I) (0.3 mM for Mg(II), 0.7 M for Na(I)).[32]

Figure 10 shows the effect of different ions, at 0.5 mM concentration, on the aggregation of the nanoparticles. At this
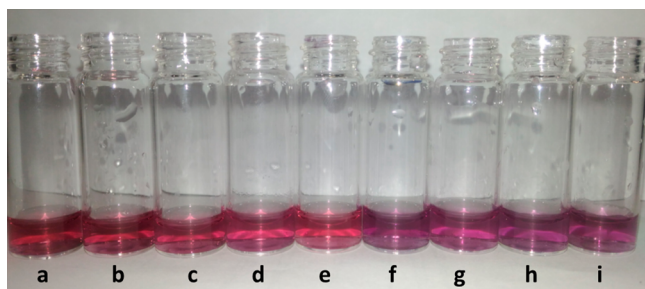
Figure 10. Nanoparticle solutions ([PGNPs] = 75 nM) with the respective presence of Na^+ (b), K^+ (c), Li^+ (d), Al^{3+} (e), Zn^{2+} (f), Ca^{2+} (g), Pb^{2+} (h), and Mg^{2+} (i) with the concentration 0.5 mM. Vial a is the blank.

concentration, only bivalent ions induce the color change of the solution. This is due to the greater interaction between the doubly charged cations and the negative polyanionic nanoparticles. The ion Al(III), with three negative charges, is not able to interact with the nanoparticles since at neutral pH it is mainly present in solution as negatively charged aluminates. Hence, the nanoparticles realized can effectively allow students to distinguish monovalent and divalent cations in water.

Unknown Assay

After the experiments were performed, the students were asked to use the nanoparticles to analyze an unknown sample of a nitrate salt of a monovalent of divalent cations. The samples to analyze were given to the students in a corked and numbered test tube. The students, following the instructions given to them, prepared a solution with a concentration about 8 g/L of the assigned salt and added 20 μL of this solution to 1.00 mL of a 75 nM nanoparticle suspension. Considering the different molar weight of the salts, the concentration of the different ions in the test samples varied from 0.5 to 2.5 mM. This did not influence the assay since the concentration's gap between the aggregation concentrations found for divalent and monovalent cations (Figure 9) is larger. Hence, by observing the occurrence of a change of the color of the solution, the students were asked to establish the nature of the cation, whether it is bivalent or monovalent.

◼ DISCUSSION

This experience, besides proving to be highly interesting for the students, touched different areas of chemistry and allowed the students to use the skills acquired in their courses to deal with a nonstandard problem.

First of all, a new recent area of chemistry was introduced, leading the students to synthesize monolayer protected nanoparticles. These are self-assembled hybrid systems whose synthesis and study involved concepts of chemical thermodynamics and inorganic and organic chemistry, but also of math

and geometry (as in the determination of the nanoparticle surface−volume ratio). The purification and characterization of the nanoparticles offered the possibility to appreciate novel uses of familiar techniques, such as UV−vis spectroscopy, and to approach new analytic techniques like GPC (gel permeation chromatography), TEM, and TGA (thermogravimetric analysis). Current educational projects are mainly focused on the applications of nanomaterials as produced, with scarce examples of complete purifications and characterizations.

Considering the laboratory activity, some students encountered a few difficulties at the beginning, mainly because of the unfamiliar procedures they had to use and because of the high level of attention required by the preliminary cleaning procedures. As a matter of fact, some groups did not succeed in their first synthesis attempt because of insufficient attention paid to the cleaning steps or, in one case, because they used the same pipet tip for different substances. However, this did not discouraged them; rather, they were more motivated than before to carry out the experiment, and finally, they succeeded. Unsuccessful first attempts and repetitions allowed the students to gain more confidence with the procedures and to understand the level of attention required by a complex synthetic procedure.

The groups obtained nanoparticle samples with similar average dimensions, ranging from 5 to 9 nm with an average diameter close to 7 nm. Differences are mainly due to different delays between the preparation of the starting solution for the synthesis and the addition of the reducing agent, and to the fact that the synthesis and monolayer assembly did not occur in degassed solutions and in inert atmosphere. However, this size dispersion turned out to be a bonus for the impact of the activity, allowing the groups to perform different size and concentration calculations (see the advice for teachers in the Supporting Information).

◼ CONCLUSION

The assigned questions, confirming that the activity proposed, managed to capture the students' attention, getting them to participate and focus on all the steps. In the end, despite some sporadic errors, they overall did a good job.

Detailed analysis of the tests results confirms the good outcome. The average number of correct answers was 74%; 14% were partially correct, and only 12% resulted totally wrong (Figure 11). The best results were obtained in questions 7 and
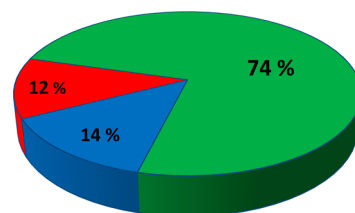
Figure 11. Overall outcome of the survey: correct answers (green), partially correct (blue), and wrong (red).

10 (see the advice for teachers in the Supporting Information), regarding the interpretation of the color changes that can be observed during the experiment. The questions with the higher percentage of wrong answers were 8 and 11, about the sensor behavior analysis with different ions and the need for the glassware washing method. The questions about the

calculation parts were solved mostly in a correct way, and the errors were on the measurement units.

As for the recognition of the charge nature of the unknown sample, only 2 out of 22 groups failed the test. This was probably caused again by incorrect glassware washing; in fact, in these cases a monovalent ion was confused with a divalent one.

Overall, the cost of the experience, for a class of 22 students, was around 68 € per student. This high cost can be amortized by repeating the experience more times, since several reagents are sold in larger quantities than needed so they would last for many experiences. As a matter of fact, when the cost is calculated on the quantities of chemicals actually used, it drops to 3.4 € per student.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.9b00179.

> Detailed procedure of the experiment for the students, including a list of chemicals and materials for the experiment, and hazards (PDF, DOCX)

> Other considerations, TGA analysis, students' results, evaluation rubric, expected answers, and results of students' assessments (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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