Poly (N-Isopropylacrylamide)-co-(Acrylic Acid) Microgel/Ag Nanoparticle Hybrids for the Colorimetric Sensing of H2O2

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Poly (N-Isopropylacrylamide)-co-(Acrylic Acid) Microgel/Ag Nanoparticle Hybrids for the Colorimetric Sensing of H₂O₂

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ABSTRACT

Poly (N-isopropylacrylamide)-co-(acrylic acid) (pNIPAm-co-AAc) microgels composed of Ag nanoparticles (Ag NPs) have been synthesized and employed for the colorimetric sensing of H_2O_2. Each pNIPAm-co-AAc microgel, which exhibited a diameter of ~800 nm, contained multiple Ag NPs (diameter of ~5 nm), and solutions of these hybrid materials showed a UV-vis absorption band at ~400 nm. This is due to the excitation of the Ag NP surface plasmon. We go on to show that the intensity of this absorption band is dependent on the concentration of H_2O_2 in solution. Specifically, in the presence of H_2O_2 the magnitude of the absorption peak dramatically decreases in a linear fashion over the concentration range of 0.30 to 3.00 μM H_2O_2 (r^2=0.9918). We go on to show that the response is selective for H_2O_2 and can still function in complex mixtures, e.g., we showed that the response is still robust in milk samples. While Ag NPs themselves can exhibit similar responses, this system has many benefits including sample processing and long term stability -- i.e., Ag NPs are destabilized in solutions of a certain pH, and aggregate readily. Our microgel/Ag NP hybrids have been shown to be extremely stable and are easily purified prior to use by simple centrifugation/washing protocols. This system is simple and straightforward to use, is low cost, and can be used in complex media, which makes it practical for analyzing complex biological and environmental samples.

KEYWORDS: *Ag nanoparticles, hybrid microgels, colorimetric sensor, hydrogen peroxide*

INTRODUCTION

H_2O_2 is one of the most important intermediate species involved in many environmental and biological processes, and is ubiquitous in various industrial processes and medicine. [1-3] Because of this, the detection and quantitation of H_2O_2 in samples is of extreme importance. Multiple detection methods and schemes such as fluorimetry, [4] spectrophotometry, [5] chemiluminescence [6] and titrimetry [7] have been employed in the past for the detection of H_2O_2. Among them, many biosensors have been developed on the basis of electrocatalysis of immobilized enzymes as a result of H_2O_2 reduction. [8] However, the enzyme-based H_2O_2 sensors have some disadvantages. For example, the enzymes are expensive, the activity is dependent on environmental conditions, and they suffer from a short shelf life. [9] With the development of nanotechnology, nanomaterials for sensing H_2O_2 have been receiving increased attention with many advantage including enhanced catalytic activities and electron transfer, and stability. Among metals, silver nanoparticles (Ag NPs) display excellent electrocatalytic activity towards the reduction of H_2O_2. [10-12] It has also been shown that Ag NPs are useful at enhancing electron transfer for the reduction of H_2O_2, believed to be a result of larger paths for charge transport channels. [13] Furthermore, Ag NPs have many features such as highly sensitivity, biocompatibility and cost effectiveness when compared to other noble metal nanoparticles. [14]

While Ag NPs have some advantages, they suffer from instability in water, and tend to aggregate. This hinders their long-term storage and processibility, ultimately limiting their application. Therefore, it is important to protect the Ag NPs from aggregation if they are to have future utility. The aggregation of metal nanoparticles can be prevented either by electrostatic or steric stabilization, [15-17] and many methods have been developed to solve these problems by using stabilizing agents or some protecting carrier systems. [18-21] For example, Filippo et al. [22] and Guascito et al. [23] have used polyvinyl alcohol to stabilize Ag NPs and used for H_2O_2 sensing. Zhao et al. [8], Wang et al. [24] and Sophia et al. [25] employed PVP (polyvinyl pyrrolidone) as protecting agent for Ag NPs to be used in H_2O_2 sensing. Recently, poly (N-isopropylacrylamide) (pNIPAm) copolymer microgels have been used successfully to stabilize Ag NPs. Dong et al. [26] used poly (N-isopropylacrylamide)-co-(acrylic acid) (pNIPAm-co-AAc) microgel particles with different

crosslinking densities as microreactors for the in situ fabrication of Ag NPs. Khan et al. [18] fabricated Ag NPs in poly (N-isopropylacrylamide-co-methacrylic acid) microgels for rapid degradation of 4-nitrophenol in aqueous medium. Finally, Ballauff et al. synthesized poly(styrene) core pNIPAm shell particles, with the shell doped with Ag NPs and studied their ability to reduce 4-nitrophenol as a function of temperature. [27] While many have generated Ag NPs in pNIPAm-based microgels, there have been no reports on their use as a colorimetric sensor for H_2O_2 in solution.

As established above, the determination of H_2O_2 concentration in solutions is of importance for many reasons. There are many established methods for conducting such an analysis, such as fluorimetry (FL), chemiluminescence (CL) and electrochemical methods. While this is the case, these techniques involve the use of expensive equipment that must be operated by skilled operators. In this submission, we developed a pNIPAm-co-AAc/Ag NPs hybrid microgel-based system for conducting such measurements by the use of a simple UV-Vis spectrophotometer. Not only is the system easier to use, and the Ag NPs easier to process, compared to the others mentioned above, but the microgel-based systems were shown to be very sensitive to H_2O_2 concentration in solution, even when using complex media, e.g., milk samples.

MATERIALS AND METHODS

Chemicals: The monomer N-isopropylacrylamide (NIPAm) was purchased from TCI (Portland, OR) and purified by recrystallization from hexanes (ACS reagent grade, EMD, Gibbstown, NJ). N, N'-Methylenebisacrylamide (BIS) (99%), acrylic acid (AAc) (99%), ammonium persulfate (APS) (98%), silver nitrate, sodium borohydride and hydrogen peroxide were obtained from Sigma-Aldrich (Oakville, Ontario) and were used as received. All other chemicals were of analytical grade. Deionized (DI) water with a resistivity of 18.2 MΩ•cm was obtained from a Milli-Q Plus system from Millipore (Billerica, MA), and filtered through a 0.2 μm filter prior to use.

Synthesis of pNIPAm-co-AAc microgels: pNIPAm-co-AAc microgels were synthesized utilizing established procedures. [28] Specifically, the monomer mixture was comprised of 75% NIPAm and 20% AAc with 5% BIS as the crosslinker. The monomer, NIPAm (10.54 mmol), and the crosslinker, BIS (0.703 mmol), were dissolved in DI water (99 mL) with stirring in a small beaker. The mixture was filtered through a 0.2 μm filter affixed to a 20 mL syringe into a 250 mL, 3-necked round bottom flask. The flask was then fitted with a temperature probe, a condenser/N_2 outlet, stir bar, and a N_2 inlet. The monomer solution was purged with N_2 gas for about 1 h with stirring, while the

temperature was allowed to reach 70 ° C. AAc (2.812 mmol) was added to the heated mixture with a micropipette. An aqueous solution of APS (0.046 g in 1.0 mL) was delivered to the monomer solution with a transfer pipette to initiate the reaction, and reaction was continued for 3 h. Following completion of the reaction, the reaction mixture was filtered through glass wool to remove any large aggregates. The coagulum was rinsed with deionized water and the reaction solution was diluted to about 100 mL. Aliquots of these particles (12 mL) in centrifuge tubes were centrifuged at a speed of 10000 relative centrifugal force (rcf) at 20 ° C to produce a pellet. The supernatant was removed from the pellet of particles, which were then resuspended to their original volume (12 mL) using deionized water. This process was completed a total of five times to remove the unreacted monomer and linear polymer from the microgels. Finally, pellet of particles in centrifuge tubes was collected and suspended to 30 mL by adding deionized water for the next synthesis step.

Synthesis of pNIPAm-co-AAc /Ag NPs hybrid microgels: Hybrid microgels were synthesized by reduction of silver ions within the polymer network of pNIPAm-co-AAc microgels. Briefly, 5 mL of a previously prepared and purified microgels solution and 35 mL of deionized water were added into a three-necked round bottom flask (100 mL) equipped with N_2 inlet and condenser. The pH of this solution was adjusted to 8.62 by the addition of 0.01 M NaOH. Then 0.05 mL of an aqueous 0.1 M $AgNO_3$ solution was added and stirred for 30 minutes at 25 °C followed by the drop wise addition of 0.01 g of $NaBH_4$ dissolved in 5 mL of deionized water. The solution color immediately changed from colorless to yellow, and was further stirred for one hour. Then the resultant mixture was centrifuged at a speed of 10000 relative centrifugal force (rcf) at 25 °C to produce a pellet composed of the pNIPAm-co-AAc/Ag NP hybrid microgels. The centrifuge process was completed a total of five times and the final centrifuged pellet was diluted to 10 mL with deionized water for analysis.

Characterization: UV-Vis spectra of the as prepared Ag NP hybrid microgel solutions were collected using a 8452A Diode Array Spectrophotometer (Hewlett Packard, USA). Transmission electron microscopy (TEM) images were recorded using JSM-1200EX II TEM (JEOL, Ltd., Tokyo, Japan) system. The purified Ag NP hybrid microgels were deposited onto a TEM grid with a thin layer of carbon. Scanning electron microscopy (SEM) images were obtained with a JSM-6010LA JEOL (Peabody, MA) SEM. An energy-dispersive X-ray (EDX) (Philips, Roanoke, VA, U.S.A.) was used to confirm the compositions of the Ag NPs in the hybrid microgels.

Sensor of H_2O_2: For the quantification of H_2O_2 in solution, multiple reaction solutions were

analyzed. Each reaction solution contained 200 μL of the pNIPAm-co-AAc/ Ag NP hybrid microgels, and various amounts of a diluted H_2O_2 solution was added to result in a specific H_2O_2-concentration. After mixing via gentle vortexing the solutions were allowed to remain for 1 minute. After 1 minute the solutions were all diluted to 3.00 mL with deionized water and added to a 1 cm path length quartz cuvette and the UV-vis spectrum collected from 200 nm to 700 nm. The spectra revealed a strong absorption at 400 nm, and the intensity at this wavelength was related to the solution H_2O_2 concentration.

Analysis of a Milk Sample: To verify the utility of this method for H_2O_2 sensing in complex fluids, a local milk sample (from Edmonton, Alberta, Canada) was analyzed. Before analysis, the sample was prepared as follows: 5.00 mL of milk or 5.00 mL of milk spiked with a specific amount of H_2O_2 was added with 20 mL of methanol (containing 0.1 M sodium acetate)   respectively. After sonication for 10 min, each sample mixture was equilibrated for 30 min to allow the proteins to precipitate. Every solution was further centrifuged at 10000 rcf for 10 min. After that, 20 mL of n-hexane was added to the each supernatant solution to extract fat and the aqueous portion was collected for analysis, respectively. Specifically, 200 μL of the pNIPAm-co-AAc/ Ag NP hybrid microgels and a certain volume of aqueous pretreated sample were mixed and allowed to incubate for 1 min, the sample was then diluted to 3.00 mL with deionized water and analyzed via a UV-vis spectrometer.
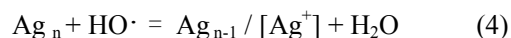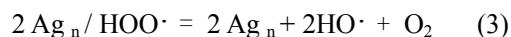
RESULTS AND DISCUSSION

pNIPAm-co-AAc microgels were used as microreactors for the in situ synthesis of highly dispersed and stable Ag NPs, using $AgNO_3$ as a precursor and $NaBH_4$ as a reducing agent. The Ag ion loading was done at pH 8.62, which was shown to be the optimal pH (see supporting information). As can be seen in the SEM image in Figure 1(a), microgels could be synthesized, and exhibit a spherical shape. The Ag composition of the microgels was confirmed using EDX. As can be seen in Figure 1(b), the EDX spectrum of the microgels (the exact location of spectrum collection is shown in Figure 1(a)) showed very strong peaks, which are indicative of Ag. To further confirm that the microgels contained Ag NPs, TEM images were collected. As can be seen in Figures 1(c, d), the microgel network contained Ag NPs with diameters of ~ 5 nm. Another image of the Ag NPs in the microgels can be seen in supporting information. These data are evidence that the microgel network structure provides an ideal scaffold for the nucleation and growth of Ag nanoparticles.

We went on to investigate the optical properties of solutions of the pNIPAm-co-AAc/Ag NP hybrid microgel solutions. The UV-Vis spectra of the pNIPAm-co-AAc/Ag NP hybrid microgels and the pNIPAm-co-AAc microgels were collected, and as can be seen in Figure 2, the solutions containing the pNIPAm-co-AAc/Ag NP hybrid microgels exhibited a strong absorption at ~ 400 nm, while the other solution didn't exhibit an absorption in this region. The absorption at ~ 400 nm is attributed to the surface plasmon resonance of the Ag NPs. This has also been confirmed by Wu et al. who explained that the broad plasmon band near 400 nm refers to the surface plasmon absorbance band of Ag NPs immobilized in the microgels. [29] We would like to point out here that the Ag NPs in the microgels were extremely stable, compared to Ag NPs in solution without microgels for stabilization. Specifically, we have shown that the Ag NPs in the microgels were stable when stored in the dark at room temperature, while the Ag NPs synthesized in the same manner, but without the microgels, aggregated and settled out of solution within 5 minutes, see supporting information. Furthermore, Ag NPs in the microgels were also stable at the different temperature (Figure 3).

After characterization of the pNIPAm-co-AAc/Ag NP hybrid microgels, we went on to prove their sensitivity to the presence of H_2O_2. As can be seen in Figure 4, the color of the pNIPAm-co-AAc/Ag NP hybrid microgel solution changed from yellow to colorless upon the introduction of H_2O_2, making the solution concentration 1 mM H_2O_2. We note here that bubbles were generated due to the generation of oxygen as a result of the catalytic reaction between Ag and H_2O_2. The proposed reaction mechanism is shown below, as presented by Jiang and coworkers: [30]

$$Ag_n + H_2O_2 = Ag_n / HOOH \qquad\qquad (1)$$

$$Ag_n / HOOH = Ag_n / HOO\cdot \qquad\qquad (2)$$

$$2\,Ag_n / HOO\cdot\ =\ 2\,Ag_n + 2HO\cdot\ +\ O_2 \qquad (3)$$

$$Ag_n + HO\cdot\ =\ Ag_{n-1} / [Ag^+] + H_2O \qquad (4)$$

Since the pNIPAm-co-AAc/Ag NP hybrid microgels were shown to be sensitive to H_2O_2, we went further and characterized the system's response to various concentrations of H_2O_2. To accomplish this, the same amount of pNIPAm-co-AAc/Ag NP hybrid microgels was added to solutions of various concentrations of H_2O_2 and the UV-vis spectrum collected. As can be seen in Figure 5, the absorbance value at 400 nm decreases as the solution concentration of H_2O_2 increased.

Importantly, it is noted that the baseline for the curves is stable from sample to sample. From the UV-vis spectra, we were able to plot the variation in absorbance at 400 nm as a function of H_2O_2. From the data in Figure 6, it can be seen that the response is extremely linear in the range of 0.3-3.0 μM H_2O_2 (r^2=0.9918). The effect of pH on the catalytic reaction has been studied (Figure 7), and the results showed that the pH value did not affect the UV-vis absorbance significantly over a wide range. The absorbance of the catalytic reaction was also shown to be stable at different temperatures (Figure 3).

Finally, we went on to evaluate the selectivity of the pNIPAm-co-AAc/Ag NP hybrid microgels to H_2O_2. To investigate the selectivity, we exposed pNIPAm-co-AAc/Ag NP hybrid microgels to individual solutions containing 50 μM of: glucose, ascorbic acid, uric acid, Cu^{2+}, Na^+, Ca^{2+}, Fe^{3+}, Mg^{2+}, and K^+. Figure 8 reveals that the absorbance values did not change considerably (by comparing the absorbance at 400 nm before and after exposure) in response to these interfering species, while it changed greatly in the presence of 1 μM H_2O_2.

Based on the above results, it is clear that the pNIPAm-co-AAc/Ag NP hybrid microgels can be used to quantify the amount of H_2O_2 in aqueous solutions. Next, we wanted to show that the pNIPAm-co-AAc/Ag NP hybrid microgels could be used to quantify H_2O_2 in complex media. To evaluate this, we have performed the above sensing strategy in a milk sample. The milk samples spiked with different concentrations of H_2O_2 were treated as detailed in the Experimental Section above to remove protein and fat from the samples, and the aqueous portion of the treated sample was collected for testing. The results from three separate test at each indicated concentration are shown in Table 1. As can be seen, H_2O_2 could be detected in each sample that was spiked, and the recoveries (amount H_2O_2 detected compared to the original amount of H_2O_2 in the milk) were on average 90.9% and 92.0 % with standard deviations below 4.5%, which confirms satisfactory accuracy and precision of the proposed method. This method may have great potential applications for hydrogen peroxide assay in food and biological samples.

Table 1 Results of hydrogen peroxide assay in milk samples

Sample	Conc. spiked H_2O_2 (μM)	Detected (μM, average value)	Average recovery (%, n=3)	Standard deviation (%, n=3)
	0	not detected	/	/
Milk	0.88	0.81	92.0%	3.7%
	1.76	1.60	90.9%	4.4%

CONCLUSIONS

In conclusion, we have demonstrated that a simple colorimetric sensor for the determination of H_2O_2 in aqueous samples, as well as complex media, could be generated. The system is made by generating Ag NPs inside the polymer network of pNIPAm-co-AAc microgels, which affords solutions with a yellow color and a strong UV-vis absorption at 400 nm. We showed that the absorbance at 400 nm decreased in the presence of H_2O_2, leading to the solution color disappearing. This was due to the H_2O_2-induced autocatalytic oxidization of the Ag NPs. We also showed that the absorbance at 400 nm was linearly dependent of H_2O_2 concentration over a wide range of concentrations, and was selective to H_2O_2. Finally, showed that the method could be used to detect H_2O_2 in milk samples. This approach represents a fast and inexpensive method for H_2O_2 detection, and has clear advantages over Ag NPs themselves including enhanced stability, long shelf life, and easy processibility. These systems may find use for analyzing medical samples, for environmental monitoring and for food quality control.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Figure Captions:

Figure 1. (a) SEM, (b) EDX spectrum, and (c, d) TEM for the generated pNIPAm-co-AAc/Ag NP hybrid microgels.

Figure 2. UV-vis absorption spectra for (red) pNIPAm-co-AAc/Ag NP hybrid microgels and (black) pNIPAm-co-AAc microgels.

Figure 3. The effect of temperature on the absorbance of ($\bullet$, red) pNIPAm-co-AAc/Ag NP hybrid microgel solutions and ($\blacksquare$, black) after addition of H_2O_2 (total concentration 3.00 mM).

Figure 4. Photographs of solutions containing pNIPAm-co-AAc/Ag NP hybrid microgels (a) before and (b) after the addition of H_2O_2.

Figure 5. UV-vis absorption spectra of solutions containing pNIPAm-co-AAc/Ag NP hybrid microgels before, and after the addition of the indicated amounts of H_2O_2.

Figure 6. The variation of the absorbance at 400 nm as a function H_2O_2 concentration.

Figure 7. The effect of pH value on the catalytic reaction between the pNIPAm-co-AAc/Ag NP hybrid microgel after addition of H_2O_2 (total concentration 3.00 mM).

Figure 8. The selectivity of the Ag NPs hybrid microgels toward H_2O_2. 1. H_2O_2 (1μM), 2. ascorbic acid (50 μM), 3. Glucose (50 μM), 4. uric acid (50 μM), 5. Ca^{2+} (50 μM), 6. Fe^{3+} (50 μM), 7. Cu^{2+} (50 μM), 8. Mg^{2+} (50 μM), 9. Na^{+} (50 μM), 10. K^{+} (50 μM).
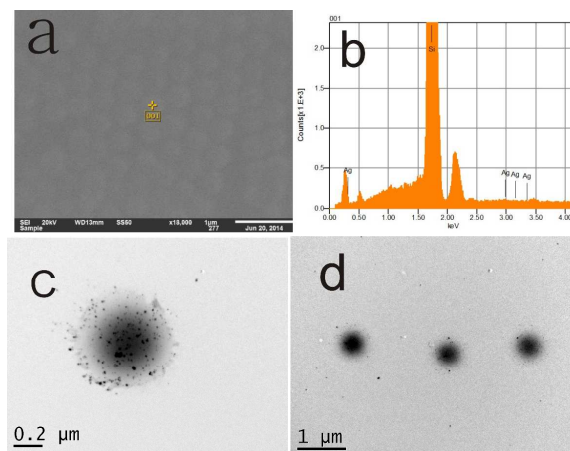
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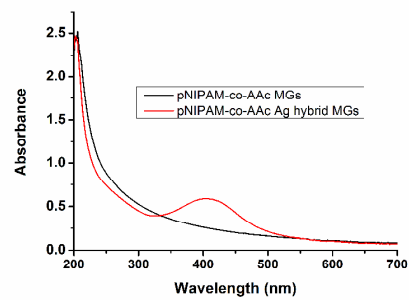
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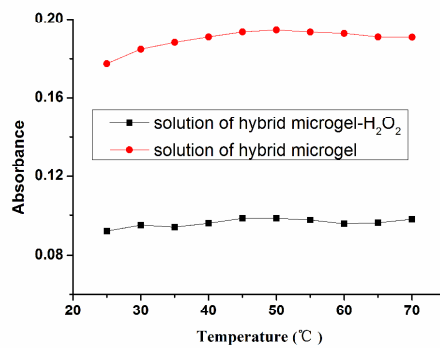
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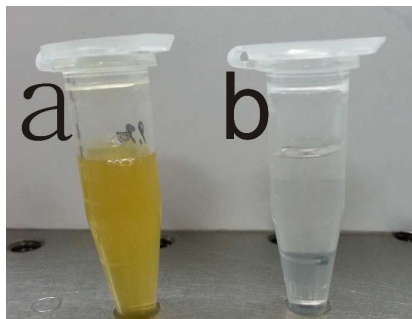
Figure 4.
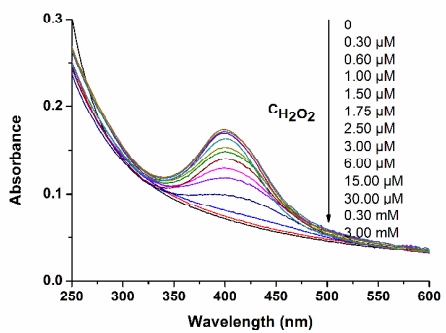
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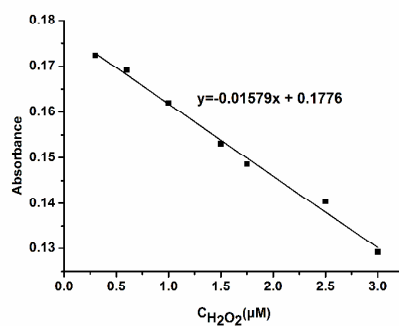
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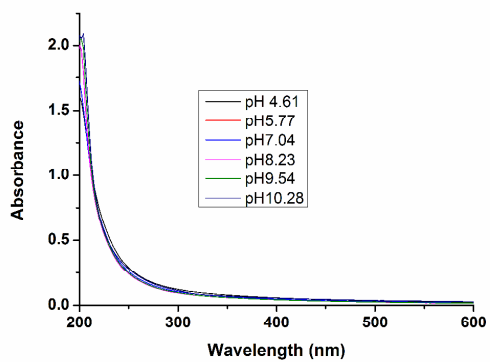
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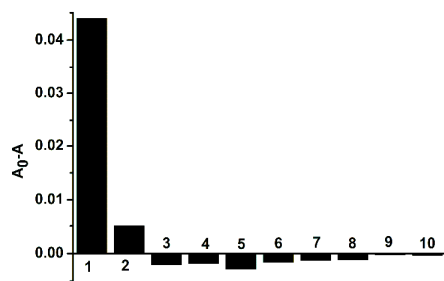
Figure 8.

Graphic abstract