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The effects of glucose and glucose oxidase on the Uv-vis spectrum of gold nanoparticles: A study on optical biosensor for saliva glucose monitoring

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ABSTRACT

In this study we have investigated the effect of glucose and glucose oxidase (GOD) on the absorption spectrum of gold nanoparticles (Au NPs) with 10 − 13 nm diameter, in order to improve optical methods of glucose monitoring using surface plasmon resonance of these particles. Different concentrations of glucose solution in water were prepared in the range of human saliva intensity. Two procedures are applied to study glucose effects on the particles; mixing the glucose to the nanocolloid and then adding the GOD, and reversely mixing the glucose and GOD solutions and then pouring in to the nanocolloid. Two different results were obtained that are analyzed based on optical properties. In each method, the effect of glucose and GOD on the size and Uv–vis spectrum of gold nanoparticles has been investigated. Results were interpreted by the physical concept of surface Plasmon resonance (SPR) of Au NPs. This study can open new insight about optical glucose sensing based on surface plasmon of metal nanoparticles.

1. Introduction

Diabetes is one of the critical factors for death and physical disability. This disease can harm the body and bring disabilities like kidney failure, blindness, high blood pressure, and heart disease [1]. Determining the amount of Glucose concentration in blood is a crucial issue for diabetes treatment. Further, low glucose known as hypoglycemia can cause permanent brain damages [2,3].

Measuring blood glucose has been widely studied because of its importance for health care providers in medical process. Several methods have been presented based on biochemical and physical reactions of glucose with surrounding solution or environment such as electrochemical, fluorescence and colorimetric, but few methods developed are done easily, and at low cost. Today, a wide range of techniques has been promoted but efforts to improve the monitoring methods continue [4].

One of the most common and well known chemical instruments is oxidizing glucose using an enzyme called glucose oxidase (GOD). It can be used in electrochemical and photonic devices of glucose monitoring [4]. GOD is one of the well-known glycoprotein which contains 17 % of carbohydrates with the average diameter of 8 nm, and semi-spherical shape. Also, GOD is one of the three known enzymes that possess orthophosphates proteins. Because of its unique properties like dispersibility in water, resistance to precipitation and stability against proteases, make it favorable to use in different circumstances especially at room temperature [5,6]. Anions and cations can disable GOD at high and low values of pH [7]. Also, low intensities of heavy metallic ions like Ag, Pb and Hg in the solution can hinder GOD which is advantageous for biosensor devices [8]. All these properties make GOD be efficient for blood or saliva glucose monitoring. Measuring the amount of blood glucose by monitoring human's saliva is an established method. Viscosity of the human's saliva can be different can be different for individuals which depend on their diet and life style. Noteworthy, because of its simplicity, non-invasive, and being painless of this method, it can be a good alternative case for other methods in order to monitoring the concentration of blood Glucose in human's body [9].

On the other hand, gold nanoparticles (Au NPs) are well-known transition metal particles that their chemical activity varies in different sizes. In 1987, Haruta et al. [10] showed that Au NPs with the diameter less than 5 nm are the most effective catalysts for producing nanomedicines [11]. Au NPs is widely used in different disciplines such as biosensors [12], biology [13], DNA [14], genetics [15], physiotherapy of cancer cell and tumors [16], and targeted drug delivery [17]. Furthermore, Au NPs can be used as amplifier layers of signal and surface plasmonic resonance (SPR) applications [18]. In addition, for the excellent features of Au NPs such as facile synthesis route, special surface ratio, great chemical stability and compatibility, these particles has been appropriate in biosensing devices [19]. Optical properties of Au

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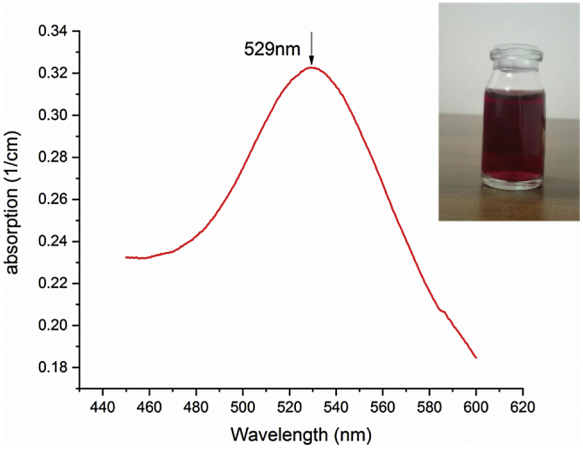

Fig. 1. Uv–vis spectrum of Au NPs.

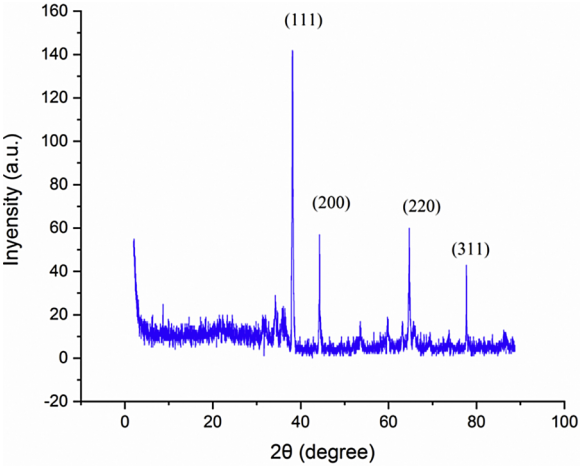

Fig. 2. XRD pattern of the particles.

NPs like SPR depend sensitively on the geometrical properties including size, shape and also the surrounding matrix and composition [20]. Au NPs has been extensively used in biosensors because of its local conductivity and special SPR [12]. These particles are used in glucose monitoring in both the electrochemical and optical methods [4,21].

Among the transducers that are used for developing biosensors, the optical ones are more suitable for analysis saliva because of its transparency [22]. In this way, colorimetric experiments like Lambert and Borchet [23], or spectrophotometric techniques that based on near infrared Raman (NIR/Raman) [24] and Clarke's electrode [25] can be mentioned. These methods are suitable for glucose monitoring because of their non-invasive route [23]. On the other hand, measuring the blood glucose through colorimetric method in the presence of Au NPs has been reported in other works. Subsequently, X. Liu et al. performed a colorimetric experiment to detect the glucose based on the enzymatic etching of gold nanorods [26]. Among the different sensors that based on optical-fiber, SPR-based sensors have raised many attentions. Technology of the SPR biosensors have presented in 1991 [27]. In 2010, G. Chandrasekar et al. found that hydroxide peroxide (H_2O_2) causes anisotropic shape transformation of gold nanorods (NRs) that influences the surface plasmon [28].

The oxidation process of glucose is catalyzed by GOD and the produced H_2O_2 can etch Au NRs to elliptical or even round shapes. Correspondingly, it shifts the plasmon resonance peaks which are sensitive to the size and shape and this technique is utilized to measure glucose concentration. Physical depiction of surface plasmon resonance of gold nano-rods GNRs is studied in [29].

Recently, some efforts for glucose monitoring using Au NPs has been done. The effects of glucose concentration on the absorption of gold nanocolloids have been studied by N. Albusta et al. [30]. Results showed that there is a linear relationship between the glucose concentration and the amplitude of absorption at entire the wavelength range.

In this study we have investigated the effect of glucose and GOD on the Uv–vis spectrum of Au NPs in order to find a method to glucose monitoring using these particles. Different concentration of glucose in water was used in the range of the intensity of the human saliva.

Two procedures are applied to test glucose effect on the particles; 1) glucose solution was mixed with the Au NPs and then GOD was added, 2) the glucose and GOD solutions were mixed together and poured into the Au NPs solution. The results of the two approaches, which were obtained by investigating optical properties of the different solutions, were different.

2. Experimental procedure

In this investigation Au NPs was synthesized through Turkevich method [31] that based on resuscitation gold atoms. GOD (50Ku), sodium citrate, Hydrogen Tetrachloroauric acid ($HAuCl_4$), and double distilled water were purchased from Sigma-Aldrich and were used without further purification. In order to synthesize Au NPs, 0.2 g of sodium citrate was poured in 20 mL distilled water and stirred for 20

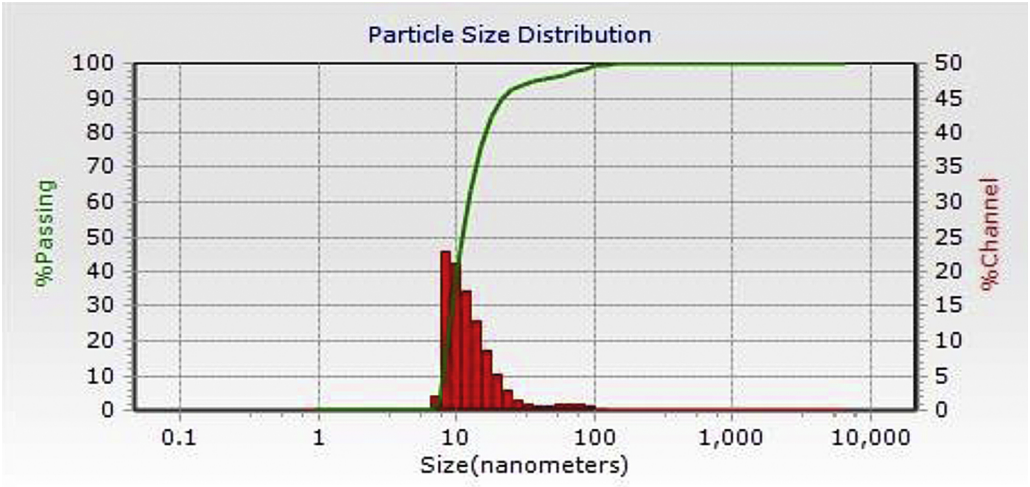

Fig. 3. DLS of synthesized Au NPs.

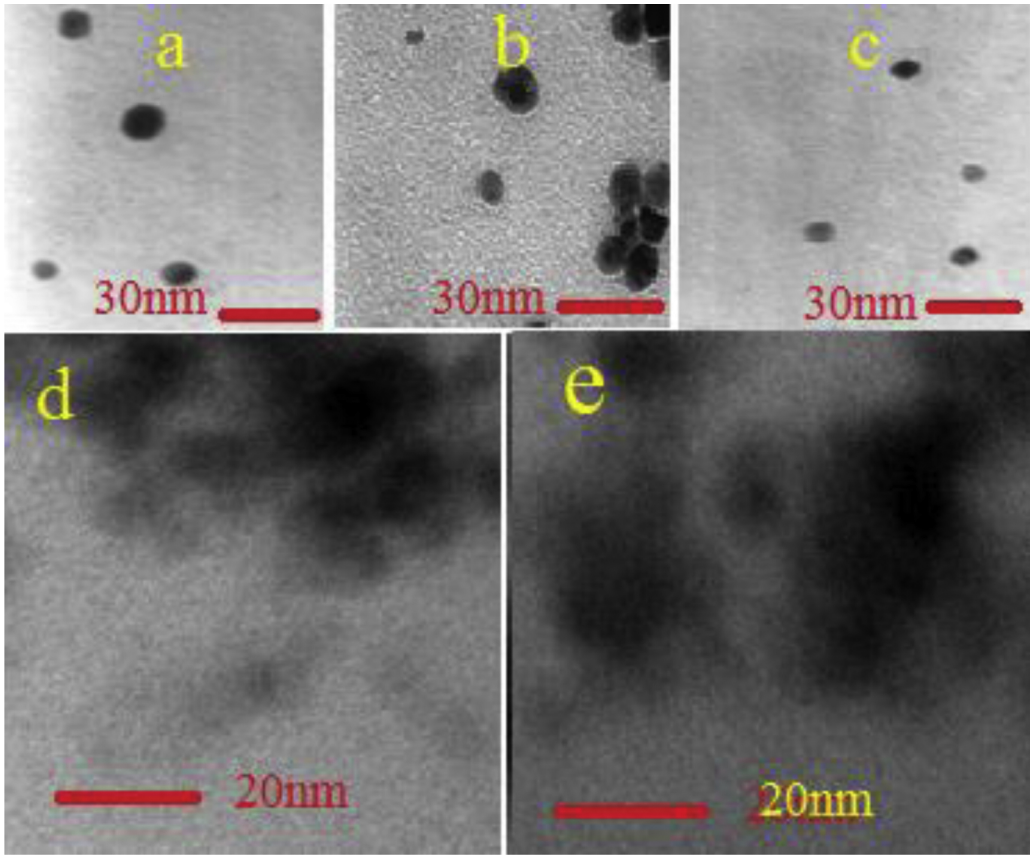

Fig. 4. TEM images of a-synthesized Au NPs. b- Separated individual particles produced by procedure.1.
c- Individual particles produced by procedure.2. D- Agglomerated produced by procedure.1.
e- Agglomerated produced by procedure.2.

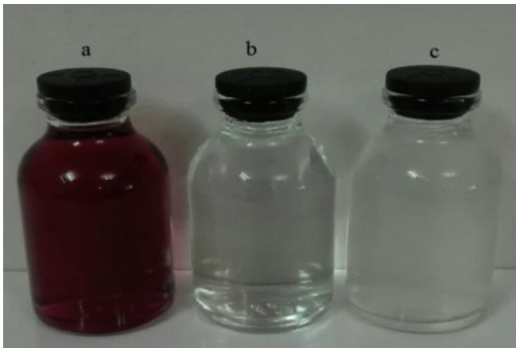

Fig. 5. Au nanocolloid (a) and solutions of Glucose (b) and GOD (c).

min at 50 °C. After that, 6.77 mg ($2 \times 10^{-5} mol$) of $HAuCl_4$ was dissolved in 20 mL distilled water and was placed on a heating mantel for 20 min at 100°C till its volume reached at 15 mL. After that, 2 mL of the sodium citrate solution (which contains 0.02 g or $7.98 \times 10^{-5} mol$ sodium citrate) was added drop-wise into the $HAuCl_4$ solution during 4 s and placed on heating mantel for 8 min at about 100°C. The color of the final solution was turned to red. By controlling the rate of the adding drops the size of the Au NPs were changed and solutions with different particle sizes were produced. By injection of sodium citrate solution within 4 s, the smallest and most dispersed particles are obtained. The color of the particles was ruby red (inset Fig. 1) as shown with the Uv–vis spectrum in Fig. 1. X-ray diffraction (XRD) pattern of the prepared powder of the colloid shows performance of Au NPs particles (Fig. 2).

Also, the surface plasmon resonance (SPR) peak at 529 nm indicates the formation of Au NPs. Dynamic light scattering (DLS) analyze has been carried out and reveals formation of 10 − 13 nm particles (Fig. 3) which is in agreement with TEM image (Fig. 4.a).

Citrate solution is usually used to resuscitate gold ions. In Turkovich method, in addition to its regenerative role, sodium citrate acts as coating agent and keeps the particles from aggregation due to the formation of a layer of adsorbed citrate anions on the surface of the Au NPs. The final colloid was exhibits excellent stability for a long period of time and no aggregation was observed even after several weeks.

After that, glucose solutions with different concentrations were prepared. In order to simulate the real condition, 4 solutions with various concentrations (90, 100, 110, 120 mg mL^{-1}) were prepared

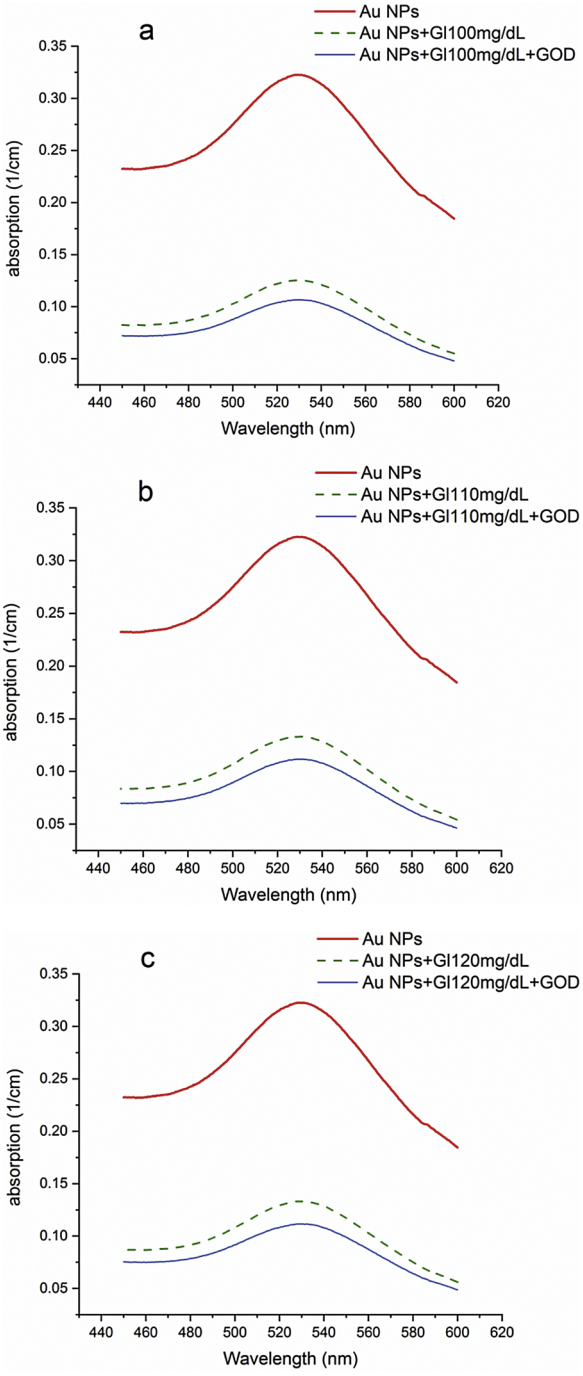

Fig. 6. Uv–vis of Glucose solution; a-100 mg/dl b-110 mg/dl c-120 mg/dl.

which are in the range of the concentration of glucose in human saliva. Water was used as the solvent. Also, 15 mg of GOD has been solved in 50 mL distilled water. The color of Au nano-colloid, glucose and GOD solutions are shown in Fig. 5.

3. Results and discussion

After preparation of the solutions, the effects of reaction between glucose, GOD and Au NPs were studied through two procedures with different orders of solutions mixing.

3.1. Procedure1

In this way, 1 mL Au nano-colloid was mixed with 1 mL glucose solution with concentration of 100 $mgdL^{-1}$. The Uv–vis spectrum and DLS analyses were taken from the solution. Then, 0.5 mL of GOD was poured in the solution and relaxed at least for 2 min to react with other agents. Again, the Uv–vis spectrums were taken from the solutions. This process is also performed for other glucose concentrations (110,120 $mgdL^{-1}$).

At any step, pouring a solution to the colloid reduces the NPs concentration. As we know, the absorption coefficient of a solution could be described as

$$\alpha = N \cdot \sigma \tag{1}$$

where N is the particles concentration (number per unit volume) and σ is the absorption cross section of the particles. To compare the absorption spectra of the particles, the curves are normalized to a unique volume. It reflects the effects of N in the Eq.1 and comparison is made on the particles optical cross section. For this, after adding glucose the data are multiplied by 2 and by adding GOD, are multiplied by 2.5. The normalized Uv–vis spectra are plotted in Fig. 6.

A noticeable effect in this procedure is the decrease in the amplitude of absorption by adding glucose and a little more decrease is observed by adding GOD solution, as shown in figure. According to Eq.1, decreasing the absorption can results from decreasing N or σ. In this condition, that N doesn't change, the only way to this reduction is decrease the optical cross section.

It has been detected that glucose can reduce the size of Au NPs and there is a linear relation between the glucose concentration and this decrease [30]. Decrease the particles size leads to reducing the amplitude of the absorption, according to Eq.1. Fig. 6 verifies the results of ref. [30], but changes in glucose concentrations are very low in our case (in comparison with ref [30]) and the variations in absorption are negligible in these concentrations.

As shown in Fig. 6, the peak of the SPR remains fixed during the process which has been confirmed in ref [30]. Since the peak of SPR shifts if the particles size become below 10 nm [32], it could be concluded that the size changes is negligible and small corrosion has been occurred. These effects of size reducing are more sensible for optical cross section (rather than the peak of SPR), for particles above 10 nm. For example, consider a 13 nm Au NP shrinks to 10 nm. The SPR peak reduces about 0.1 nm (ref [32].), but the cross section reduces to 60 % of the initial size ($\sigma \propto \pi r^2$). These results are in accordance with Fig. 6.

Also, the corrosion is a time dependence effect. Gradual oxidation of Au NPs can enhance by time [28]. More corrosion may occurs at long times but the saliva glucose measuring should take a few minutes. TEM images of the individual Au NPs show a relative decrease in the diameter (Fig. 4.b.).

It can be helpful to study the stability of the colloid. After 24 h, DLS and TEM experiments have been done. It has been shown that DLS measures the hydrodynamic diameter of the particles which contains

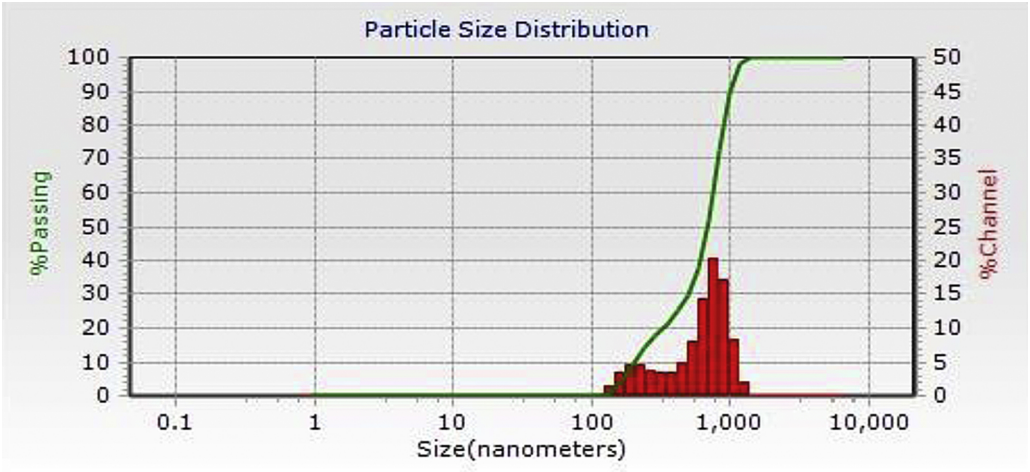

Fig. 7. DLS [Au NPs + glucose 100 mg/dl] (agglomeration after one day).

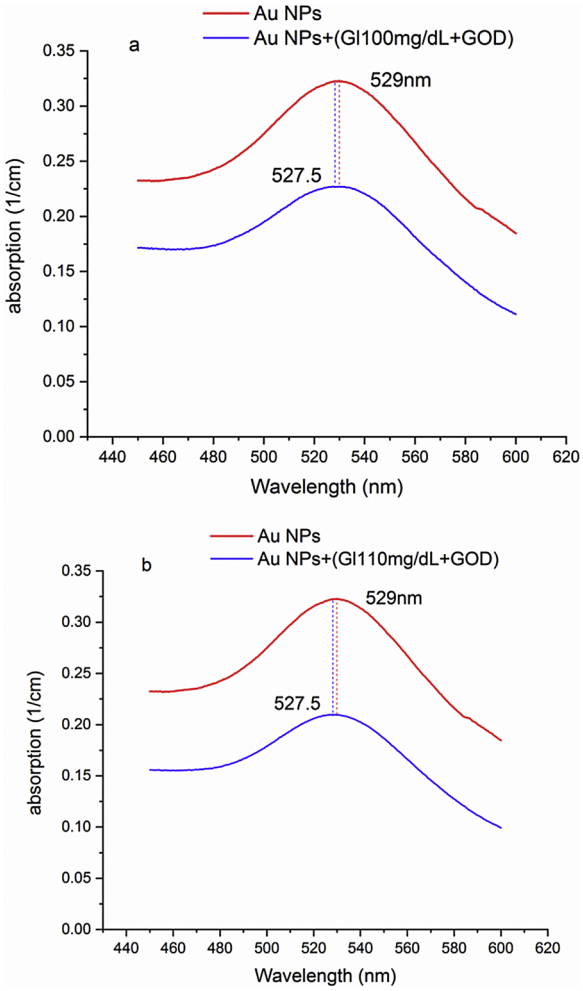

Fig. 8. Uv–vis of procedure 2 (peak shift = 1.5 nm).

the surrounding agents, but TEM gives the exact diameter of the particles [33,34]. In Fig. 7, DLS measurements show that the dimension increases dramatically by adding glucose. It can be attributed to aggregation of the NPs by adding glucose (and GOD) and also enhancing the amount of surfactant. TEM images confirm agglomeration of the particles after one day (Fig. 4.d).

3.2. Procedure2

In this step the glucose and GOD solutions were mixed and after a few seconds were poured in to the nanocolloid. First, 1 mL of glucose solution was mixed with 0.5 mL GOD solution and was stirred for 2 min. Then 1 mL of Au NPs solution was added drop-wise to it. Glucose and GOD have opportunity to react and the created electrons corrode and etch Au NPs. As the particles size decreases, the peak of SPR shifts to shorter wavelengths [32]. After 5 min, the Uv–vis spectrum was taken from the solution. This process is also performed for the three glucose concentrations (100, 110 and 120 $mgdL^{-1}$). The results of the Uv–vis spectrometer of two cases are shown in Fig. 8.

As shown in Fig. 8, the peak of SPR experiences a blue shift (about 1.5 nm) which indicates that the particles size reduces below 10 nm. It shows that the rate of size reduction is more than procedure 1. Besides, the amplitude of absorption reduces that as mentioned before, is due to reduction in cross section. TEM image in Fig. 3.c confirms the diameter of the particles reduces below 10 nm.

After 24 h, DLS experiment has been carried out, as shown in Fig. 9. Diagram indicates the agglomeration of the particles and also performance of heavy surfactant around the particles. In Fig. 4.e, TEM image has been offered that is in agreement with DLS results.

Finally, Fig. 10 shows the dependence of the absorption amplitude (at the peak of SPR) to the glucose concentration. This curve shows a nonlinear sensitivity of absorption on the glucose concentration that is fitted to the following equation:

$$\alpha = -0.0002055n^2 + 0.04142n - 1.86 \quad (2)$$

where n is the glucose concentration (mg/dL). This relation can easily give the glucose concentration using a simple absorption measurement of a green light beam.

Experiments are repeated for other sizes of gold NPs, but the best results are obtained for 10 – 13 nm particles. As mentioned before, this size is a critical diameter that if particle shrinks a little (below 10 nm), confinement size effects appears and beside reduction of cross section, blue shift of SPR occurs. For example, if we use 20 nm NPs, by adding glucose or glucose oxidase and reducing 2 – 3 nm of diameter, only the absorption cross section reduces and no shift in SPR will be observed.

4. Conclusion

In this work, gold nanoparticles were first synthesized by reduction method. The size of the Au NPs is 10 – 13 nm and color of the particles is ruby red. The effect of glucose and glucose oxidase on the Uv–vis spectrum of gold nanoparticles has been investigated. Different concentrations of glucose in water are prepared with the same range of

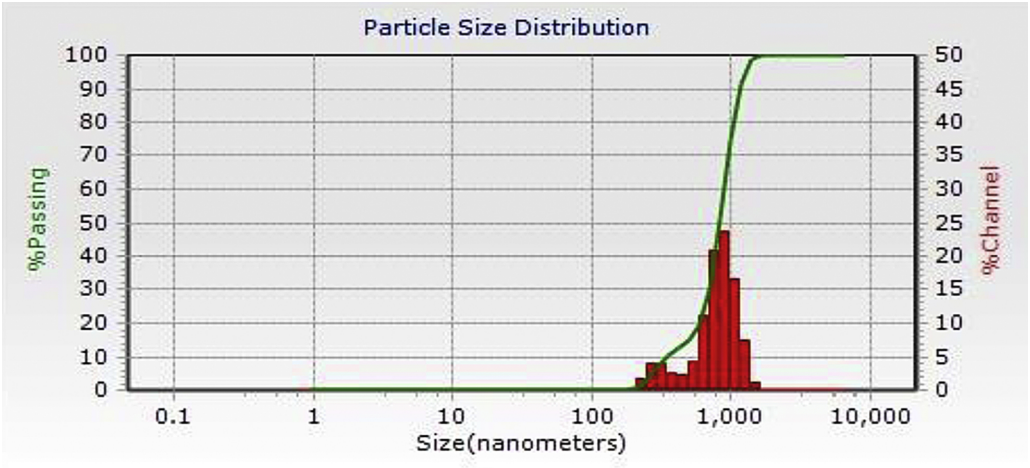

Fig. 9. DLS procedure 2 for glucose 110 mg/dl.

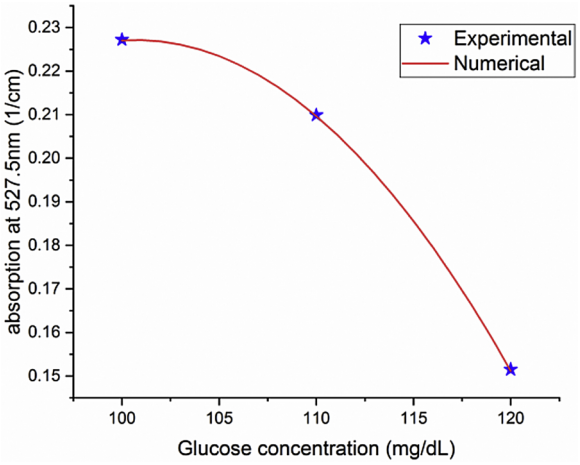

Fig. 10. Dependence of absorption peak on glucose concentration.

human saliva. First, glucose solutions are poured in gold nanocolloids and the Uv–vis, TEM and DLS analyze were done. Then, GOD solution is added to the mixture. It was found that no peak shift is observed in the peak of SPR, but the amplitude of absorption reduces in all the visible range. In the last procedure, a mixture of GOD and glucose is added to the gold nanocolloid and a 1.5 nm blue shift is observed at the peak. These effects depend to the glucose concentration and are related to the surfactant agents on the particles surface and also the order that they reached the particle. It was found that the mixture of glucose and GOD can reduce the particles size below 10 nm where both the absorption peak and amplitude are influenced. This study can open new sights in using plasmonic nanoparticles in optical monitoring of saliva glucose and glucose biosensors.

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