# Measurement of Glucose Concentration by Colorimetric Method Based on Aggregation of Gold Nanoparticles

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**Abstract**

**Metal nanoparticles, especially gold nanoparticle, is one of the nano materials investigated in many fields because of easy preparation and good biocompatibility. In this report, characteristic of the gold nanoparticles by pH or ion concentartion and measurement of glucose concentration by colorimetric mathod based on the aggregation of gold nanoparticles are described. Aggregation of unmodified gold nanoparticles is strongly influenced by ion concentration, but not influenced by pH. The concentration of glucose can be measured by colorimetric based on the aggregation of unmodified gold nanoparticles by reaction of glucose and glucose oxidase. The colorimetric method can be used to detected 1 mM glucose within 10 min. The detection method of glucose proposed by the authors does not require any expensive instrument. It is worthy of note that this rapid and simple method is one of the ideas developing of sensor using gold nanoparticles.**

**Keywords:** gold nanoparticles; glucose; aggregation; glucose oxidase; colorimetric method.

## 1 Introduction

Recently the applications of metal nanoparticles as they have unique physical and chemical properties associated with size quantization effect have been studied in bioanalysis and analytical chemistry (1-3). Especially, gold nanoparticles attract great scientific and technological interest because of large surface to volume ratio introducing surface functionality and localized surface plasmon resonance (LSPR) absorbance in the visible region (4. 5). The color of gold nanoparticles solution changes depending on interparticle distance and dielectric constant of the surrounding medium. The detection methods of color based on the aggregation of gold nanoparticles have been increasingly introduced in the field of biosensors. The colorimetric method using modified gold nanoparticles aggregation is widely reported in detection of nucleic acid (6), metal ion (7), proteins (8,9) and saccharide (10).

The biosensor for measurement of glucose is one of the widely researched biosensors. Qualitative and quantitative detections of glucose on the basing of enzyme reaction assays have been reported by many researchers. These sensing methods are coupled to a variety of potentiometric (11, 12), colorimetric (13, 14), piezoelectric (15) and amperometric method (16-18). There are a few reports on the colorimetric method using gold nanoaprticle aggregation to measure glucose (14, 19).

In this paper, we report the characterization of

unmodified gold nanoparticles aggregation having no influence by undergoing pH change in solution and influence by ion concentration. We also report the easy detection method of glucose by colorimetric method using gold nanoparticles aggregation based on enzyme reaction. We tested the effect of gold nanoparticle diameter and temperature of enzyme reaction on our detection method based on aggregation of gold nanoparticle. Our detection method of glucose does detect 1 mM glucose within 10 min for the gold nanoparticles of 50 nm diameter.

## 2 Materials and Methods

### 2-1 Materials

Gold nanoparticles with different diameters were purchased from Tanaka Kikinzoku Kogyo K. K. (Tokyo, Japan). Glucose oxidase from *Aspergillus sp.* was purchased from Toyobo Co., Ltd. (Osaka, Japan). D(+)-Glucose was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Disodium hydrogenphosphate and sodium dihydrogenphosphate for phosphate buffer (PB) were also purchased from Wako Pure Chemical Industries, Ltd. Other Chemicals for preparation of buffer solutions were purchased from Wako Pure Chemical Industries, LTD. (Osaka, Japan) and Nakarai Tesque, Inc. (Kyoto, Japan).

### 2-2 Apparatus

The gold nanoparticles aggregation was measured by Elx800 micro plate reader from BioTek Instruments, Inc. (Vermont, USA) as $O.D._{620}$. UV-vis spectroscopic measurements were performed by U-3000 spectrophotometer from Hitachi High-Technologies Co., Ltd. (Tokyo, Japan).

### 2-3 Gold nanoparticles preparation

Gold nanoparticles with average diameter of 20, 30, 40 and 50 nm were prepared by centrifugal operation (8,000 g for 15 min at 4 °C) for exchanging buffer solution. The gold nanoparticles were pulse-sonicated for a few seconds, and were added to pure water. After mixing, the gold nanoparticles were collected by the same process as describe above. After pulse-sonication, the gold nanoparticles were diluted with pure water to $O.D._{520}$ = 1.7 - 3.0.

### 2-4 Preparation of different pH buffer

The 20 mM hydrochloride / potassium chloride buffer was used as pH 1 and 2 buffer. The pH 3 buffer was 20 mM glycine / hydrochloride buffer. The 20 mM citric acid buffer was used as pH 4 and 5 buffer. The 20 mM potassium phosphate buffer was used as pH 6 and 7 buffer. The pH 7 potassium phosphate buffer was also prepared 100, 150 and 200 mM to observe the aggregation of gold nanoparticles as influence of buffer concentration. The pH 8 buffer was 20 mM trizma hydrochloride buffer. The 20 mM glycine / sodium hydroxide buffer was used as pH 9 and 10 buffer.

### 2-5 Effect of pH on gold nanoparticles aggregation.

The reaction of glucose and glucose oxidase produces gluconic acid, thus pH of the reaction solution lowers. We checked the effect of pH on aggregation of gold nanoparticles. The 300 μL of diameter 20 nm gold nanoparticles and 100μL of pH 1-10 buffer (20 mM) were mixed. The color change of mixed solutions as aggregation of gold nanoparticles was observed by the naked eye.

### 2-6 Effect of buffer concentration on gold nanoparticles aggregation.

The aggregation of gold nanoparticles was strongly influenced by ion concentration in solution. We evaluated the effect of ion concentration on the aggregation using potassium phosphate buffers. The 300 μL of diameter 20 nm gold nanoparticles solution and 20, 100, 150 and 200 mM phosphate buffer (100μL) were mixed. The color change of mixed solutions as a result of aggregation of gold nanoparticles was observed by the naked eye.

### 2-7 Measurement of glucose concentration

The diameter 40 and 50 mm gold nanoparticles solutions ($O.D._{520}$ = 2.8, $O.D._{520}$ = 3.1) were used to observe the aggregation as glucose and glucose oxidase reaction at room temperature of 26 °C. The 50 μL of diameter 40 nm gold nanoparticles solution was added to 100 μL of pure water. The 1, 5, 10, 25 and 50 mM glucose solution (20 μL) and 162 unit/ml glucose oxidase solution (20 μL) were mixed the gold nanoparticle mixture. The effect of the prepare temperature on aggregation of gold nanoparticles by glucose and glucose oxidase reaction was tested using the diameter 50 nm gold nanoparticles at temperature of 35 °C. The degree of aggregation of gold

nanoparticles was measured by micro plate reader as $O.D._{620}$. The test at different temperature was also the same procedure as that described above for room temperature of 26 °C. The diameter 30 and 50 nm gold nanoparticles solutions ($O.D._{520}$ = 3.4, $O.D._{520}$ = 3.1) were used for recording the UV-vis absorption spectrum after mixing gold nanoparticles, glucose and glucose oxidase solution. The diameter 30 nm gold nanoparticles solution (500 μL) was added to 1000 μL of pure water. The 25 and 500 mM glucose solution (200 μL) and 162 unit/ml glucose oxidase solution (200 μL) were mixed the gold nanoparticle solution. The color of the mixture of gold nanoparticles and enzyme reaction solution was measured by spectrophotometer at temperature of 30 °C. The diameter 50 nm gold nanoparticles solution also prepared by the same procedure as described above except for glucose concentration and temperature.

**3 Result and discussion**

3-1 Effect of pH to aggregation on gold nanoparticles

After exchanging buffer solution to pure water in diameter 20 nm gold nanoparticles solution, the gold nanoprticles solutions (300 μL) are added to pH 1-10 buffers (100 μL). The aggregation of gold nanoparticles can not be observed expect for pH 1 buffer solution by the naked eye (Fig.1). The color of mixed solution of gold nanoparticles and pH 1 buffer solution turned from red to pink. The pH of mixed solutions are also measured. The pH of mixed solutions is 3.4 – 9.1. The pH of gold nanoparticles solutions that are not exchanged solution to pure water indicated about 5 – 6. The aggregation of gold nanoparticles was not influenced by pH of solution.

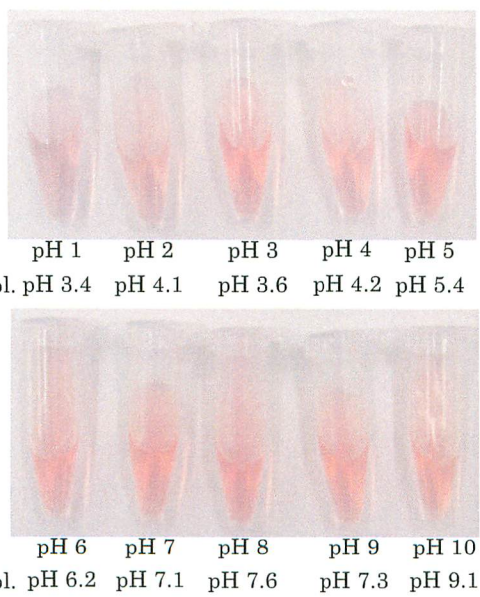


Fig.1 Photographs of gold nanoparticles solutions after mixing at various pH buffers.

3-2 Effect of ion concentration to aggregation on gold nanoparticles

The diameter 20 nm gold nanoparticles solutions (300 μL) are mixed with 20, 100, 150 and 200 mM phosphate buffers (100 μL) are evaluated that effect of ion concentration on the aggregation (Fig.2). The 20 mM phosphate buffer mixed solution does not show any different color compared with pure water mixed. The color of 100 mM phosphate buffer mixed solution turns deep red. The mixed solution of 150 mM phosphate buffer shows violet. The color of mixed solution turns clear and can observe dark gray particles at 200 mM phosphate buffer. The color of mixed solution changes form red to dark gray. It is suggested that gold nanpparticles aggregation depending on ion concentration in buufer. We use 20 mM different pH buffers to obseve the effect of pH in solution on the aggregation of gold nanoparticles. The 20 mM buffers does not influenced the aggregation of gold nanoparticles. However, in case of pH 4 citric acid buffer (200 mM) mixed with gold nanoparticles solutions, the color of mixed solution also shows the same result as that of 200 mM phosphate buffer mixed (data is not shown).

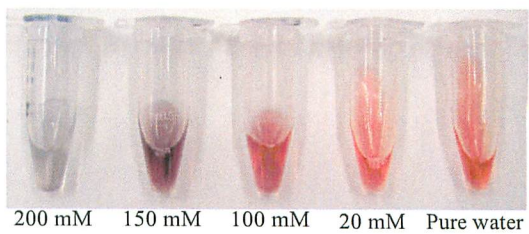


Fig.2 Photographs of gold nanoparticles solutions after mixing at various concentrations of potassium phosphate buffers.

3-3 Measurement of glucose concentration

The diameter 40 and 50 nm gold nanoparticles solutions are used to observe the aggregation as glucose and glucose oxidase reaction at room room

temperature of 26 °C (Fig.3). The color of 40 nm gold nanoparticles solution mixed with various concentrations of glucose and glucose oxidase turns from red to blue after 50 min reaction. 60 min after mixed, the color of 50 mM glucose mixed goled nanopartticles solution turns blue. The mixed solution of 25 mM glucose mixed shows violet at 70 min. Then, the color of 25 mM mixed solution turns from violet to blue at 80 min. The 10 mM glucose mixed gold nanopparticles solution shows blue at 180 min. The color of 5 mM glucose mixed gold nanoparticles solution does not show any difference compared to the control at 180 min. (Fig.3 A). The change of color of mixed solution depends on reaction time and glucose concentration. This phenomenon supports that the aggregation of gold nanoparticles is based on enzyme reaction. The color of 50 nm gold nanoparticles mixed with 5 mM glucose shows blue at 10min after mixed. 30 min after mixed, the color of gold nanoparticles solution mixed with 1 mM changes from red to violet (Fig.3 B). The color of 50 nm gold nanoparticles solution turns faster than 40 nm gold nanoparticles solution. It suggests that the aggregation of gold nanoparticles is affected by surface area of gold nanoparticle. The larger surface area is attacked more times by ion, and touched another gold nanoparticles.

The color change of 50 nm gold nanoparticles solution mixed with glucose and glucose oxidase solution is shown at reaction temperature 26 °C to 37 °C. The color of 1mM glucose mixed gold nanoparticles solution turns red to deep red at 10 min (Fig.4) The 5 and 50 mM glucose mixed gold nanoparticles solution turns violet color at 0 min. The color of 5 and 50 mM glucose mixed gold nanoparticles solutions shows clear including gray particles (Fig.4 A). The change of color 50 nm gold nanoparticles solutions mixed with glucose and

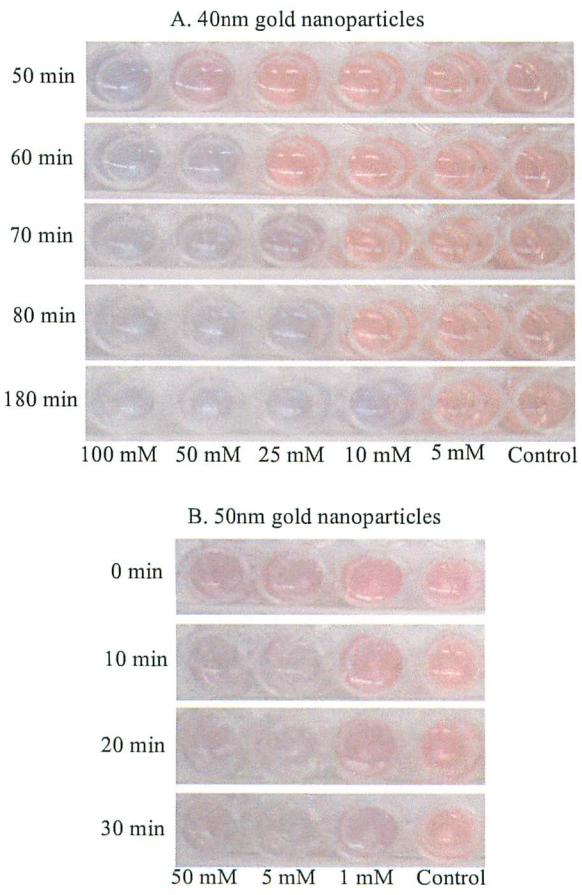


Fig.3 Color change of gold nanoparticles solution mixed with glucose at room room temperature of 26 °C.

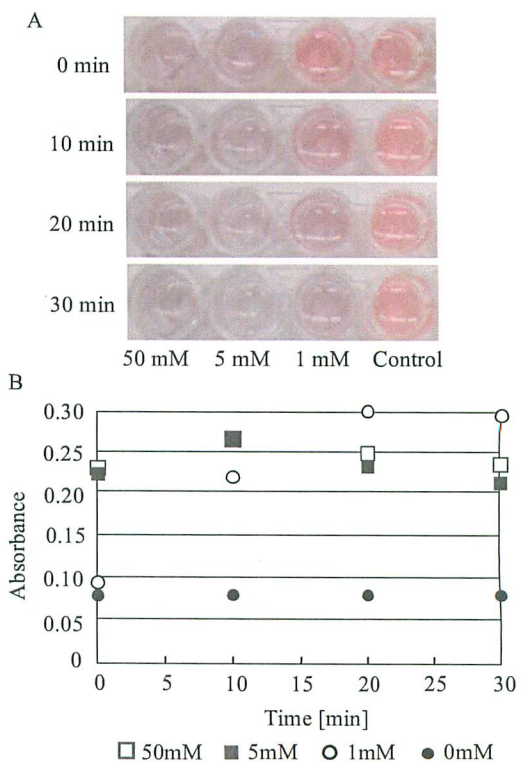


Fig.4 A: Color change of 50 nm gold nanoparticles solution mixed with glucose at room temperature of 37 °C. B: The time course of absorbance change at various glucose concentrations in mixed solution.

glucose oxidase solution at 37 °C is measured by by micro plate reader as $O.D._{620}$. As a result, the gold nanoparticles solution mixed pure water to use as control does not change the color of mixed solution, and the 5 and 50 mM glucose mixed gold nanoparticles solution immediately change the color of solutions (Fig.4 B). High temperature promotes the change of color of gold nanoparticles solutions depending on enzyme reaction.

The diameter 30 nm gold nanoparticles solutions are mixed with 25 and 500 mM glucose and glucose oxidase solution at temperature of 30 °C. The color change mixed solution is measured by spectrophotometer. The absorbance spectra of solutions are recorded under the wavelength of 450 – 800 nm (Fig.5). We can not find obvious difference in the plasmon resonance peak intensity of gold nanoparticles solution mixed with pure water to use as control (Fig 5 C). The absorbance spectra from 600 – 800 nm for 500 mM gulucose mixed gold nanoparticles solution decreases at 30 min. Then, the

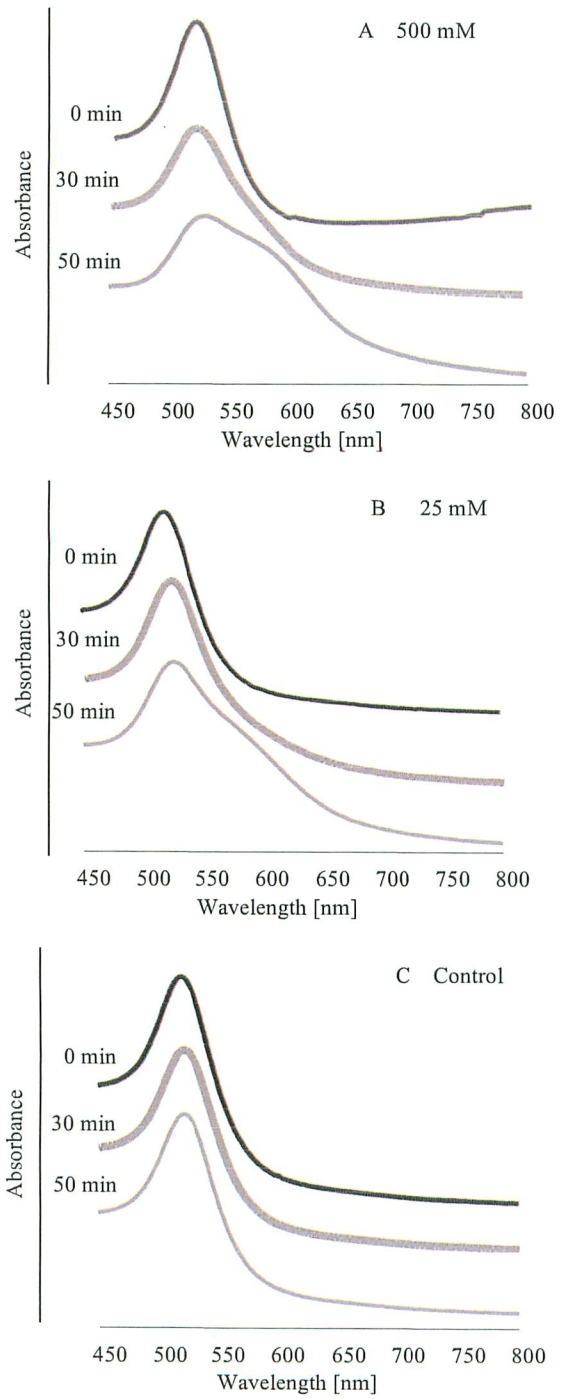


Fig.5 Absorption spectra of various concentrations glucose mixed 30 nm gold nanoparticles solution at temperature of 26 °C.

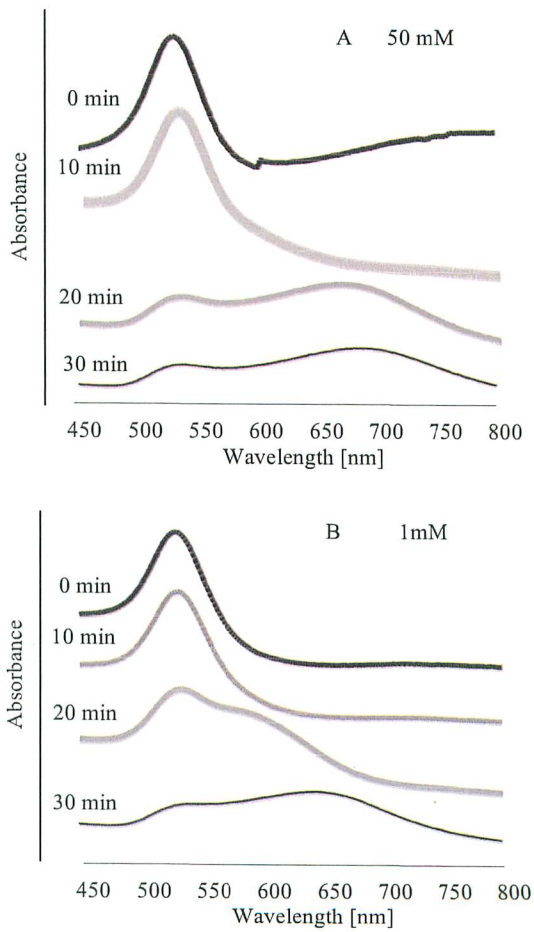


Fig.6 Absorption spectra of various concentrations glucose mixed 50 nm gold nanoparticles solution at temperature of 35 °C.

peak at 520 nm decreased at 50 min (Fig.5 A). The peak at 520 nm also decreases at 50 min in 25 mM glucose mixed gold nanoparticles solution (Fig.5 B). However, the rate of decreasing is lower than the absorbance spectra for 500 mM glucose mixed golid nanoparticle solution. 50 nm gold nanoparticles solutions are also measured the color change at mixed 1 and 50 mM glucose by spectrophotometer (Fig.6). The peak at 520 nm in 1 and 50 mM glucose mixed gold nanoparticles solutions decreases after 20 min (Fig.6 A, B). The absorbance spectra of 50 nm gold nanoparticles solution mixed with pure water and glucose oxidase solution does not show any difference at 0 min (data is not shown). The peak shift at 520 nm due to near-field coupling that occurred when the interparticle distance decreases (20). The absorbance peak at around 600 – 700 nm should be attributed to the electric dipole-dipole interactions and the coupling between the plasmons of neighboring particles in the aggregates (8, 21), indicating the aggregation of gold nanoparticles. Compared with 30 nm gold nanoparticles at 30 °C and 50 nm gold nanoparticles at 35 °C, the absorbance spctra of 50 nm gold nanoparticles mixed solutions changes at early time after mixed and lower concentration of glucose. The results support that the aggregation of gold nanoparticles is due to enzyme reaction of glucose and glucose oxidase.

## 3 Conclusions

The aggregation of unmodified gold nanoparticles is affected by ion concentration and not influenced by pH. We could detect 1 mM glucose within 10 min colorimetric method based on the aggregation of 50 nm gold nanoparticles. The diameter of gold nanoparticles influences the aggregation compared with different diameter gold nanoparticles. These results are worthy of developing sensor using gold nanoparticles.


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