The 21St Iranian Conference on Optics & Photonics &
7th Iranian Conference On Photonics Engineering
13-15 January 2015, Shahid Beheshti University

پایش پیوسته نانوذرات طلا با استفاده از میکروسکوپ عدسی نور- گرمایی

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چکیده - در این تحقیق، یک سیستم میکروسکوپ عدسی نور- گرمایی معکوس مجهز به فلوسل طراحی شد و اندازه گیری غیرمستقیم سیگنال پلاسمونیک نانوذرات طلای ساخته شده به روش کاهش سیترات با پدیده عدسی نور- گرمایی انجام گردید. اندازه گیری با استفاده از یک لیزر تهییج متوالی با طول موج ۵۳۲ نانومتر و لیزر پراب ۶۵۲ نانومتر انجام شد. برای این منظور تغییر شدت نور در مرکز پرتو پراب با استفاده از یک فتودیود پایش شد. نتایج حاصله نشان داد که پایش پیوسته نانو ذرات طلا در حجم میکرو و نانو در محدوده ۱/۶-۶/۴ نانوذره در حجم کانونی پرتو تهییج با موفقیت انجام شد. عدم تکرار پذیری سیگنال عدسی نور- گرمایی نانوذرات طلا در آب با استفاده از افزایش سرعت فرکانس مودولاسیون پرتو تهییج و بکارگیری فلو سل با سرعت جریان تزریق بهینه، برطرف گردید.

کلید واژه- میکروسکوپ عدسی نور- گرمایی معکوس، نانوذرات طلا، پایش پیوسته، پلاسمونیک.

Online Monitoring of Gold Nanoparticles by Photothermal Lens Microscopy

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Abstract-*In this paper, we have developed an inverted photothermal lens microscope with a flow through optical cell to detect the gold nanoparticles (AuNPs) prepared by trisodium citrate reduction. The measurements were made using a continuous-wave excitation (wavelength, 532 nm) and probe laser beams (wavelength, 652 nm).The changes in the transmitted probe beam's center intensity were detected with a photodiode. The indirect detection of the plasmonic signals of AuNPs was done by using thermal lens phenomena. The results show the possibility of online determination of AuNPs in micro and nano volume in the range of 1.6-6.4 particles per the focal volume of the excitation beam. The irreproducibility of AuNPs's thermal lens signal in water is eliminated by increasing in the modulation frequency of excitation beam and employing flow optical cell with an optimum flow velocity of sample injection.*

Keywords: AuNPs, Inverted Photo Thermal lens Microscope, Plasmonic.

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1 Introduction

Gold Nanoparticles are mainly used as substrates for enhancement of sensitivity or as stable labels for bio molecule detection [1]. Therefore, the quantitative measurement of Gold nanoparticles in a controllable way is highly important for nanoparticles-based analytical applications. UV-Vis Spectrophotometry is the most common detection method for quantitative measurement of AuNPs [2,3]. But recently, researches go through single-nanoparticle detection [4]. So, a highly sensitive detection system is required.

Photo Thermal Lens Microscopy (PTLM) as a highly sensitive detection method have been used, it is a sort of photothermal spectrometry done under an objective lens of a microscope. The photothermal effect is based on photo absorption and subsequent heat release, and thus it can be applied to almost all target compounds by simply selecting a suitable excitation beam wavelength [5,6].

Duo to the wide application of AuNPs in biomolecules and drug detection [3], on line monitoring of that by a sensitive method is highly important. Therefore in this work the online monitoring of AuNPs with an inverted thermal lens microscope is done.

2 Experimental

2.1 Instrumentation

The excitation beam was a 50mW diode solid-state laser with an emission line of 532nm which was modulated with a mechanical chopper. The modulation frequency was 500Hz. A 5 mW laser 652 nm were used as probe beam. The two beams were coaxially aligned with a 50% beam splitter and introduced into an inverted microscope. Magnification and numerical aperture of the objective lens were 3X and 0.08, respectively. After passing through the sample in the optical flow cell 60µL with the path length of 1mm, the excitation laser was cut by an interference filter. The probe beam was diverged and after passing through a pinhole was detected by a photodiode connected to a lock-in-amplifier. The time constant of the lock-in amplifier was 10 ms.Figure 1 shows a schematic diagram of designed PTLM system.

The sampling was done with the optimum flow velocity of 10µl/min.

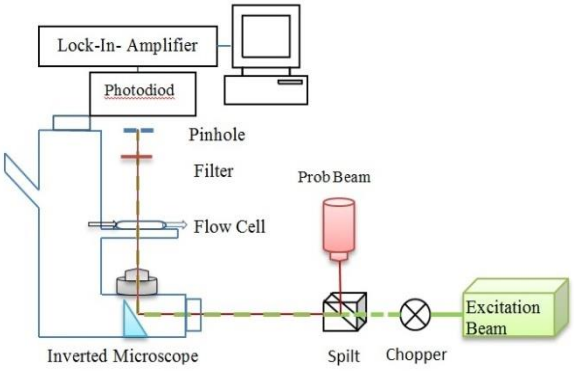


Figure 1.Schematic diagram of the experimental setup.

2.2 Preparation of AuNPs

Colloidal gold nanoparticles were prepared through the classical citrate reduction method reported previously [7]. Briefly, in a 250mL round bottom flask equipped with a condenser, 50 mL of 400ppm $HAuCl_4$ was brought to a rolling boil with vigorous stirring. Then 5.0 mL of 0.04M sodium citrate solution was quickly added into the solution with stirring .The color of solution became deep red, boiling was continued for 20 minutes. Then the solution was cooled down to room temperature and stored at 4 ℃. As illustrated in Figure 2 and 3, the gold colloid was characterized by UV-Vis spectra with maximum absorption of 520nm and DLS revealed a particle diameter of 13nm.

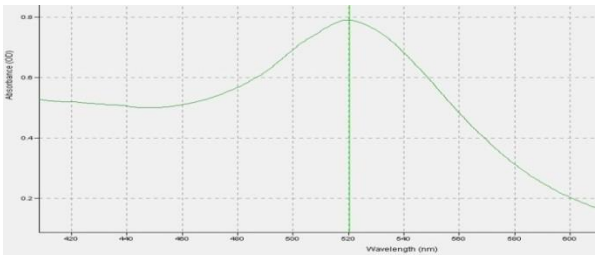


Figure 2: The UV-Vis spectra of AuNPs.

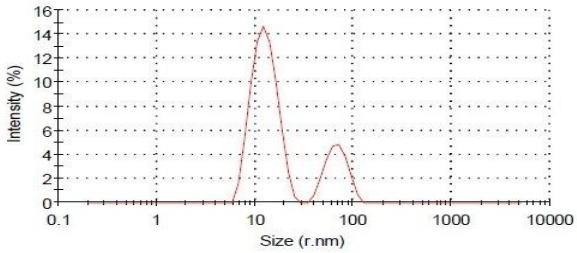


Figure 3: The size distribution of AuNPs.

Additionally, the concentration of the stock solution of AuNPs was determined to be 6.67 nM according to the Beer's law using an absorption

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coefficient of 2.7 × $10^8 cm^{-1}$ M^{-1} at 520 nm for Au NPs of 13 nm diameter [7].
Gold nanoparticle solutions were diluted and placed into a plastic optical cell (to avoid the adsorption of colloid gold particles, which was relatively high for glass cells). For making calibration curves, series of samples were prepared by successive dilution of AuNPs solutions and the on-line photo thermal lens measurement of each concentration was done for 30 s and average of them was calculated.

3 Result and Discussion

Thermal lens signals were obtained by constructed PTLM. Then, amplitudes of thermal lens signals for a steady state thermal lens were measured as followed:

$$TLM_{signal} = \frac{I_0 - I_\infty}{I_\infty} = -\frac{2.303(dn/dT)}{\lambda k} PA \quad (1)$$

Where I_0 and I_∞ are, the intensity of probe beam during the photothermal lens effect at initial time (t=0), and steady-state time (t= ∞), respectively. *P* is the power of pump laser, dn/dT is the change in solvent refractive index with temperature, λ is the laser wavelength, *k* is the thermal conductivity and *A* is the absorbance of the sample.
It was concerned that the pulse heights signals in water were quite different. This might be due to the high heat conductivity of water. By increasing the modulation frequency of excitation beam to 500Hz this irreproducibility was improved.
The detection volume of the PTLM can be estimated on the basis of the theory of thermal lens [8]. The detection volume is equal to the focal volume of the excitation beam, where the thermal lens is generated. The focal volume is calculated by the following equations:

$$d = 1.22 \times \lambda / NA \quad (2)$$
$$I_c = \pi (d/2)^2 / \lambda \quad (3)$$

Where *d*, λ and I_c are diameter, wavelength, and confocal length of the excitation beam, respectively [9].
From the Eqs. (2) and (3), in this work, the focal volume is calculated to be 1.6 fL ($f=10^{-15}$). Therefore, the expected number of molecules in the detection volume can be calculated by the product of the detection volume, the concentration of sample and Avogadro's number.
The AuNPs concentrations used in this work was in the range of 1.67-6.67nM which was corresponded to 1.6-6.4 nanoparticles per the focal volume of the excitation beam. The time constant of the lock-in amplifier was 10 ms. The physical meaning of this expected number is the time average of the numerous events during a measuring times.
As illustrated in figure 4, the on-line measurement of AuNPs signal shows that increase of AuNPs concentration cause decrease of Lock-in-amplifier signal which is corresponded to the basic of photothermal lens theory [6].
In Figure 5, the calibration curve was plotted for different concentrations of AuNPs under the optimum conditions. The graph exhibits a linear behavior in the range of 1.6-6.4 nanoparticles/ focal volume of excitation beam. The signal measurement was performed 10 times for water as a blank sample and the limit of detection (LOD) was calculated by following equation (4).

$$LOD = \frac{3 \times standard\ deviation\ of\ the\ blanksignals}{slope\ of\ the\ calibration\ curve} \quad (4)$$

The achieved LOD and RSD (n=10) for AuNPs were 0.7 nM and 3.43% respectively.

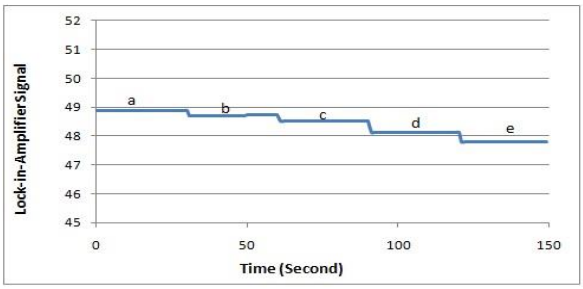


Figure 4. The On-line measurement of a series of AuNPs concentration. a:1.67nM, b:2.50nM, c:3.33nM, d:5.00nM, e:6.67nM

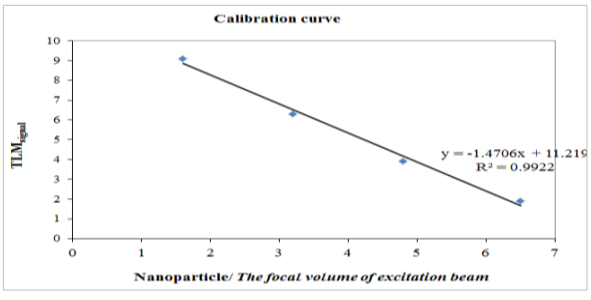


Figure 5: Calibration curve for different concentration of AuNPs

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Therefore, we demonstrated that the photothermal signals from countable particle could be obtained by the PTLM and their quantitative determination was possible.

4 Conclusion

We measured the thermal lens signal of AuNPs by PTLM with concentration range of 1.6-6.4 and detection limit of 0.73 nanoparticles per the focal volume. This measurement method is useful for detecting biomolecules which have the ability of coupling with AuNPs. Since the difficulty of sampling in micromolar and nanomolar scale, we utilized the online sampling which was quite compatible with PTLM system.

Acknowledgement

The authors are grateful to the support from the Iran National Science Foundation (INSF).

References

[1] K. Mawatari, M. Tokeshi and T. Kitamori,"Quantitative Detection and Fixation of Single and Multiple Gold Nanoparticles on a Microfluidic Chip by Thermal Lens Microscope"*Analytical sciences.* Vol. 22, No. 5, pp. 781-784, 2006.

[2] G. Shen, H. Xu, A. S. Gurung, Y. Yang and G. Liu,"Lateral Flow Immunoassay with the Signal Enhanced by Gold Nanoparticle Aggregates Based on PolyamidoamineDendrimer"*Anal Sci*. Vol. 29, No. 8, pp.799-804, 2013.

[3] S. L. Zhu, J. B. Zhang, L. Y. L. Yue, D. Hartono, and A. Q. Liu,"Label-free protein detection via gold nanoparticles and localized surface plasmon resonance"*Advanced Materials Research*, Vol. 74, 95-98, 2009.

[4] D. Yin, E. J. Lunt, M. I. Rudenko, D. W. Deamer, A. R. Hawkins and H. Schmidt,"Planar optofluidic chip for single particle detection, manipulation, and analysis"*Lab on a Chip.* Vol. 7,No. 9, pp. 1171-1175, 2007.

[5] Y. Kikutani, K. Mawatari, K. Katayama, M. Tokeshi, T. Fukuzawa, M. Kitaoka and T. Kitamori,"Flowing thermal lens micro-flow velocimeter" *Sensors and Actuators B: Chemical.* Vol. 133, No. 1, pp. 91-96, 2008.

[6] N. Shokoufi, S. Madarshahian, *Thermal lens spectrometry: Techniques and Instrumentation*, LAP Lambert Academic Publishing, pp. 43-47, 2012.

[7] F. Zhang, L. Zeng, Y. Zhang, H. Wang and A. Wu,"A colorimetric assay method for Co2+ based on thioglycolic acidfunctionalized hexadecylrimethyl ammonium bromide modified Aunanoparticles (NPs)"*Nanoscale*. Vol. 3, pp.2150–2154, 2011.

[8] S.E. Bialkowski, *Photothermal Spectroscopy Methods for Chemical Analysis*, Wiley, New York, 1996.

[9] M. Tokeshi, M. Uchida, K. Uchiyama, T. Sawada and T. Kitamori,"Single- and countable-molecule detection of non-fluorescent molecules in liquid phase"*Journal of Luminescence*. Vol. 83–84, *pp.* 261-264, 1999.

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