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

طیف بینی فتوترمال لنز برهمکنش نانوذرات طلا با مولکولهای زیستی گوگرددار

نادر شکوفی*، عاطفه عباسی عهد و کاظم کارگشا*

پژوهشکده فناوری پاک، پژوهشگاه شیمی و مهندسی شیمی ایران، تهران، ایران

چکیده - حساسیت، خواص نور گرمایی و مصرف کم حلال ویژگی های برجسته فتوترمال لنز میکروسکوپی برای اندازه‌گیری مولکول‌های زیستی می باشد. در این تحقیق از یک سیستم فتوترمال لنز میکروسکوپ هم محور مجهز به یک لیزر تهییج متوالی با طول موج 532 نانومتر و یک لیزر پراب 652 نانومتر، جهت بررسی برهمکنش نانوذرات طلا با کاپتوپیریل به عنوان نمونه ای از مولکول زیستی گوگرد دار استفاده گردید. نانوذرات طلا به جهت رزونانس پلاسمون سطحی در ناحیه مریی جذب قوی دارند. با افزودن مقادیر کم کاپتوپیریل تجمع اتفاق می افتد و محلول نانو ذره بتدریج تغییر رنگ می یابد و در نتیجه آن سیگنال فتوترمال لنز تقویت می شود. این تقویت مربوط به کاهش هدایت حرارتی نانوذرات طلا در اثر اتصال به مولکول های کاپتوپیریل می باشد. نتایج حاصله نشان داد که تغییرات در سیگنال فتوترمال لنز متناسب با تغییرات غلظت کاپتوپیریل در محدوده mgL^{-1} 2-1/. می باشد.

کلید واژه- پلاسمونیک، فتوترمال لنز میکروسکوپ، مولکول زیستی، نانوذرات طلا.

Photo Thermal Lens Spectrometry of Gold Nanoparticles Interaction with Thiol Containing Biomolecules

Nader Shokoufi*, Atefeh Abbasi Ahd and Kazem Kargosha*

Department of Green Technologies, Chemistry & Chemical Engineering Research Center of Iran (CCERCI), Tehran, Iran

Abstract-*Sensitivity, thermooptical properties and low sample consumption are major properties that highlighted photothermal lens microscopy (PTLM) for detection of biomolecules. In this work, we utilized a coaxial photo thermal lens microscope equipped with a continuous-wave excitation (wavelength, 532 nm) and probe (wavelength, 652 nm) laser beam to investigate the interactions of gold nanoparticles (GNPs) with Captopril as an example of thiol containing biomolecules. GNPs due to their surface plasmon resonance (SPR) possess the absorption in visible range. Following the addition of trace Captopril, the color of gold colloid solution gradually changes. As a consequence the Photothermal lens signal enhanced. The changes of PTLM signal were found to be proportional to the concentration of Captopril over the range of 0.1–2 mgL^{-1}.*

Keywords: Biomolecules, Gold nanoparticles, Photothermal lens microscopy, Plasmonic.

1 Introduction

The photothermal lens technique is based on measurement of the temperature rise that is produced in an illuminated sample as a result of nonradiative relaxation of the energy absorbed from a laser. Photo Thermal Lens Microscopy (PTLM) is a sort of photothermal spectrometry done under an objective lens of a microscope.
In PTLM, the excitation wavelength of commercially available detector is around 500 nm, which would limit the applicability of PTLM. To extend the application area, PTLM with a UV laser excitation has been developed for detecting various analytes without derivatization [1,2]. However, the excitation with the focused UV laser can decompose analytes by photochemical reactions, resulting in the reduction of the detection sensitivity. To overcome these problems Gold nano particles has been used.
The characteristic color changes accompanying the surface properties and the aggregation formation of the GNPs have been exhaustively utilized in the sensing strategy for various analytes such as inorganic ions [3], DNA [4], and proteins[5]. For the selective sensing of these compounds, functional molecules including recognition sites are attached onto the GNPs surface to induce the aggregate formation. On the other hand, negatively charged GNPs bind amine, cyanide, and thiol groups onto their surfaces [6,7], so that the semi-selective sensing can be accomplished on the basis of these functional groups-triggered aggregates. In this work we utilized a PTLM to investigate the interactions of gold nanoparticles with Captopril as an angiotensin converting enzyme inhibitor.

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

2.2 Preparation of GNPs

Colloidal gold nanoparticles were prepared through the classical citrate reduction method reported previously [8]. All glassware used in this experiment were cleaned with aqua regia (3:1 HCl/HNO_3) and rinsed with tap water and deionized water, and dried prior to use. Briefly, in a 250mL round bottom flask equipped with a condenser, 50 mL of 400ppm HAuCl4 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 then stored at 4 °C. The gold colloid was characterized by UV-Vis spectra with maximum absorption of 520nm and DLS revealed a particle diameter of 13nm.
Additionally, the concentration of the GNPs in the testing solution was determined to be 6.7 nM according to the Beer's law using an absorption coefficient of $2.7 \times 10^8 cm^{-1} M^{-1}$ at 520 nm for GNPs of 13 nm diameter [8].
GNPs 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).

2.3 Preparation of Captopril

Cap standard (Zhejiang Huahai Pharmaceuticals Company of China, Beijing) was directly dissolved in water to prepare stock solution of 2.0×10^{-2} mol L^{-1} and stored at 0–4°C. The working solutions were then prepared by appropriate dilution of this stock solution. All other reagents were of analytical grade, and doubly distilled water was used throughout.

2.4 General Procedure

About 2mL of 3.4nM GNPs solution was pipetted into a 5 mL baloon, then 2mL of Captopril working solution were added. The mixture was then diluted to 5 mL. The mixture was rapidly directed to an optical flow cell to be detected with a Photothermal lens microscope.

3 Result and Discussion

Following the addition of trace Cap, the colour of gold colloid solution gradually changes. It is demonstrating that the aggregation of gold nanoparticles occurs in the presence of Cap. As it is illustrated in UV-Vis spectrum of figure 1, there is not a drastic change in the absorbance around 532nm, it shows a red-shift to the longer wavelength. While in the same wavelength of 532nm, Photothermal lens signal enhancement of GNPs is observed in consequence of adding Cap concentration.

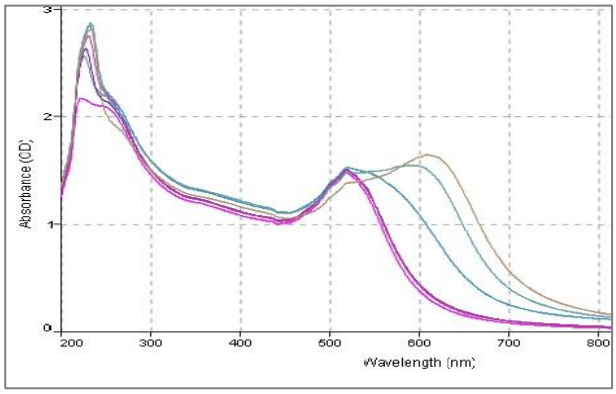

Figure 1: Red-shift absorption in consequence of GNPs aggregation.

Thermal lense signal enhancement goes through the thermooptic behaviour of GNPs. Density of Cap molecule binding to the surface of GNPs has been previously reported [9] from the equation (1):

$$d = C_{cap} V N_0 / \pi n_p D^2 \qquad (1)$$

Where C_{cap} is the Cap concentration, V the volume of the sample, n_p the number of gold nanoparticle in the sample, D the diameter of gold nanoparticle and N_0 is the Avogadro constant.

We calculate that there are about 4.1×10^3 Cap molecules binding to the surface of a 13-nm diameter gold nanoparticle. The thiol portion of Cap covalently binds to the surface of the gold nanoparticle and hence forms the core-shell super assembly, which decrease the thermal conductivity of GNPs. From equation 2 it is deduced that this decrease of thermal conductivity will result the increase of PTLM signal.

The thermal lens signal is measured as follow:

$$(2)\ TLM_{signal} = \frac{I_0 - I_\infty}{I_\infty} = -\frac{2.303(dn/dT)}{\lambda k} PA$$

Where I_0 and I_∞ are, the intensity of probe beam during the thermal lens effect at initial time (t=0), and steady-state time ($t=\infty$), 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.

As it is illustrated in Figure 2, different concentrations of Cap are used to construct the calibration curves. There is linear relationship between the intensities and the Cap concentrations over the range of 0.1–2 mgL^{-1}.

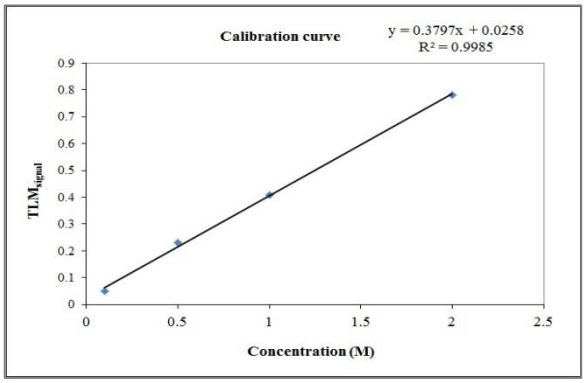

Figure2: Calibration curve for various concentrations of CAP.

As it is shown in table 1, this range of linearity is near from that of RLS spectroscopy method and the obtained detection limit of 100 μgL^{-1} is 10 fold lower than non-labelled UV 250nm detection.

labelling	Detection System	Linear range ($mg\ L^{-1}$)	Detection limit (μgL^{-1})	Ref
AuNPs	PTLM	0.1–2	100	
AuNPs	RLS	0.1–1.7	32.0	[9]
(p-BPB)	UV260		100	[10]
-	UV250		1000	[10]

Table 1: The comparative table of Coptopril detection with different method of detection.

4 Conclusion

Photothermal lens microscopy was successfully used to investigate the process of colloidal gold aggregation induced by a kind of thiol containing pharmaceutical Cap. The results show a minimum of 100 μgL^{-1} of Cap detection under certain conditions. Therefore, this method may have potential applications in determining thiol-containing substances.

Acknowledgements

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

References

[1] S. Hiki, K. Mawatari, A. Hibara, M. Tokeshi, T. Kitamori, "UV excitation thermal lens microscope for sensitive and nonlabeled detection of nonfluorescent molecules", *Anal. Chem*. Vol. 78, pp. 2859-2863, 2006.

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

[3] S.Y. Lin, C.H. Chen, M.C. Lin and H.F. Hsu, "A Cooperative Effect of Bifunctionalized Nanoparticles on Recognition: Sensing Alkali Ions by Crown and Carboxylate Moieties in Aqueous Media" *Anal. Chem*. Vol.77, pp. 4821-4828, 2005.

[4] A. Charrier, N. Candoni and F. Thibaudau, "DNA detection method based on the two-dimensional aggregation and selective desorption ofA nanoparticle probes" *J. Phys. Chem. B.* Vol. 110, No. 26, pp. 12896-12900, 2006.

[5] 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.

[6] R.G. Freeman, K.C. Grabar, K.J. Allison, R.M. Bright, J.A. Davis, A.P. Guthrie, M.B. Hommer, M.A. Jackson, P.C. Smith, D.G. Walter and M.J. Natan, "Self-Assembled Metal Colloid Monolayers: An Approach to SERS Substrates" *Science magezine*. Vol. 267, No. 5204, pp. 1629-1632, 1995.

[7] Z. Zhong, S. Patskovskyy, P. Bouvrette, J.H. Luong and A. Gedanken, "The Surface Chemistry of Au Colloids and Their Interactions with Functional Amino Acids" *J. Phys. Chem. B.* Vol. 108, No. 13, pp. 4046, 2004.

[8] F. Zhang, L. Zeng, Y. Zhang, H. Wang and A. Wu, "A colorimetric assay method for Co2+ based on thioglycolic acid functionalized hexadecyl rimethyl ammonium bromide modified Au nanoparticles (NPs)" *Nanoscale*. Vol. *3*, pp. 2150–2154, 2011.

[9] Z. De Liu, C. Z. Huang, Y. F. Li and Y. F. Long, "Enhanced plasmon resonance light scattering signals of colloidal gold resulted from its interactions with organic small molecules using captopril as an example" *Analytica chimica acta.* Vol. 577, No. 2, pp. 244, 2006.

[10] G. Donáth-Nagy, S. Vancea & S. Imre, "Comparative Study of Captopril Derivatization Reaction by LC-UV, LC-MS and CE-UV Methods" *Croatica Chemica Acta*, Vol. 84, No. 3, pp. 423-427. 2011.