

## Comparing signal amplification of thiocyanated Gold nanoparticles in the presence of different ions

N. Mah-Heidari<sup>1</sup>, J. Rashidiani<sup>1</sup>, F. Dashtestani<sup>2</sup>, R. Ali Taheri<sup>1</sup>, H. Sedighian<sup>3</sup>,  
Kh. Eskandari<sup>1\*</sup>

<sup>1</sup> Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

<sup>2</sup> Department of Biology, Electronic Branch, Islamic Azad University, Tehran, Iran

<sup>3</sup> Applied Microbiology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Received: 2 May 2017; Accepted: 5 July 2017

---

**ABSTRACT:** Detecting is the most important section in all kinds of sensors. In this regard, the amplification of surface plasmon resonance intensity of gold colloids nanoparticles (GNPs) was studied in the presence of several ions. GNPs were synthesized and then capped by thiocyanate and characterized via DLS and TEM image. In the next step the effect of different concentrations of ions such as iron, copper and iodide, for the suitable amplification of surface plasmon resonance intensity of GNPs, were investigated. This amplification studies were followed by UV-Vis and fluorescence spectroscopy. The results also could be observed by the naked eye. It was showed that iron ions were the best amplifier for the surface plasmon resonance of GNPs. The results lead to an extraordinary change signals of GNP peaks and developed a simple and new probe for tracking immunosensor system. This process seems to promote the sensitivity and selectivity of colorimetric biosensors rather than the previous method.

**Keywords:** Biosensor; Gold colloids nanoparticles (GNPs); Metal ions; Signal amplification; Spectroscopy; Thiocyanat

---

## INTRODUCTION

Different kind of biosensors assay have been designed for detecting bioelements such as: acoustic, optical and the other ones based on mass, viscosity and so on. (Mohanty and Koungianos, 2006). In recent years many researches has been conducted to improve biosensors sensitivity, intensity and selectivity besides that cost - effective and simplification. Many different solutions were developed however by applying nanomaterial sci-

ence approximately difficulties overcome rather than previous design (Cao, *et al.*, 2011, Pingarrón, *et al.*, 2008). One of the proper signal in biosensing is color changing properties of materials. Colorimetric sensing can define a wavelength both in a range of visible and ultraviolet spectra. UV-Vis spectroscopy is based on excited electron, when the range of UV-Vis is raised in fact it is declared an electron transfer phenomenon. There is a different technique for sensing cations and

---

(\*) Corresponding Author-e-mail: kheskandari@alumni.ut.ac.ir

anions or metal ions even there are introduced methods for detecting varied neutral molecule in this method. some examples of cations sensors are pH indicator and fluorescence. there are analogue examples in addition to applying chromoionophores for anion tracking (Maeda and Anzenbacher, 2012). Gold colloids nanoparticle (GNPs) is a good candidate in colorimetric signal. Colorimetric properties of GNPs emerged when their size reduce down to nanometer and made them to exploit widely in designing biosensors. GNPs can be easily conjugated to many biological marker and act as a label as well for immunological approach (Hutter and Maysinger, 2013, Lei and Butt, 2010). Additionally, GNPs have been used as a sensors in many researches in order to detect various agents. Since, the most outstanding characteristic of GNPs is their Surface plasmon resonance (SPR) and thus made them possible to change colors in different wavelength. According to this optical property many simple visible colorimetric sensors based on GNPs were designed (Yue, *et al.*, 2016). GNPs used to determine different anion and cation in electrochemical biosensors due to its specific colorimetric and SPR properties and so on. Thiocyanate ion is one of anions which was detected by gold colloids. However thiocyanate ion has colorimetric characteristics by itself and it has been evaluated in reaction of the other anion and cations such as:  $\text{Cl}^-$ ,  $\text{F}^-$ ,  $\text{Br}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Fe}^{3+}$  and so on (Zhang, *et al.*, 2012). Zhang and et al declared a colorimetric- based sensor by GNPs which can detect thiocyanate in a fast way. They approved that after adding thiocyanate to GNPs the solution become more stable so that prevent nanoparticle from aggregation and this can observable by changing color, eventhough the other anions and cations are also considered. in another study Baschang and et al showed that thiocyanate can be involved in reducing GNPs size and making them more monodisperse (Baptista, *et al.*, 2012, Baschong, *et al.*, 1985).

This work tried to propose a new amplifying system based on thiolated GNPs (KSCN/GNPs) intracted with different ions such as  $\text{I}^-$ ,  $\text{Cu}^{2+}$  or  $\text{Fe}^{2+}$ . So, GNPs were synthesized and characterized by size-zeta DLS. KSCN/GNPs were prepared and confirmed by UV-Vis spectroscopy. Then different concentrations of above ions added to the KSCN/GNPs solution. The effect of different ions in concentration ranges on

SPR of GNPs were examined by UV-Vis and fluorescence spectroscopy. Among examined ions,  $\text{Fe}^{2+}$  ion were the best for amplification of SPR bond of GNPs. Therefore,  $\text{Fe}^{2+}$ /KSCN/GNPs would be used in biosensors as signal amplifier.

## MATERIAL AND METHOD

Hydrogen tetrachloroaurate ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ), trisodium citrate, thiocyanate (KSCN), potassium cyanide, ferrous sulfate, potassium iodide and copper nitrate were obtained from Merck. All tests were performed at room temperature ( $25^\circ\text{C}$ ). Gold nanoparticles (GNPs) distribution size were determined by DLS instrument (Malvern, England). All data were collected via UV-Vis spectroscopy (Cecil, England) in the wavelength from 520 nm to 600 nm And absorption spectra was also carried out by Fluorescence spectroscopy (Cary Eclipse, Australia). Transmission Electron Microscopy (TEM) images were recorded for more characterization (Zeiss-EM10C-105 100 KV German).

### Preparation of GNPs and KSCN/GNPs

GNPs with an average diameter of 32.5 nm were synthesized in room temperature based on the classic method in literature (Mashhadizadeh, *et al.*, 2008). Briefly, 50  $\mu\text{l}$  of  $\text{HAuCl}_4$  stock (20% w/w) was added to 20 ml of distilled water while stirring and heated up to  $70^\circ\text{C}$ . Then, trisodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ ) 1% (w/v) was added to the above solution gradually. The formation of GNPs can be observed by a change in color of solution to purple. The solution was stirred for 2 more hours. It was stored in refrigerator in  $4^\circ\text{C}$  in dark bottle. For capping KSCN on GNPs, 0.2  $\text{mg}\cdot\text{ml}^{-1}$  KSCN solution in distilled water was prepared and its different volumes (0, 5, 10, 20, 30 (5mM) were added to 1 mL of GNPs. The mixed solution pipetted for several times and incubated for 10 minutes in room temperature for better adsorption.

### Preparation of $\text{I}^-$ , $\text{Cu}^{2+}$ or $\text{Fe}^{2+}$ (1mM)/ KSCN/GNPs (0.4mM)

Copper nitrate ( $\text{CuNO}_3$ ), potassium iodide (KI) and Iron(II) sulfate ( $\text{FeSO}_4$ ) in concentration of 0.01  $\text{g}\cdot\text{ml}^{-1}$ , 0.016  $\text{g}\cdot\text{ml}^{-1}$ , and 0.019  $\text{g}\cdot\text{ml}^{-1}$  in deionized water were

prepared respectively. For comparing the effect of these salts on the GNPs absorption, they were added to KSCN/GNPs in different concentrations as 0, 5, 10, 20, 30  $\mu$ l. Then, the changes of the KSCN/GNPs absorption peaks were recorded via UV-Vis spectroscopy.

## RESULT AND DISCUSSION

GNPs and KSCN/GNPs were prepared and characterized. Then The effect of different ions ( $I^-$ ,  $Cu^{2+}$  and  $Fe^{2+}$ ) on the SPR of GNPs were investigated by UV-Vis and fluorescence spectroscopy.

### Characterization of GNPs and KSCN/GNPs

The size and zeta potential of GNPs were determined by zeta-sizer DLS instrument. As it was shown in (Fig. 1), the size of GNPs was around 10 nm and the zeta potential was -32.2 mV.

The negative zeta potential of GNPs related to citrate ions which is adsorbed on the surface of GNPs during synthesis. Different concentrations of KSCN were

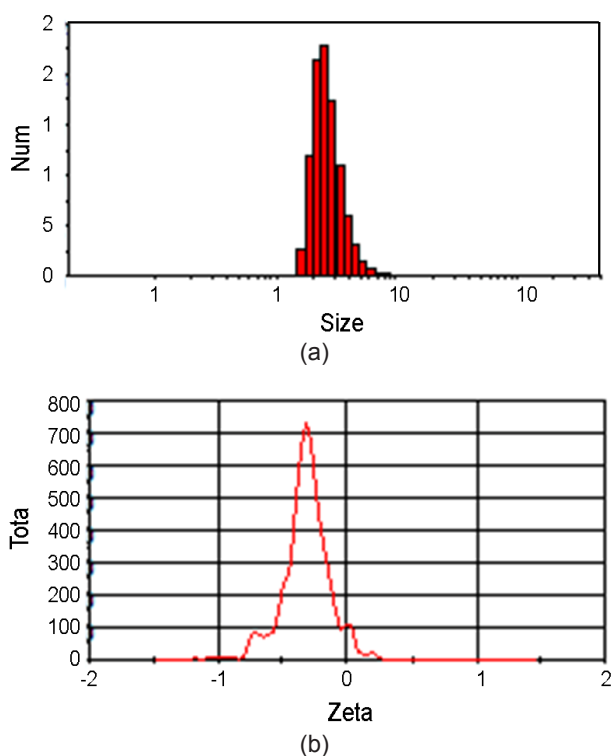


Fig. 1. DLS data. (a). Size distribution of GNPs (average 35.25 nm) (b). Zeta potential of GNPs (-32.2 mV).

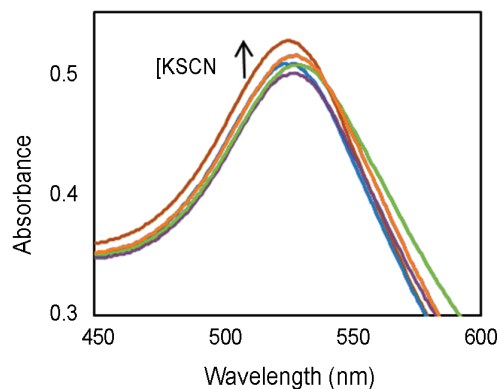


Fig. 2. UV-Vis spectra of GNPs. Different concentration of KSCN (0.5, 10, 20 and 30  $\mu$ l from 5mM) were added.

added to GNPs and the results were monitored by UV-Vis spectroscopy (Fig. 2). The data were shown that by increasing KSCN concentration, the SPR of GNPs at 530 nm was increased. It indicated that KSCN was attached to GNPs through its thiol group and changed the peak.

### Effect of $I^-$ , $Cu^{2+}$ or $Fe^{2+}$ on SPR band of GNPs

To evaluate the effect of ions such as  $I^-$ ,  $Cu^{2+}$  and  $Fe^{2+}$  on the SPR of KSCN/GNPs different concentrations of these ions were added to KSCN/GNPs. The UV-Vis spectra were recorded for addition of each ion separately (Fig. 3). As shown in this ( Fig. 3-A), by adding  $Cu^{2+}$ , the intensity of the SPR of GNPs were barely decreased (0.007). However, as (Fig. 3-B) demonstrated, in addition of  $I^-$  no significant intensity were observed (0.005 decrease). As it was obvious by increasing the  $Fe^{2+}$  concentration, strong amplification in SPR intensity of GNPs was happened (Fig. 3-C). So,  $Fe^{2+}$  is good candidate as colorimetric amplifier. According to the SPR of GNPs in (Fig. 3-C), the most changes were occurred in different  $Fe^{2+}$  concentrations up to 70  $\mu$ l addition. More than this concentration there was no changes in SPR band. It means that KSCN/GNPs were saturated by  $Fe^{2+}$ .

TEM image of KSCN/GNPs is shown in Fig. 4-A. As it can be seen KSCN/GNPs is well formed and dispersed. For better characterization of  $Fe^{2+}$ /KSCN/GNPs formation were done by recording TEM image (Fig. 4-B). As it is obvious via TEM image, after conjugation of  $Fe^{2+}$  to KSCN/GNPs the nanoparticles aggregates a little.

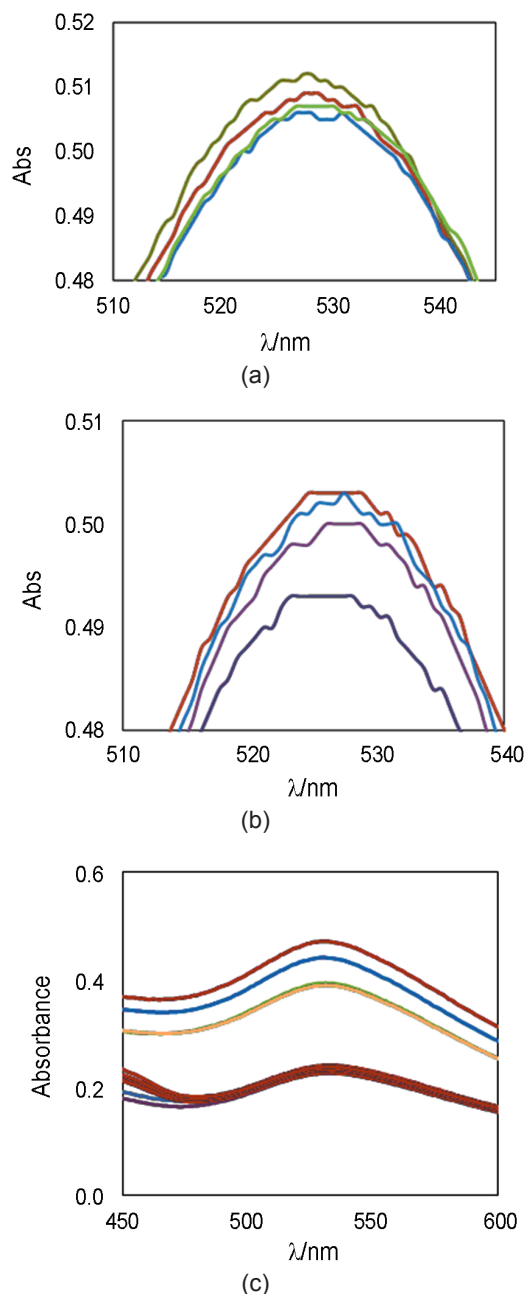


Fig. 3. UV-Vis spectra of KSCN /GNPs in the presence of different ions. (A)  $\text{Cu}^{2+}$  (0,10, 20, 30  $\mu\text{l}$  from 1mM) (B) KI (0,10,20,30 $\mu\text{l}$  from 1mM) and (C)  $\text{Fe}^{2+}$  (0,10,20,30,40,50,60 ,70,80,90,100  $\mu\text{l}$  from 1mM).

**Fluorescence studies of  $\text{Fe}^{2+}$ /KSCN/GNPs**

Fluorescence spectroscopy was used for better investigation of  $\text{Fe}^{2+}$  ion's effect on the amplification of GNPs' SPR intensity band. The excitation peak of GNPs was recorded in 310 nm. Then KSCN (30  $\mu\text{l}$ , 1 mM) was added to quvette and then different volumes of  $\text{FeSO}_4$  (10, 20, 30, 40, 50, 60  $\mu\text{l}$ ) were added to solution. The results were shown in (Fig. 5). As it can be seen, at first by adding 30  $\mu\text{l}$  KSCN a rarely

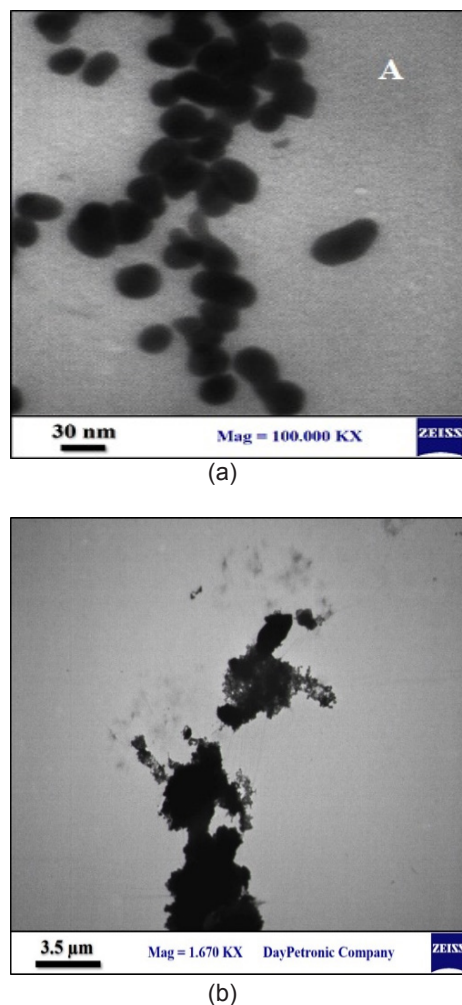


Fig. 4. TEM images of A: KSCN/GNPs (before conjugation) and B:  $\text{Fe}^{2+}$ /KSCN/GNPs (after conjugation).

changes in emission spectram of GNPs was observed. In the following, an increase intensity in emission was reported by adding 10  $\mu\text{l}$  of  $\text{Fe}^{2+}$ , however an suprising change was observed by increasing the concen-

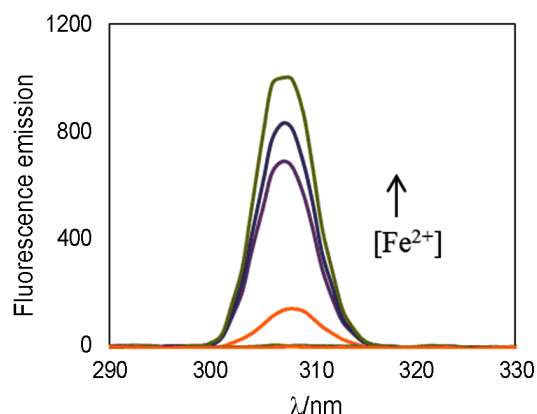


Fig. 5. Fluorescence emission spectra of KSCN/GNPs: increasing peak by adding different concentration of  $\text{Fe}^{2+}$  to the solution.

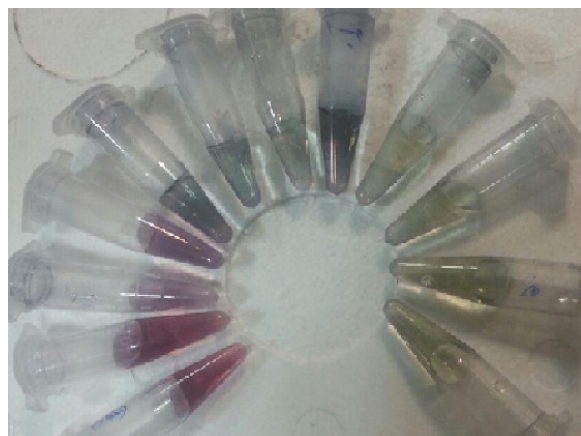


Fig. 6. KSCN/GNPs in different concentration of  $Fe^{2+}$  left to right (A-L). GNPs (A), KSCN/GNPs (B),  $Fe^{2+}$ /KSCN/GNPs (C-L) 10 , 20, 30, 40, 50, 60, 70, 80, 90 and 100  $\mu$ l, 1mM.

tration of  $Fe^{2+}$ . By increasing the  $Fe^{2+}$  concentration, no enhancement in emission peaks were observed. It could be concluded that high level of  $Fe^{2+}$  may be quenched fluorescence emission of GNPs. By adding 40  $\mu$ l  $Fe^{2+}$  the fluorescence intensity peak of GNPs was

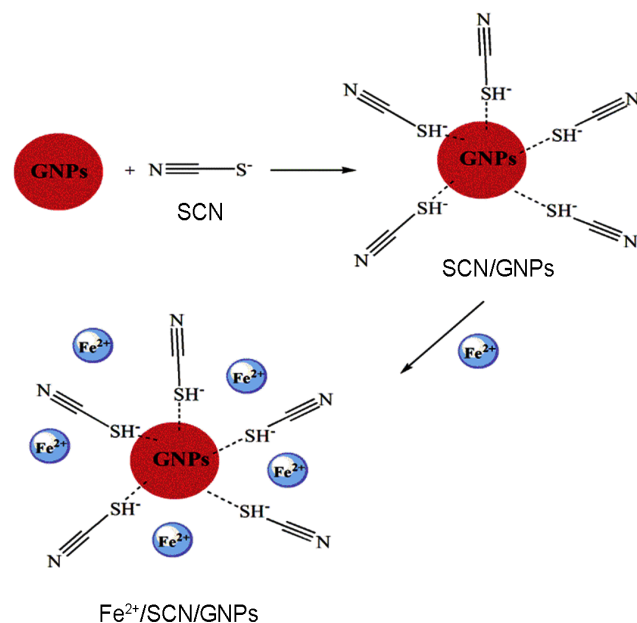


Fig. 7. Schematic illustration of showing the reaction between GNPs and KSCN and  $Fe^{2+}$ .

completely quenched. All data were repeated twice for

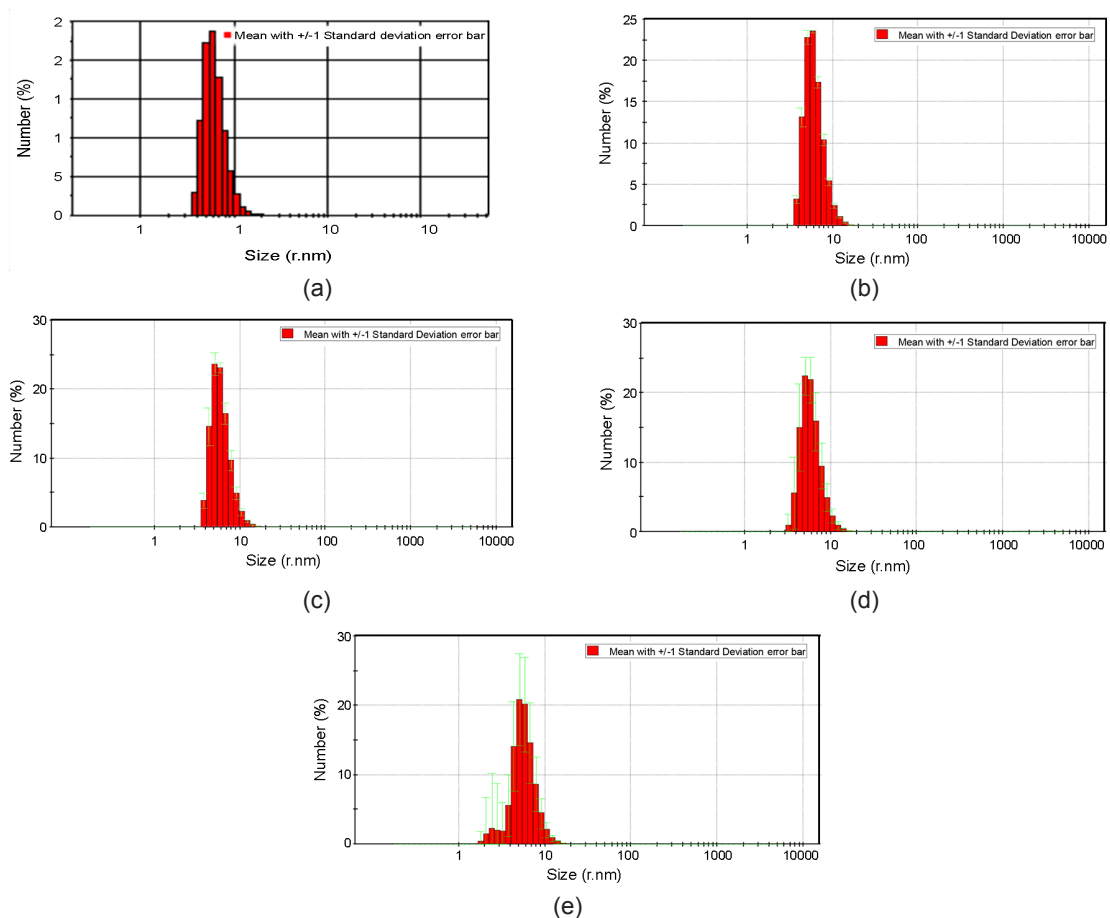


Fig. 8. DLS distribution changes of GNPs/SCN- in different concentration of  $Fe^{2+}$ . (A) GNPs numerical distribution Z- average 500  $\mu$ l 35.25 nm. (B) GNPs 500  $\mu$ l /SCN- 30  $\mu$ l 34.71 nm. (C) GNPs /SCN-/  $Fe^{2+}$ 10 $\mu$ l 34.9 nm. (D) GNPs /SCN-/  $Fe^{2+}$ 60  $\mu$ l 37.5 nm. (E) GNPs /SCN-/  $Fe^{2+}$ 100  $\mu$ l 41.5 nm.

sure and the results were shown the same.

### Naked eyes of Fe<sup>2+</sup>/KSCN/GNPs

The color changes of adding different concentrations of Fe<sup>2+</sup> to KSCN/GNPs were observed by the naked eyes (Fig. 6).

The changes in color of Fe<sup>2+</sup>/KSCN/GNPs formation are because of aggregation which is shown in TEM image (Fig. 4-B). So Fe<sup>2+</sup>/KSCN/GNPs would be a good candidate for colorimetric recognition in sensing. In (Fig. 7) An Shemtic reaction between KSCN/GNPs and Fe<sup>2+</sup> ions is shown.

The extensive changes of AuNps/SCN-/Fe<sup>2+</sup> size is obviously shown by DLS and it is another strong reason to choose Fe<sup>2+</sup> over the others. It is displayed huge changes of complex size by adding different concentrations of Fe<sup>2+</sup> (Fig. 8).

### Bonding formation of Fe<sup>2+</sup>/KSCN/GNPs

KSCN attached to GNPs via thiol atom of SCN. By adding Fe<sup>2+</sup> to the SCN/GNPs the intraction between Fe<sup>2+</sup> and SCN through thiol and nitrogen atom may be occured. So, Fe<sup>2+</sup> can increase SPR bond of GNPs. This bonding formation would be happened for Cu<sup>2+</sup> ions too. But this bonding is not efficient enough and thus Cu<sup>2+</sup> ions could not change the SPR bond a lot. I<sup>-</sup> ions may not be formed such bonding with GNPs and hence it can not shift the SPR of GNPs at all. The most changes was seen by Fe<sup>2+</sup> so, it is preferred to use this ions to the other which was mentioned above for further experiment. Differnet selected ions comparison data in a same range was collected in (Table 1) to better understanding of Fe<sup>2+</sup> preference for continuing experiments. Another explantion due to previ-ous expriments is that Cu<sup>2+</sup> ions bridge just two units

with nitrogen group and the others coordinated with sulphate so that this cation is not as enough strong as Fe<sup>2+</sup> to make vast changes in attaching with SCN (Ra-madan and El-Naggar, 1996), The very few changed peaks of I<sup>-</sup> in reaction with thiocyanate can be stated that even high concentration of this anions makes no differents changed based on its very similar charge transfer to the spectrum of thiocyanate, therefore no extensive changes were seen in the UV-Vis spectrum. The nature of dimine ligand substituent of Fe<sup>2+</sup> and nuclephilics anion like cynide and its tendency of ions pairing is far much more than two mentioned ions (Blandamer, et al., 1984).

## CONCLUSIONS

On according to the results ,different Anions and Cations were assayed. After conducting different ex-periment, this work showed that the other ions such as Copper and iodide can not be a proper option for further experiments. Finally, To have an perfect result in following researches, FeSO<sub>4</sub> was preferred.in this report, it is claimed that FeSO<sub>4</sub> must be conducted a great satisfactory outcome in order to design a higher sensitive colorimetric biosensor for detecting vibrio cholera and the other immune body targets.

## ACKNOWLEDGEMENT

Financial supports provided by the Research Council of the Baqiyatallah University of Medical Sciences are gratefully appreciated.

## REFERENCES

- BAPTISTA, P.V., ROSA, J., DORIA, G., CONDE, J., VEIGAS, B., ALMEIDA, C., ASSUNCAO, M. & GIESTAS, L., (2012). Noble metal nanoparticles for biosensing applications. *J. Sensors.*, 12: 1657-1687.
- BASCHONG, W., LUCOCQ, J. & ROTH, J., (1985). Thiocyanate gold: small (2-3 nm) colloidal gold for affinity cytochemical labeling in electron mi-

Table1. Optimization of different ions volumes for approach- ing better absorption SPR peak of GNPs

Concentration (µl)	KSCN (Abs)	Cu <sup>2+</sup> (Abs)	I <sup>-</sup> (Abs)	Fe <sup>2+</sup> (Abs)
0	0.524	0.524	0.524	0.524
5	0.523	0.512	0.524	0.500
10	0.515	0.509	0.503	0.472
20	0.516	0.506	0.503	0.442
30	0.515	0.507	0.501	0.450

- croscopy. *J. Histochem. Cell Biol.*, 83: 409-411.
- BLANDAMER, M.J., BURGESS, J., DUCE, P.P., PAYNE, K.S., SHERRY, R., WELLINGS, P. & TWIGG, M.V., (1984). Kinetic and spectroscopic intimations of intermediates in nucleophilic attack at diimine complexes of iron (II) and of molybdenum (O). *J. Transit. Metal Chem.*, 9: 163-173.
- CAO, X., YE, Y. & LIU, S., (2011). Gold nanoparticle-based signal amplification for biosensing. *J. Anal. Biochem.*, 417: 1-16.
- HUTTER, E. & MAYSINGER, D., (2013). Gold-nanoparticle-based biosensors for detection of enzyme activity. *J. Trends. Pharmacol. Sci.*, 34: 497-507.
- LEI, K.F. & BUTT, Y.K., (2010). Colorimetric immunoassay chip based on gold nanoparticles and gold enhancement. *J. Microfluid. Nanofluidics.*, 8: 131.
- MAEDA, H. & ANZENBACHER, P., (2012). *Colorimetric Sensors: a book of Supramolecular Chemistry. From Molecules to Nanomaterials.*
- MASHHADIZADEH, M.H., ESKANDARI, K., FOROUMADI, A. & SHAFIEE, A., (2008). Self-Assembled Mercapto-Compound-Gold-Nanoparticle-Modified Carbon Paste Electrode for Potentiometric Determination of Cadmium (II). *J. Electroanalysis.*, 20: 1891-1896.
- MOHANTY, S. P. & KOUGIANOS, E. 2006. Biosensors: a tutorial review. *J. Ieee Potentials.*, 25, 35-40.
- PINGARRÓN, J.M., YÁÑEZ-SEDEÑO, P. & GONZÁLEZ-CORTÉS, A., (2008). Gold nanoparticle-based electrochemical biosensors. *J. Electrochim. Acta*, 53: 5848-5866.
- RAMADAN, A. & EL-NAGGAR, M., (1996). Synthesis, characterization and demonstration of superoxide dismutase-like activity of copper (II) chloride, bromide, nitrate, thiocyanate, sulphate, and perchlorate complexes with 2-methyl-amino pyridine. *J. Inorg. Biochem.*, 63: 143-153.
- YUE, G., SU, S., LI, N., SHUAI, M., LAI, X., AS-TRUC, D. & ZHAO, P., (2016). Gold nanoparticles as sensors in the colorimetric and fluorescence detection of chemical warfare agents. *J. Coord. Chem. Rev.*, 311: 75-84.
- ZHANG, J., YANG, C., WANG, X. & YANG, X., (2012). Colorimetric recognition and sensing of thiocyanate with a gold nanoparticle probe and its application to the determination of thiocyanate in human urine samples. *J. Anal. Bioanal. Chem.*, 40: 1971-1981.

#### AUTHOR (S) BIOSKETCHES

**Naimeh Mah-Heidari**, M.Sc., Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran, *Email: n.mahheidari@gmail.com*

**Jamal Rashidiani**, PHD Researcher, Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran, *Email: jrashidiyani@gmail.com*

**Fariba Dashtestani**, PHD Researcher, Department of Biology, Electronic Branch, Islamic Azad University, Tehran, Iran, *Email: d\_fariba@yahoo.com*

**Hamid Sedighian**, PHD Candidate, Applied Microbiology Center, Baqiyatallah University of Medical Sciences, Tehran, Iran, *Email: osedighian@gmail.com*

**Khadijeh Eskandari**, Assistant Professor, Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran, *Email: kheskandari@alumni.ut.ac.ir*

**Ramezan Ali Taheri**, PHD Researcher, Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran, *Email: r.a.taheri@gmail.com*