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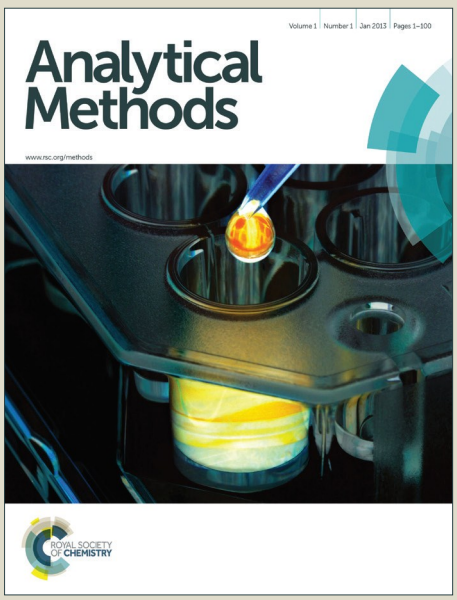

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A low-cost microcontrolled photometer with one color recognition sensor for selective detection of Pb^{2+} using gold nanoparticles

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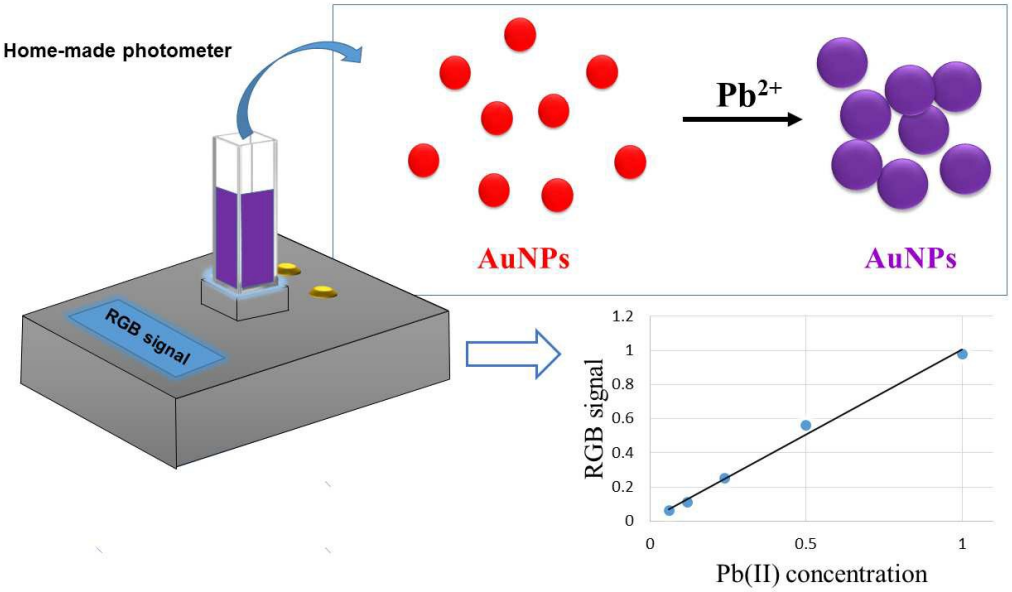

Abstract: The present work describes a microcontrolled photometer based on light-emitting-diodes (LED) for detection of Pb^{2+} using gold nanoparticles (AuNPs). The photometer makes use of a single LED as a light source, a sensor TCS230 (TAOS, USA) and an Arduino electronic card as acquisition system. On the sensor, the light from the three closely-adjoined red, green, and blue LED composing the "white" light source LED is contact-coupled to the map-illumination pointed toward the detection cell. To maintain a constant light intensity - a common white-color LED (emitting a 450–620 nm continuous spectrum) - was employed as a controllable light source. Software was written in C++ to control the photometer through a USB interface and for data acquisition. Pb(II) measurement is based on the AuNPs color change due to their aggregation provoked by Pb(II). The method showed excellent selectivity compared to other 19 metal ions (Ag^{+}, Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Hg^{+}, K^{+}, Li^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Sn^{2+}, Sr^{2+}, and Zn^{2+}). Pb^{2+} was detected with the photometer and also monitored via UV-Vis. Solutions containing Pb^{2+} in the concentration range from 0.6 to 10 mmol L^{-1} were employed to constructed the analytical curves, proving limits of detection (LOD) of 0.89 mmol L^{-1}. The sensitivity was compared to those obtained with a UV-Vis spectrophotometer at 520 nm. A repeatability of 4.11 % (expressed as the relative standard deviation of 10 measurements) was obtained. The proposed method was successfully applied to detect Pb^{2+} in spiked water samples.

Key-Words: Photometer; sensor TCS230; Pb^{2+}; Gold nanoparticles;

Introduction

Many portable instruments have been made possible by the use of clever designs incorporating smaller components such as optical fibers,[1] light emitting diodes (LED),[2] liquid crystal displays (LCD),[3] and miniature gratings for use in spectrometers. The fact that these devices are smaller and lighter gives them the huge advantage of being easily transportable to the sample site to perform *in loco* measurements. Furthermore they take up less space in a laboratory, many of them can work off batteries, often cost less than conventional benchtop instruments, require less sample and reagents producing less waste, and are often simpler to use.[4]

Low-cost powerful microcontrollers are being included as control-processing units in many types of portable and hand-held instrumentation. One of the most popular open-source microcontroller is Arduino.[5] Arduino can be programmed with the measurement algorithms, calibration function, and drift corrections needed for the full instrument operation, with the numeric final result displayed on a screen. The Arduino platform has already found applications in analytical chemistry to construct a photometer portable for determination of Fe^{3+} and Fe^{2+} in fresh water[6] and the amount microorganisms in liquid culture.[7] Usually, the basic Arduino microcontroller (version "Uno") comprises an ATmega chip that contains 14 digital inputs/outputs and 6 analogue inputs. It can be powered by battery or computer (via USB port). Finally, the scripts can be written in a language derived from C using free integrated development environment software and uploaded to the Arduino printed circuit board (PCB) via the USB port.

The recent advancements in the field of nanotechnology have opened up new arenas for the applications of nanomaterials including the development of ultrasensitive detection in the analytical sciences.[8–10] One potential approach is the use of noble metal

nanoparticles (e.g. gold and silver) as colorimetric probes.[10,11] Such a use relies on their excellent and distinctive optical properties such as high visible-region extinction coefficients and distance-dependent optical properties, which allow to visualize trace level targets simply by the naked eye. For example, Huang and Chang developed a new gold-nanoparticle based sensor for rapid determination of Hg^{2+} in aqueous environmental samples and in batteries.[12] Li and Li[13] reported the development of a highly sensitive and selective colorimetric detection method for cysteine using AuNPs. This assay relies upon the distance-dependent optical properties of gold nanoparticles, the self-assembly of cysteine on gold nanoparticles, and the interaction of a 2:1 cysteine/Cu^{2+} complex. In addition, the nanoparticles give high sensitivity for the detection of metal ions due their optical properties exhibit shape,[14] strong size,[15] and interparticle distance dependences.[16]

Lead is a non-ferrous metal widely used in batteries, paints, gasoline and different alloys. Wastewater generated during the processing of lead-acid batteries contains Pb^{2+}, which is very toxic to the environment and to living beings. Although standard techniques such as atomic absorption spectroscopy (AAS),[17] anodic stripping voltammetry,[18] inductively coupled plasma atomic emission spectroscopy (ICP-AES),[19] or fluorescence spectrum[20] are efficient in determining Pb^{2+} levels in water, these techniques are often costly, labor-intensive, time-consuming, require complex and expensive instruments and specialized personnel to carry out the operational procedures. For this reason there are ongoing researches on the development of rapid, low-cost and friendly-use techniques suitable for *in situ* assays of heavy metals. In this paper, we present a detailed explanation of the electronics of a home-made field-portable photometer that accurately determines light absorbance. We tested the performance of the photometer by measuring the amount of Pb^{2+} using AuNPs as colorimetric probes.

Since Pb^{2+} determination is relevant for studies involving aquatic environment, the instrument setup and the control software may be easily designed to carry out measurements *in loco*.

Materials and methods

Colorimetric LED photometer

Microcontroller

The instrument was based on an Arduino UNO plate. The Arduino UNO has a low cost (US$ 24.95) and was programmed using the Arduino IDE 1.0.6 software. The general features of the Arduino's system are shown on Table 1.

[Insert Table 1 here]

Detector

A TCS230 was used as detector (Fig. 1a). It has 16 phototransistors for red filtering, 16 phototransistors for green filtering, 16 phototransistors for blue filtering, and 16 phototransistors without filtering (blank). Each phototransistor responds to their respective filtering color with an electrical signal proportional to the color intensity. This signal, which has a frequency proportional to the color intensity measured in each moment, is represented by a squared wave with a work cycle of 50% (Fig. 1c).

LED array

Four standard LEDs of 3.0 V and 20-25 mA with white color emission were used for irradiate the sample bucket. The LEDs were positioned on the four sides of the detector sensors (Fig. 1b).

[Insert Figure 1 here]

Photometer overview

An LCD display JHD 162A was used to show the RGB intensity values to the user according to the detector output. Two potentiometers were used for controlling the light and the saturation on the LDC display. A resistor of 10 kΩ was used to control the current. The liquid sample is placed into a plastic bucket and positioned above the LED/detector system as can be seen in Fig. 2.

[Insert Figure 2 here]

The measure button (f1) starts the white LEDs and the detector at the same time. The RGB color values are shown on the LCD display during 20 seconds, until the system restarts. The button (f2) restarts the system.

Data treatment

The RGB color intensity is provided directly by the TCS230 sensor. The Arduino code was adapted to normalize this value per 255 in order to decrease the range of signal value. After collecting the RGB intensity values from LCD display, a .TXT file was generated and loaded into MATLAB® R2012b (MathWorks, USA). The calibration was based on RGB intensity values according Eq. 1, in which the signal i behaves linearly with the concentration of AuNP with Pb(II).

$$i = g - b \quad (1)$$

where g and b are the GB (green and blue) intensity values shown on LCD display; and i is the intensity signal.

Experimental procedure

Synthesis and characterization of AuNPs

AuNPs were produced using a previously reported method[20] based on the reduction of Au^{3+} with glycerol in alkaline medium. Briefly, all glassware was kept overnight in $KMnO_4$ + NaOH solution, rinsed with deionized water, kept for 10 min in H_2O_2 + H_2SO_4 solution (1:1 v/v), again rinsed with deionized water and dried prior to use. Afterwards, determined amounts of PVP (MW = 10.000) and gold chloride were dissolved in 10 ml of water. In a separate flask, fixed quantities of glycerol and NaOH were dissolved in 10 mL of water. The glycerol-NaOH solution was then added to the $AuCl_3$-PVP solution to yield the following final concentrations: 1.0 mmol L^{-1} Au^{3+}, 0.10 mol L^{-1} NaOH, 0.10 mol L^{-1} glycerol and 10 g L^{-1} PVP. The final mixture had a deep-red color due to the formed AuNPs. Finally, the AuNPs colloidal solution had then its pH adjusted to 7 by addition of diluted HCl. Considering a quantitative transformation of gold ions into nanoparticles, the concentration of AuNPs was estimated to be 8.0 x 10^{-8} mol L^{-1} based on their average size determined from TEM results.[21] UV-Vis absorption spectra of the AuNPs were acquired with an Evolution 60S UV-Visible spectrophotometer (Thermo Scientific) spectrophotometer. Transmission electron microscopy (TEM) images were acquired with a FEI Tecnai Spirit Biotwin 12 operating at 120 kV.

Detection of Pb(II) by AuNPs

A $Pb(NO_3)_2$ stock solution was prepared at the concentration of 20.0 mmol L^{-1} from which different volumes were taken and then added to distinct aliquots of the AuNPs solution to yield Pb^{2+} in the concentration range of 0.060 mmol L^{-1} to 10.0 mmol L^{-1} . In all cases the AuNPs concentration was kept at 2.0 x 10^{-8} mol L^{-1} by

adjusting the amount of water added to each mixture. The AuNPs-Pb^{2+} solutions were then analyzed by our colorimetric LED photometer with the RGB color intensity directly recorded the TCS230 sensor. UV-Vis spectroscopy and TEM were also conducted on the samples in order to acquire spectroscopic and morphology information of the AuNPs, respectively, when in contact with Pb^{2+}. The selectivity of the method was investigated by performing experiments with the following salts: $AgNO_3$, $AlCl_3.6H_2O$, $BaCl_2.2H_2O$, $CaCl_2$, $CdCl_2$, $CoCl_2.6H_2O$, $CrCl_3.6H_2O$, $CuSO_4$, $FeCl_2.4H_2O$, $Hg_2(NO_3)_2$, KCl, LiCl, $MgCl_2.6H_2O$, $MnCl_2.4H_2O$, NaCl, $NiCl_2.6H_2O$, $SnCl_2.2H_2O$, $SrCl_2.6H_2O$, $Zn(CH_3COO)_2.2H_2O$.

Results and discussion

Mechanism of Pb(II) sensing by AuNPs

Liu *et al*.[22] used glutathione-functionalized gold nanoparticles to colorimetrically probe Pb^{2+}. The authors argued that the –COOH and $–NH_2$ groups of glutathione could bind to Pb^{2+}, which provoked aggregation of gold nanoparticles with substantial shift in the plasmon band energy to longer wavelength and a red-to-blue color change. In our case PVP was used as stabilizer of AuNPs. PVP has an amide group that could in principle chelate with Pb^{2+} and, in addition, also concentrate Pb^{2+} due to its shell-like structure[23] around the particle. Chelating properties of PVP has been observed elsewhere.[24] As shown by the UV-Vis and TEM results bellow, Pb^{2+} was able to promote size increase and aggregation of AuNPs, which is the base for Pb^{2+} detection with gold nanoparticles.

Fig. 3 shows a UV-Vis spectrum of the AuNPs produced by simple addition of NaOH-glycerol to $AuCl_3$-PVP at room temperature (red curve). Glycerol is an inexpensive chemical and readily biodegradable under aerobic conditions, therefore an

eco-friendlier alternative compared to current reducing agents such as formamide, sodium borohydride and hydrazine. Visually the AuNPs solution is deep red, which is a consequence of the resonant coherent dipolar oscillations of the electron gas (electrons of the conduction band) at the surface of nanoparticles known as surface plasmon band (SPB). The colloidal AuNPs spectrum had a maximum absorbance (λ_{Max}) at 520 nm, a value typical for spherical gold nanoparticles.[20,25] The symmetry of the band implies a fair similarity in the shape of the nanoparticles and low degree of aggregation in the solution.[20]

[Insert Figure 3 here]

The TEM image illustrated that the AuNPs (Fig. 4A) were well dispersed and spherical in shape, thus corroborating the UV-Vis results. The mean particle size calculated from the histogram of the inset of Fig. 4 was 7.4 nm ± 2.8 nm. Upon addition of 0.10 mol L^{-1} Pb^{2+} the solution turned to a light violet due to aggregation stimulated by Pb^{2+}, which was confirmed by TEM (Fig. 4B) together with broadening and shifting of the peak to 590 nm in the UV-Vis spectrum (Fig. 3, blue curve)). The mean particle size is now 8.9 nm ± 2.8 nm. Another interesting feature of the AuNPs-Pb^{2+} is the bimodal features of the particle size distribution (Fig. 4B), which is possibly due to ripening of big particles leading to the formation of extra small nanoparticles.[20]

[Insert Figure 4 here]

For the selectivity test AuNPs were exposed to 0.10 mol L^{-1} of the following metal ions: Ag^{+}, Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Hg^{+}, K^{+}, Li^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Sn^{2+}, Sr^{2+}, and Zn^{2+}. The UV-Vis spectra in Fig. 5 demonstrate qualitatively that those ions have no obvious impact on both SPB and color of AuNPs compared to Pb^{2+}, which suggests that the method is specific to Pb^{2+}.

[Insert Figure 5 here]

The selectivity of the method can be quantitatively assessed by factor *F* calculated for each metal ion according to Eq. 2:

$$F = 1 - \frac{A_{520}}{A^0{}_{A_{520}}} \quad (2)$$

where A_{520} is the absorbance at 520 nm of the AuNPs when exposed to a given metal ion and $A^0{}_{520}$ is the absorbance at 520 nm of the pure AuNPs. An *F* value far from the unity signifies that a given metal ion had little impact on the SPB of the AuNPs. As can be seen in Fig. 6 all the ion metals except Pb^{2+} generated *F* values that strongly departed from the unity, which proves that the method is quite selective to Pb^{2+}.

[Insert Figure 6 here]

Photometer results

Analytical curves were obtained for Pb^{2+} in spiked water samples using the developed photometer analyzed at five Pb^{2+} concentration levels, which show an excellent adjustment ($r^2 = 0.995$) and a measurement range from 0.6 to 10.0 mmol L^{-1} , as shown in Fig. 7. Once the signals from the LED for Pb^{2+} samples with AuNPs were registered by the photometer, the samples were immediately inserted into the other transmission cell coupled to the spectrophotometer for the registration of trade spectra (also Fig. 7). As shown in Fig. 7, the linearity of the calibration curve for Pb^{2+} was confirmed by the low lack of fit according to the experimental F value of 0.0429.[26,27] Comparing the sensitivities between the two instruments, it can be concluded that the

sensitivities were quite similar (0.171 and 0.161 for UV-Vis and photometer responses, respectively). The precision obtained for the photometer was studied at three concentration levels, namely 1 mmol L^{-1} , 2 mmol L^{-1} , and 10 mmol L^{-1} , using 9 different samples obtaining relative standard deviations between 5.50 % and 8.26 % when expressed as Pb^{2+} concentration. In addition, the LOD for Pb^{2+} photometer was 0.89 mmol L^{-1} and the limit of quantification (LOQ) was 2.69 mmol L^{-1}, as calculated from modern IUPAC recommendation.[27]

The fact that LOD and LOC were relatively high compared to the literature[28,29] may be explained as follows: i) PVP is a long polymer that involves the nanoparticles forming a shell-like structure,[30] thus high concentration of Pb^{2+} is required for it to reach the AuNPs surface, disrupt the PVP protective layer, and promote AuNPs aggregation. Usually noble nanoparticles are produced with sodium citrate or sodium borohydride and anion adsorption confers the nanoparticle a negative charge that prevents aggregation thought simple coulombic repulsion. As the citrate and borohydride anions are much smaller than PVP, the nanoparticle surface is naturally more accessible to heavy metals and lower concentrations may be used; ii) some works in the literature have achieved low detection limits by attaching specific recognition units such as DNA[28,29] and enzymes[31] to the nanoparticle surface, however the functionalization is complex and expensive. Furthermore the stability of the DNA poses problems for application in real samples. In this work we used an extremely-simple environmentally-correct route to produce stable AuNPs using glycerol and PVP as reducing and protective agents, respectively. We sought to present a home-made equipment that could be used for a relevant application, and a comparison with UV-vis results showed success in this matter.

[Insert Figure 7 here]

Conclusion

A novel photometer for Pb^{2+} detection using AuNPs as signal producer has been successfully developed. The photometer based on sensor TCS230 and an Arduino electronic card as acquisition system was a simple and low-cost assembly. These features may enable the proposed approach to be used as a stand-alone setup for Pb^{2+} determination in water. In addition, we believe that this approach may serve as a foundation for the preparation of practical nanosensors for the rapid determination of Pb^{2+} concentrations in aqueous samples, having the features required to become a field instrument.

Acknowledgements

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Legends to Figures

Figure 1: Functional block diagram of the Arduino TCS230 color recognition sensor module. (a) TCS230 sensor; (b) photodiode array; (c) current to frequency converter.

Figure 2: Overview of RGB photometer: (a) LED Array with sensor; (b) power supply and USB interface for data acquisition; (c) Arduino microcontroller for powering the sensor; (d) homemade cell; (e) display; (f1) and (f2) controller buttons.

Figure 3: UV-vis spectra of AuNPs in absence and presence of 0.1 M Pb^{2+}.

Figure 4: TEM of AuNPs in (A) absence and (B) presence of 0.1 M Pb^{2+}.

Figure 5: UV-Vis absorption spectra of AuNPs in presence of 0.1 M metal ions: (–) AuNPs pure; (–) AuNPs + 19 metal ions; (–) AuNPs + Pb^{2+}.

Figure 6: Selectivity factor for 20 metal ions exposed to AuNPs.

Figure 7: Analytical curves for a series of standard Pb^{2+} solutions complexed with AuNP in two instruments: (a) RGB photometer data are represented with blue circles; (b) Thermo scientific 60S UV-Vis spectrophotometer data are represented with green triangles, at λ = 520 nm. All measurements are made in a 1 cm path cell.

Legends to Tables

Table 1. Arduino UNO features of the colorimetric LED photometer.

Figures

Figure 1

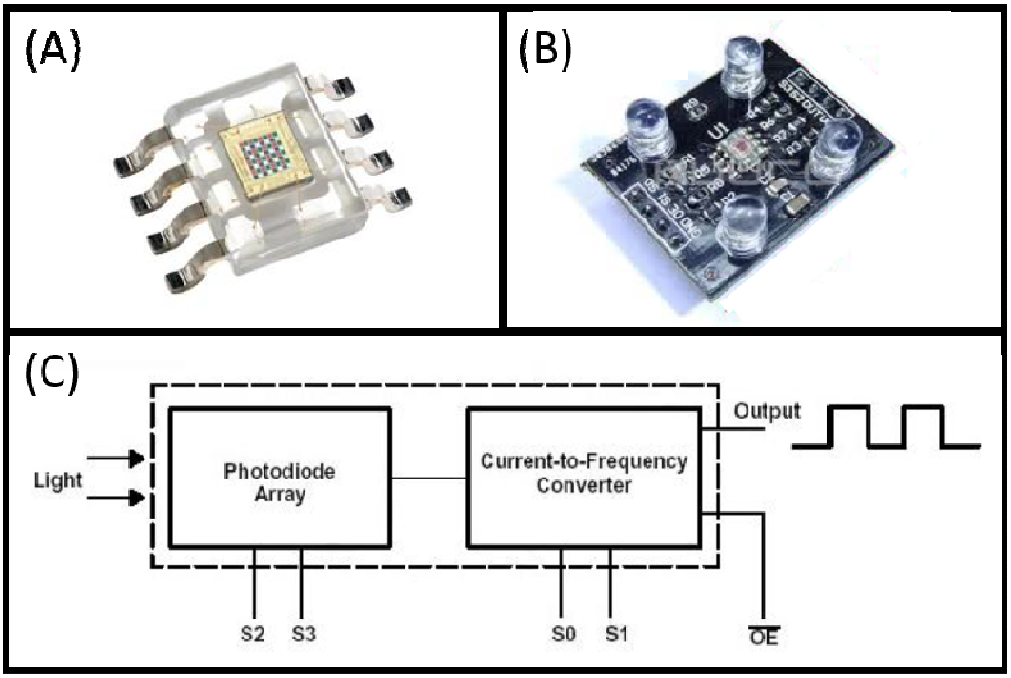

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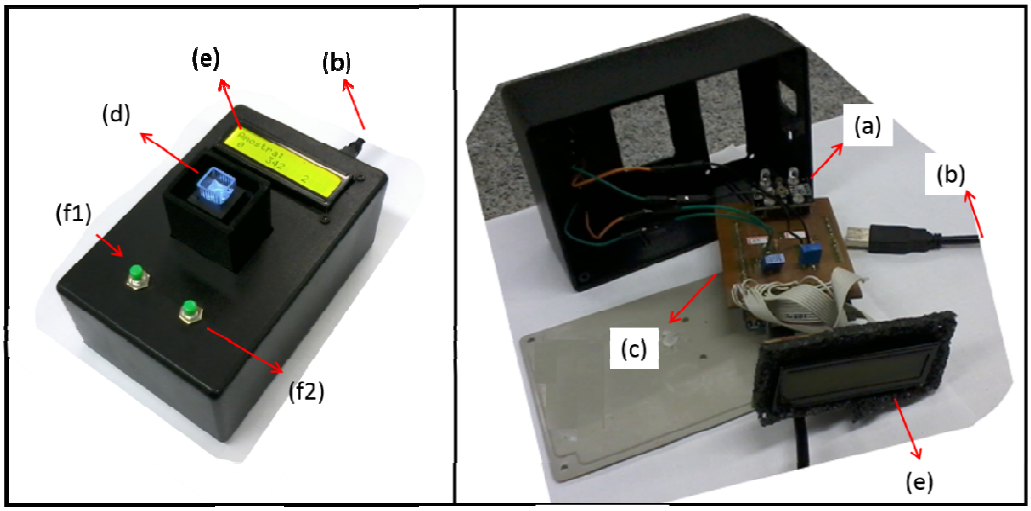
Figure 2



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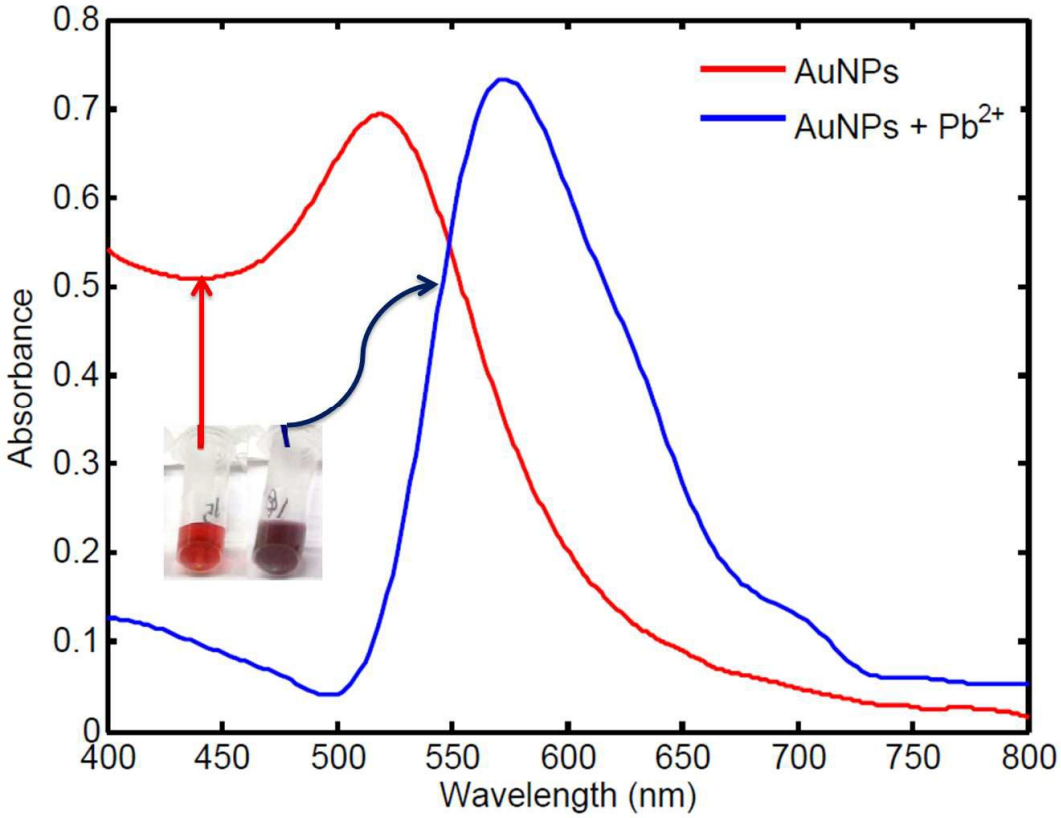

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Figure 4

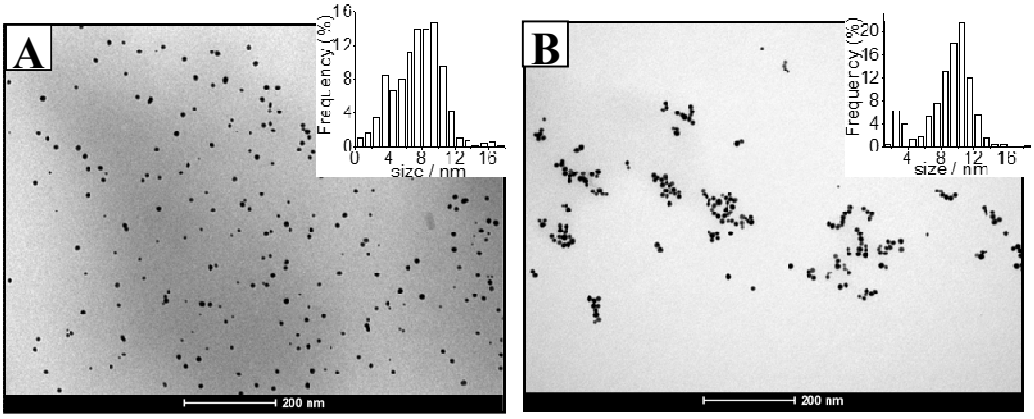

Figure 5

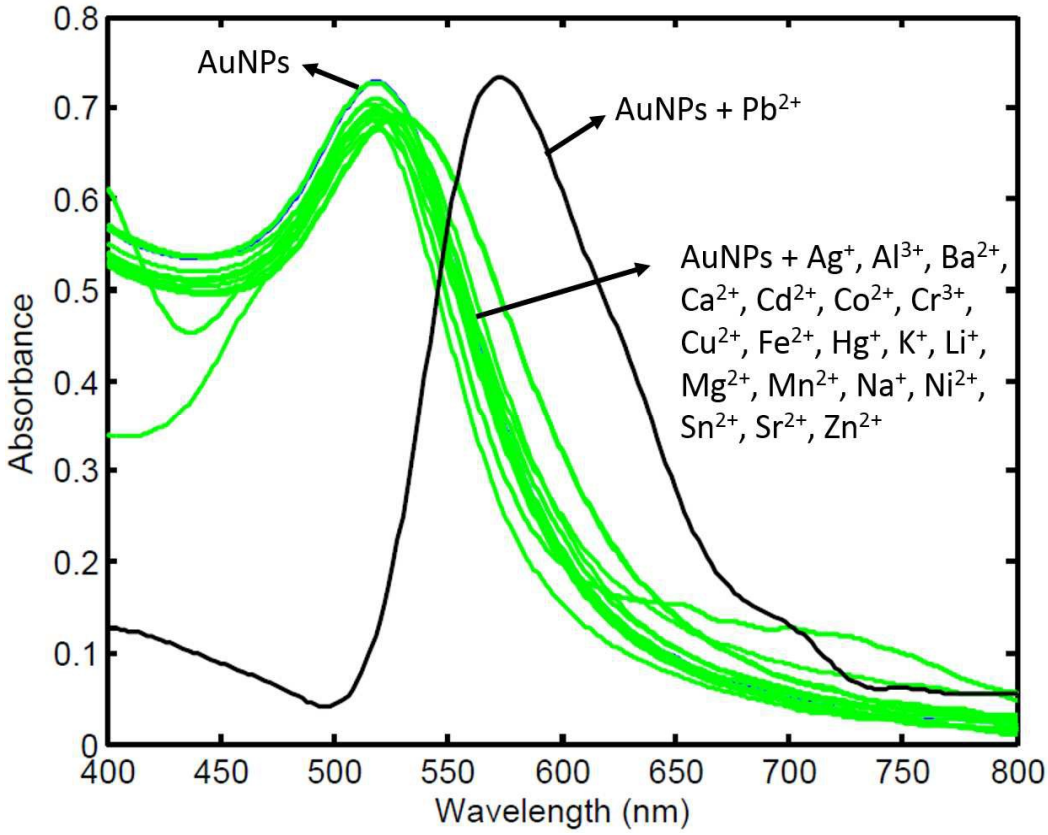

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Figure 6

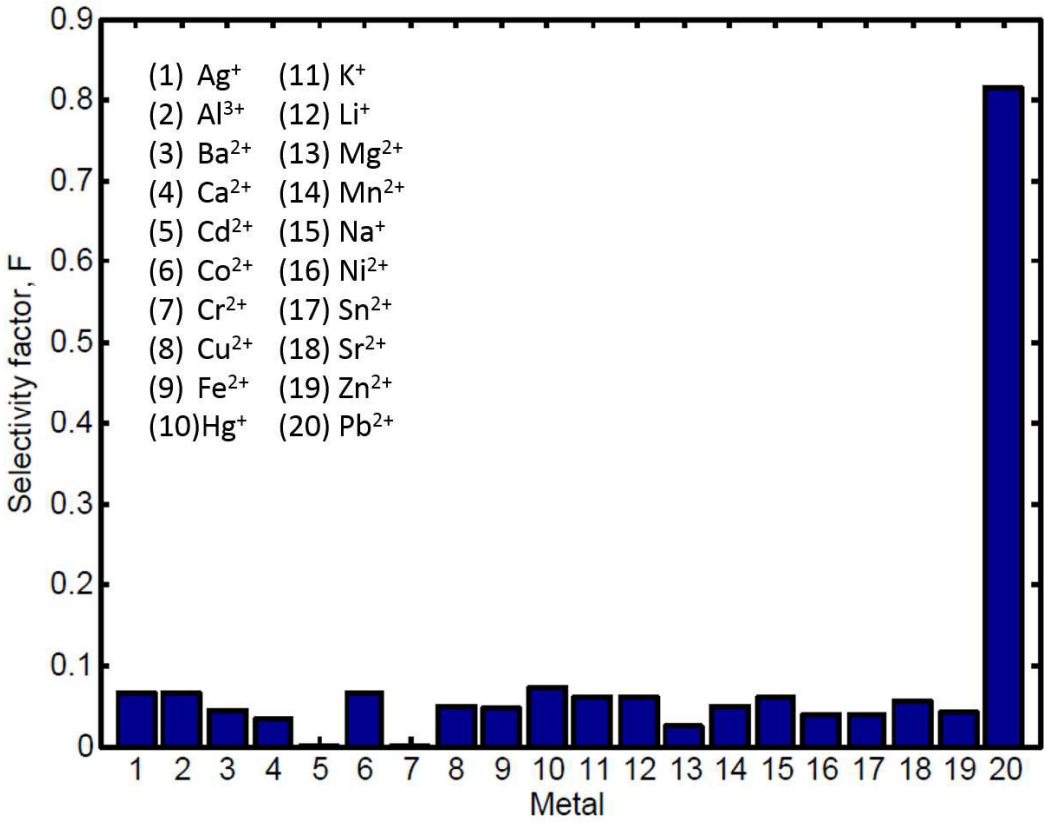
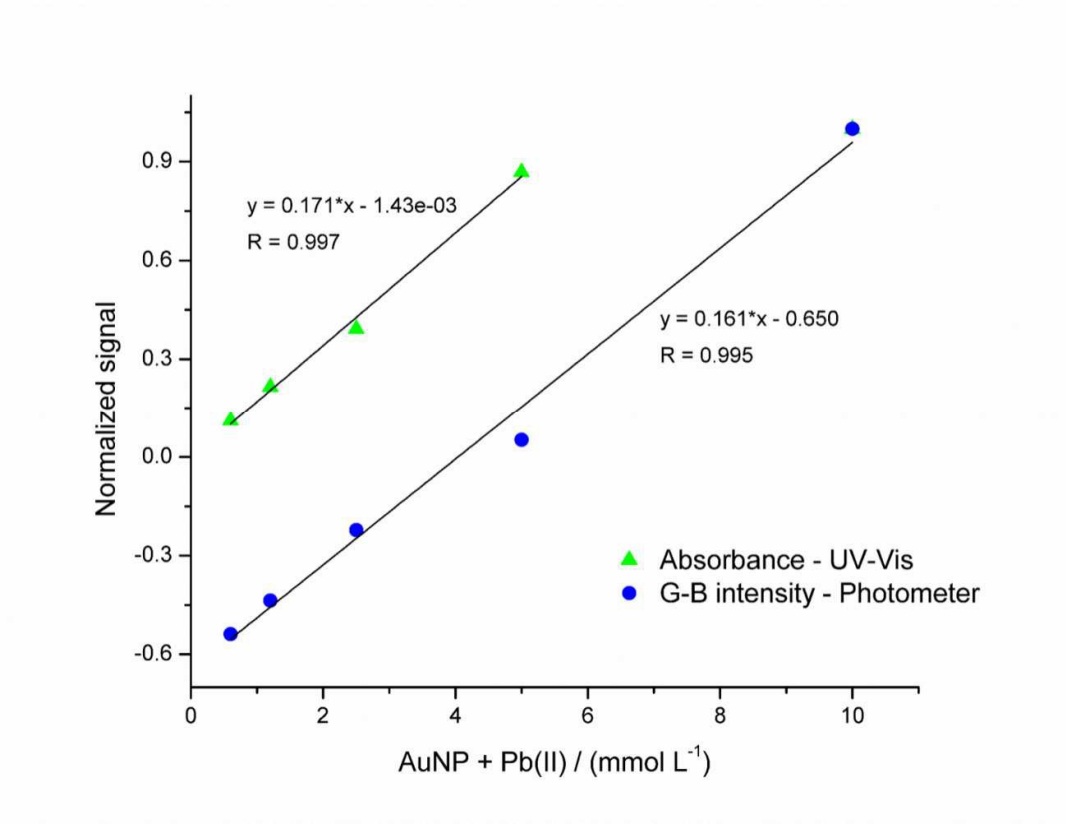


Figure 7



Tables

Table 1

Microcontroller chip	ATmega328
Working tension	5 V
Input tension (recommol L-1 ended)	7 – 12 V
Digital I/O pin	14
Analogical I/O pin	6
DC current per I/O pin	40 mA
Flash memory	32 KB
SRAM memory	2 KB
EEPROM memory	1 KB
Clock speed	16 MHz