http://dx.doi.org/10.5012/bkcs.2013.34.1.237

Chitosan-gold Nano Composite for Dopamine Analysis using Raman Scattering

Jae-Wook Lim and Ik-Joong Kang*

Department of Chemical & Bio Engineering, Gachon University, Gyunggi-do 461-701, Korea
**E-mail: ijkang@gachon.ac.kr*
Received October 31, 2012, Accepted November 30, 2012

This experiment was conducted for the purpose of developing such a sensor that can quickly sense dopamine concentration by using chitosan-gold nanoshell. Chitosan nano particles were reacted with gold nano particles so as to synthesize chitosan-gold nanoshell, and the size of the synthesized product was about 150 nm. When dopamine was reacted with chitosan-gold nanoshell, the size of it was not definitely changed, but dopamine was well reacted with chitosan-gold nanoshell, and it generated SERS (surface-enhanced Raman scattering), which led to a clear difference in the intensity of Raman scattering within the range of dopamine concentration (1 mM-10 mM). When Raman scattering was intensity marked on chitosan-gold nanoshell by employing a calibration curve according to dopamine concentration, a straight line whose margin of error was narrow was earned.

Key Words : Chitosan-gold nano composite, Dopamine sensor, SERS

Introduction

Chitosan is a biodegradable, useful and natural high-molecular substance. It can be gained through partial de-acetylation of chitin (*N*-acetyl-D-glucosamine) derived from shrimp, crab, mushroom, *etc*.[1] Chitosan is used in many researches since it has high friendliness and absorption, high heat-stability, and chemical resistance to metal ion. Ever since Richard Feynman introduced the concept of nano-particles into scientific experiments, researches have been conducted on metal nanoparticles including nano-scale colloid sol and organic metal compounds.[2-7]

In particular, gold is an interesting substance which has chemical stability and optical uniqueness.[8] As an optical characteristic, it has an absorption wavelength field in 520 nm perimeter, and it is used for bio-medical analyses *via* spectroscopy, since its absorption wavelength field may be red shift or blue shift when it combines with certain nano-particles.[9-12]

In recent days, people pay much attention to the prevention and/or treatment of brain-related diseases such as Parkinson's disease, Alzheimer's disease, Lou Gehrig's disease, cerebral palsy, dementia (AIDS dementia, vascular dementia), stroke and epilepsy. Parkinson's disease, especially, is closely connected to dopamine, a neuro-hormone and neurotransmitter; it is a representative degenerative disease, the symptoms which are caused by the lack of dopamine, and include trembling, defects in memory and motor disturbances, and it may lead to death if appropriate treatment is not sought. In fact, a considerable number of patients, mainly elderly people, suffer from this disease.[13-15] For now, as the number of elderly people increases, it is expected that Parkinson's disease patients will quickly multiply in the future. It is well known that medication with dopamine is the most effective way to cure motor disturbances caused by Parkinson's disease, and that a patient should be administered with about 100 mg/day-1500 mg/day of dopamine.[16-20]

The amount of dopamine secreted from the terminal branches of neurons in human brain can be determined, and gold nanoparticles play a very important part in using Raman laser to analyze dopamine concentration in living organisms.[21,22] Raman analysis is applicable for analyzing various substances but its scattering is normally weak. However, when a substance to be analyzed is directly combined with such metal substances as gold and silver, Raman laser generates a plasmon phenomenon on the metal surface, and from this phenomenon the rare information that could not be obtained from a general Raman analysis can now be obtained. Such analysis method is called SERS (Surface-Enhanced Raman Scattering) and is actively applied along with TERS (Tip-Enhanced Raman Scattering).[23-27]

In this research chitosan-gold nanoshell (CGN) were synthesized, and combined with dopamine, whose concentration is applicable to Parkinson's disease patients, through electronic interaction by using the nanoparticles, and identify changes in the concentration of dopamine by utilizing SERS.

Experimental Section

Reagents and Chemicals. Chemical products from Sigma Aldrich were purchased. First, TripolyPhosPhate (TPP) ($Na_5P_3O_{10}$; FW: 367.9; Practical Grade: 90.0-95.0%), potassium carbonate (K_2CO_3; FW: 138.2; Assay: 99.7%), gold chloride hydrate ($HAuCl_4$-xH_2O; FW: 343.0; Assay: 99.999%), ascobic acid ($C_6H_8O_6$; FW: 176.12; Assay: 99.0%), ammonium hydroxide, 28% in water. 99.99+% metals basis (NH_4OH; FW: 35.06), 3-hydroxytyramine hydrochloride ($C_8H_{11}NO_2HCl$ FW: 189.64), tetrakis-hydroxymethyl-phosphonium chloride (THPC) ($C_4H_{12}O_4PCl$; Assay: 80% in water). Dopamine, HPLC Water (H_2O; FW: 18)

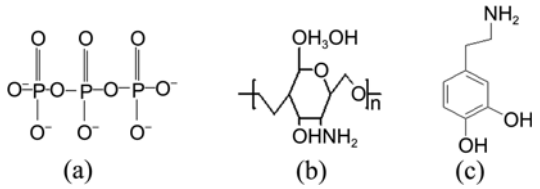

Figure 1. Molecular Structure of (a) TPP, (b) chitosan, (c) dopamine.

purchased from J. T. Baker. Acetic acid (CH_3COOH; FW: 60.05; Assay: 99.0%). and Sodium hydroxide (NaOH; FW: 38.98; Assay: 96.0%) were used as the product from Duksan Pure Chemical, Ltd.

Analytical Methods. In order to determine the size of the produced particles, DLS (BI-9000AT, Brookhaven, USA) was used. Electrophoretic Light Scattering (ELS-8000, Portal's, Japan) was used to determine the surface electric potential. To analyze the shape and size of AFM (XE-150, Park System, Korea) particles, SEM (S-4700, Hitachi's, Japan), TEM (H-7600, Hitachi's, Japan.) was used. To analyze the absorption wavelength field, UV-vis spectroscopy (HP 8453, Agilent Technologies, USA) was used. Raman (LabRam HR, He-Ne laser 633nm, Horiba company, France) was also used.

Preparation of Chitosan Nanoparticles. First, a 50 mL vial was put on a balance and the scale was set to zero, and then 0.06 g of chitosan powder was accurately measured. After taking the vial off the balance and easuring and putting 29.94 mL of distilled water onto the balance, 30 μL of acetic acid was put in a hot mixer which was being stirred at a speed of 1500 rpm for 1 h. After it was observed that dispersed chitosan particles became transparent, 20 mL of prepared 0.1 wt % TPP solution was dropped in very slowly, and let it react for 30 minutes. After the transparent solution had reacted with TPP and turned into white nanoparticles, the solution was then left alone for 2 h. Next, when this solution was moved into a 50 mL conical tube and reacted in a centrifugal separator at the speed of 5500 rpm for 15 minutes, chitosan nanoparticles could be refined. After centrifuging, supernatant was taken away and instead 29.94 mL of distilled water was put in. When the solution was dispersed with a sonicator for 1 h, chitosan nanoparticles was obtained. The solution prepared in such a manner is called chitosan nanoparticle solution.[28]

Chitosan-Gold Nanoshell. The produced chitosan nanoparticle solution was mixed with prepared HPLC-induced gold colloid at the ratio of 5:1 for 1 h. This solution was moved into a 50 mL conical tube and then centrifuged at 7000 rpm for 15 minutes. Again, after it was dispersed in distilled water, it was filtrated through a 450 nm-sized syringe filter and then sonicated for 10 minutes. Of this solution, 5 mL was taken and mixed and stirred with 5 mL of gold salt at a normal temperature at a speed of 1000 rpm. When the mixture was added with 10 μL of ascorbic acid and 30 μL of NH_4OH and stirred for 10 minutes, chitosan-gold nanoshell were obtained. Once again, after the same amounts of gold salt, ascorbic acid and NH_4OH were put in and left alone to react for 10 minutes, chitosan-gold nanoshell could be prepared.[29]

CGN-Dopamine. After chitosan-gold nanoshell solution was centrifuged at 7000 rpm for 15 minutes, the supernatant was taken away and instead distilled water corresponding to the supernatant amount was poured in. This solution was moved into a conical tube which was wrapped up with silver foil so that it might be isolated from light. After it was fully dispersed through sonication for 30 minutes, 5 mL of this solution was put into each 50 mL vial and added with 100 μL dopamine. The solution was fully stirred for 10 minutes, and then CGN-Dopamine was obtained. Figure 2 shows the whole process of the experiment in this research.

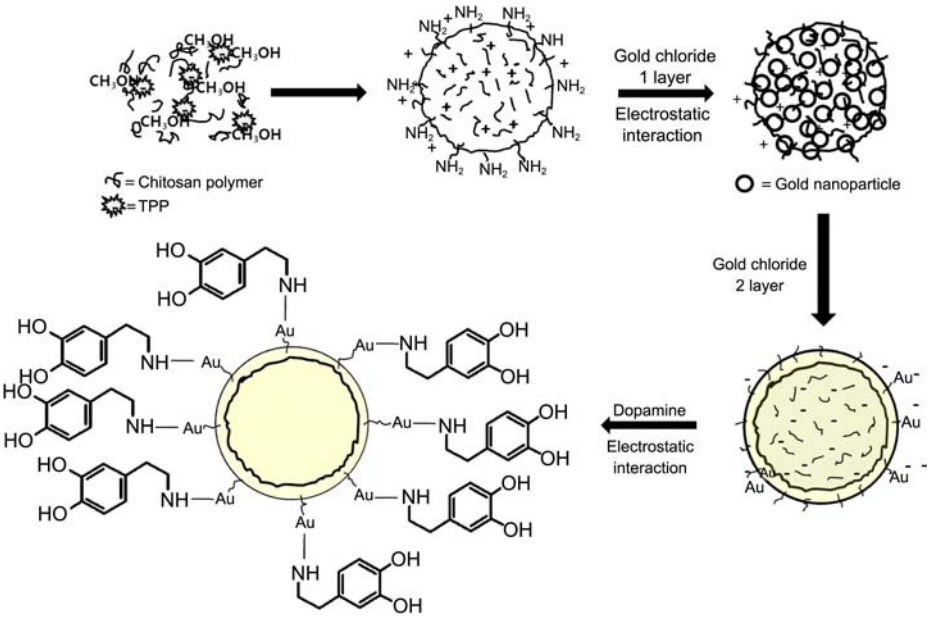

Figure 2. Schematic illustration of the fabrication process for CGN-dopamine.

Results and Discussion

Figure 3 shows the results of the measurement of zeta potential and size of chitosan of each concentration. Above all, the size of chitosan nanoparticles is very important for a drug carrier. In practicality, when the concentration of dopamine secreted from a human body is analyzed, the most suitable size of chitosan nanoparticles, which is never to be disturbed when it is applied to a human body, is more or less about 200 nm. It was confirmed that the size of the particles

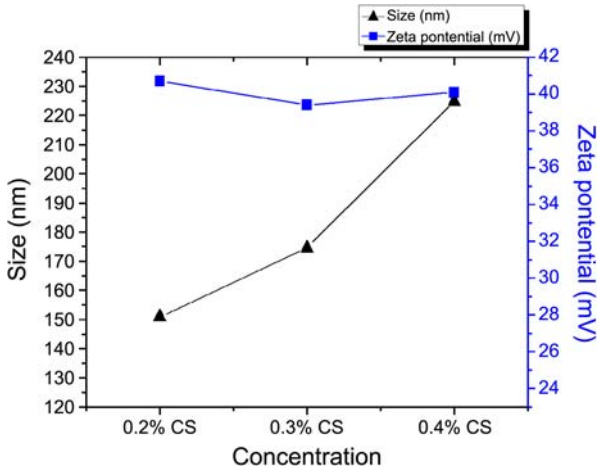

Figure 3. The size and zeta potential of chitosan nanoparticles as a function of concentration.

grew bigger as the chitosan concentration turned 0.2%, 0.3% and 0.4%. It seems that although the concentration of chitosan increased, the amount of acetic acid (30 μL) stayed stable, which was effective in cutting the molecular strand of chitosan or high molecular substance. At each concentration of chitosan, its zeta potential value was stable (about +40 mv), which implied that chitosan had been well dispersed irrespective of its size or concentration. And the value was positive because it had an amine group at its molecular terminal. The test was conducted on a sample whose size was the smallest, or 0.2% chitosan, and, from the test, chitosan nanoparticles, whose size was about 150 nm, was obtained.

Figure 4(a) shows that relatively circular chitosan nano particles are uniformly distributed and that their size is about 100-150 nm. Figure 4(b) shows that gold colloids are attached around chitosan nano particles and they are reacted in the circumference of chitosan nano particles, so that the surroundings of the particles look brighter against SEM images, compared to the particles. Figure 4(c) shows that gold salt is reacted in the conditions of Figure 4(b) to synthesize chitosan-gold nanoshell and that gold is conglomerated with each other due to their strong magnetism. An analysis was made of the particles by using TEM and it was found that chitosan-gold nanoshell whose core was chitosan and whose shell was gold was formed in Figure 4(d). Lastly, Figure 4(e) shows that dopamine was reacted with the particles of chitosan-gold nanoshell and Figure 4(e) is not as clear as Figure 4(c). This seems to be because dopamine spread in the surroundings was already shot by an electron gun of SEM and chitosan-gold nanoshell particles were well reacted with dopamine. Figure 4(f) could be earned by using TEM and it shows that, as is seen in the illustration, dop-

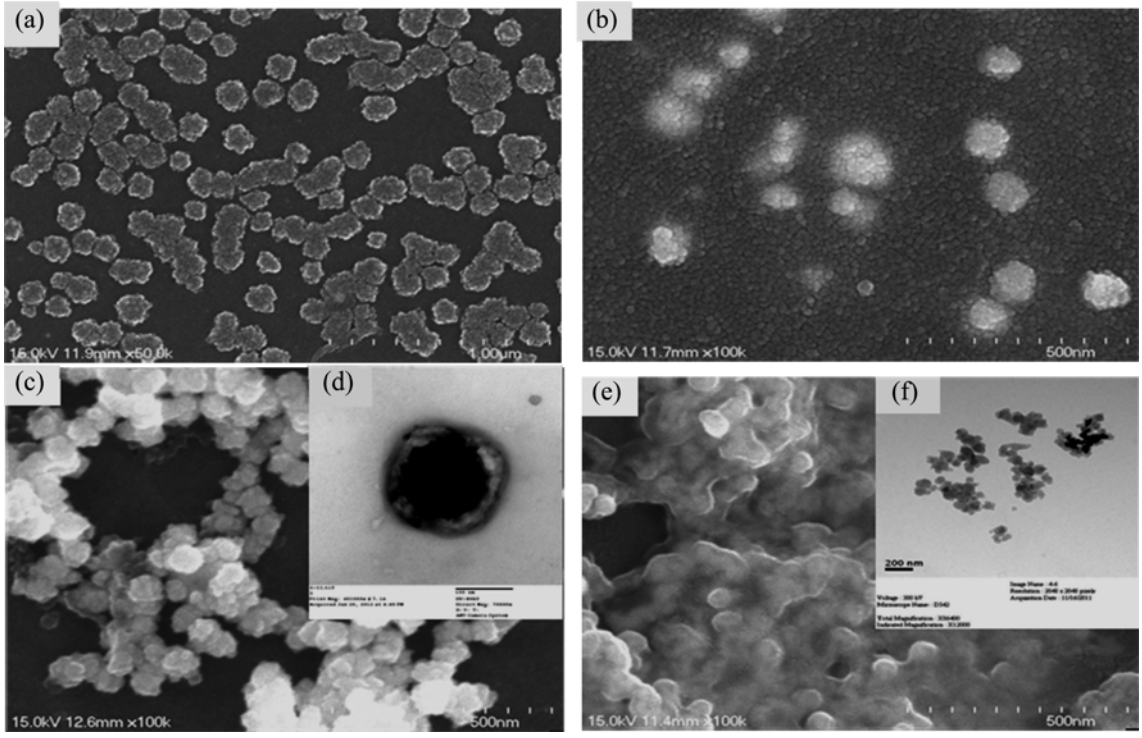

Figure 4. SEM images of (a) chitosan nanoparticles (b) chitosan-gold nano particles (c) chitosan-gold nanoshell (CGN), TEM image of (d) chitosan-gold nanoshell (CGN) (e) CGN-dopmaine and TEM image of (f) CGN-Dopmaine.

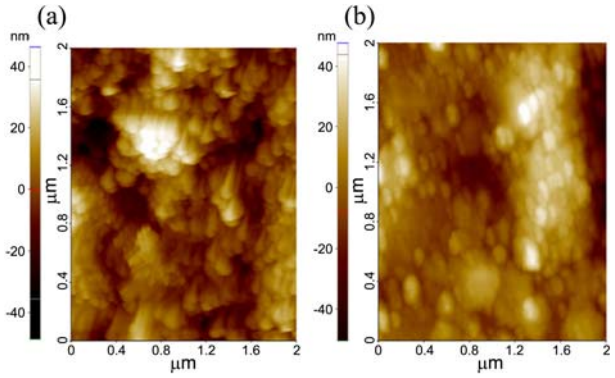

Figure 5. AFM Images of (a) chitosan-gold nanoshell (CGN) (b) CGN-dopmaine (10 mM).

amine is spread around chitosan-gold nano shell particles, which confirms that dopamine is relatively well reacted with chitosan-gold nanoshell.

Figure 5 shows the shape of Chitosan-gold nanoshell looks round, the pile of dopamine is slightly raised when dopamine is spread over chitosan-gold nanoshell, but there is no big change in the size of the particles. In other words, it seems that dopamine may be excessively spread over chitosan-gold nanoshell that is not affected by the size of dopamine. So, it is expected that dopamine will not have a great

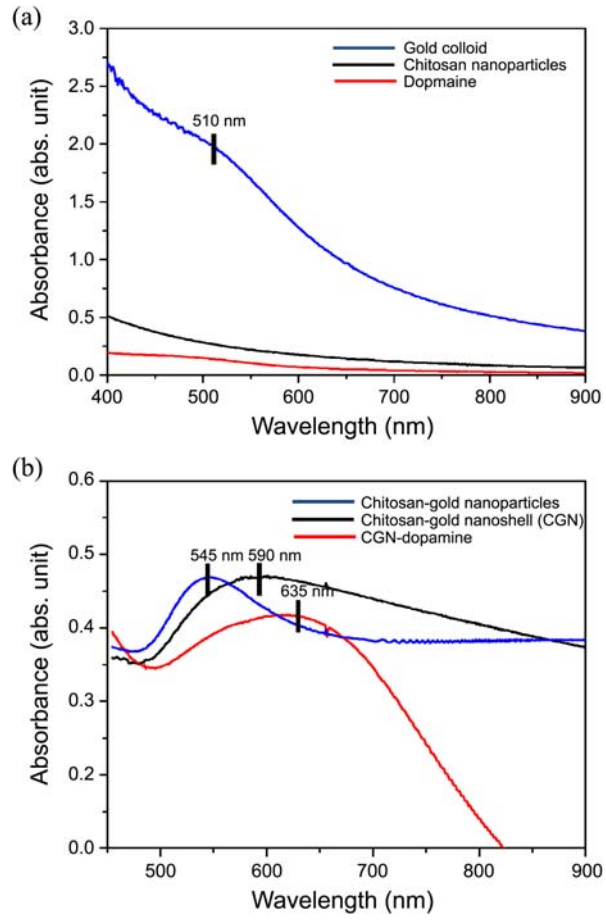

Figure 6. UV-vis spectra of (a) gold colloid, chitosan nanoparticles, dopamine (b) chitosan-gold nano particles, chitosan-gold nanoshell (CGN), CGN-dopamine.

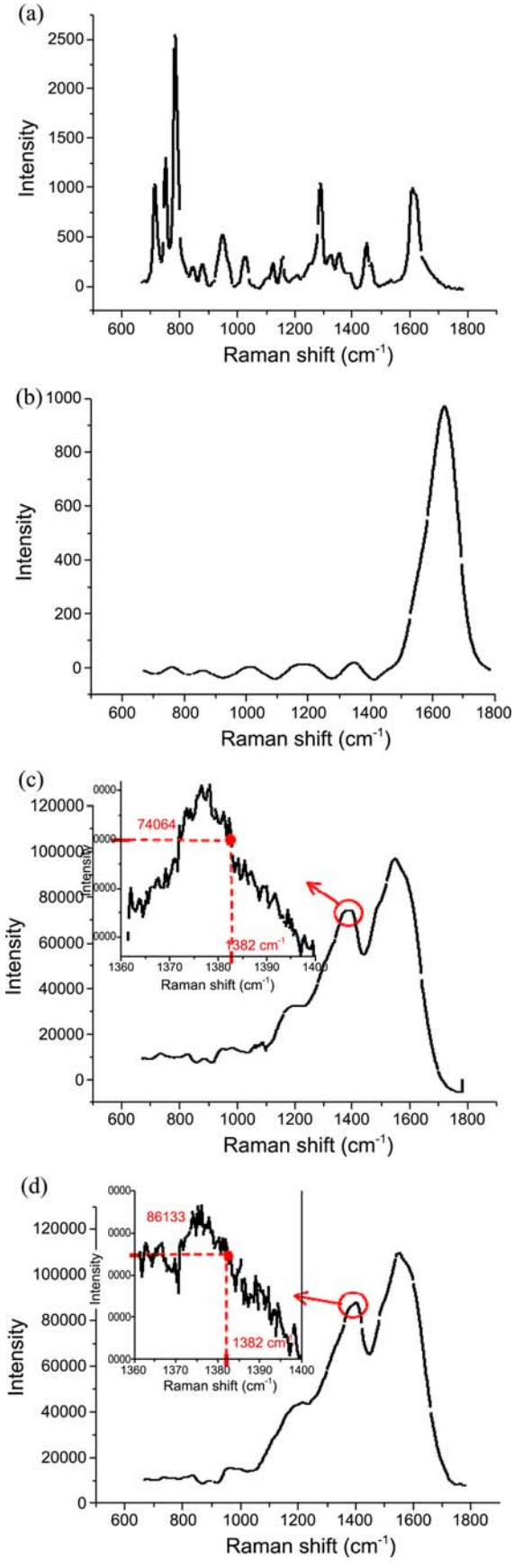

Figure 7. Raman scattering of (a) dopamine, (b) chitosan-gold nanoshell (CGN), (c) CGN-dopamine (1 mM), (d) CGN-dopamine (10 mM).

effect on the characteristics and/or nature of chitosan-gold nanoshell, which confirms that, when dopamine is coated due to the thickness of the sample according to the light & darkness of a photo, the size of chitosan-gold nanoshell remains the same.

Figure 6 shows changes from chitosan-gold nanoparticles to chitosan-gold nanoshell and CGN-dopamine. Gold has such an optical trait that it has a special absorption wavelength field. Gold is characterized by the fact that when gold reacts with a certain substance or the surface of gold is changed, there is a redshift or a blueshift. Form (a), it is identified that there is an inflection point of around 510 nm from gold colloid, which may be considered as an absorption wavelength field of gold colloid. In contrast, (a) graph demonstrates that chitosan nanopaticles or dopamine does not have any absorption wavelength field since it does not have any optical trait. From (b), it is identified that chitosan-gold nanoparticles are gold synthesized from chitosan and that the UV wavelength field is 540 nm since there is a red shift caused by reaction. When a chitosan-gold nanoparticles is formed, it has a 590 nm absorption wavelength field, from which it can be identified that there has been a change in the surface of gold layer and that the UV value has been red shifted as far as possible. Lastly, when it is identified through UV that the manufactured chitosan-gold nanoparticles is combined with dopamine, it is also known that CGN-Dopamine has a 635 nm absorption wavelength field, which demonstrates that the surface of chitosan-gold nanoparticles is well combined with dopamine.

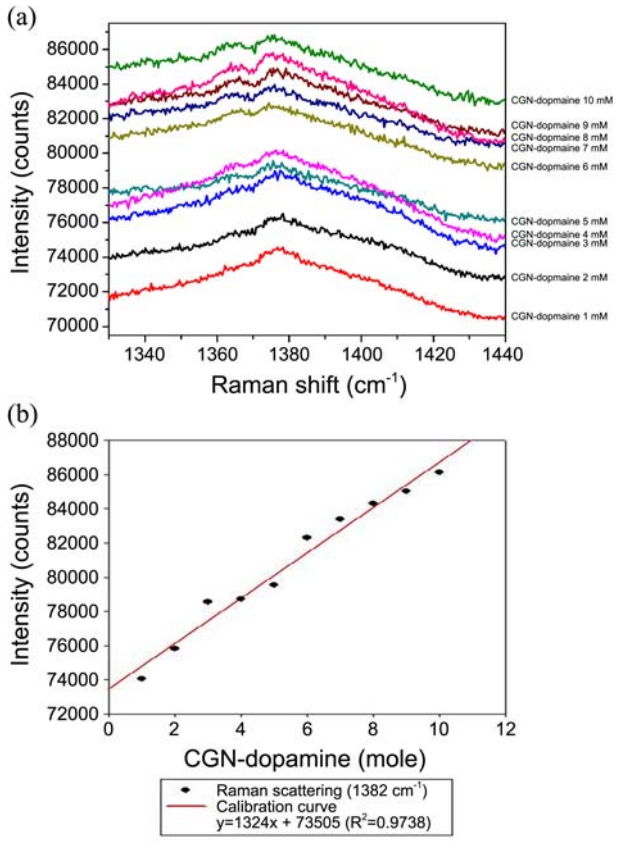

Figure 8. Raman Scattering Intensity as a Function of CGN-dopamine Concetration: (a) graph (b) concertration calibration curve at 1382 cm^{-1}.

Figure 7 shows the Raman scattering peak for the combination of dopamine with chitosan-gold nanoshell. Around 1620 cm^{-1}, the combination has a very characteristic peak of chitosan-gold nanoshell. Again, when it is combined with dopamine, there are two peaks around 1382 cm^{-1} for CGN-dopamine and 1620 cm^{-1} for CGN only. A detailed analysis is made of peaks in (c) and (d) and it is found that CGN-dopamine 1 mM has the intensity value of 74,064 around 1382 cm^{-1} and CGN-dopamine 10 mM has the intensity value of 86,133. This implies that chitosan-gold nanoshell combined with optical fiber can be an effective instrument for an analysis or be an effective bio-sensor when a precise analysis is made of changes in the intensity value according to the concentration of dopamine within the range of 1-10 mM.

Figure 8 shows Raman scattering intensity as a function of CGN-dopamine concentration. Figure 8(a) represents a graph of data earned when 1-10 mM of dopamine is reacted and (b) refers to a drawing of the data produced by using calibration curves which attests the regularity of Raman intensity. As the concentration is higher, the Raman intensity is greater, which may be expressed in the linear equation of $Y = 1324\ X + 73505$ ($R^2 = 0.9738$) by using a calibration curve. The value of R^2 is 0.9738, whose margin of error is relatively small.

Conclusions

Using chitosan-gold nanoshell, this research has determined changes in the sensitivity of Raman scattering, which reacts to 1-10 mM of dopamine concentration. It is found that the size of chitosan nanoparticles is about 150 nm. As gold nanoparticles were evenly coated around chitosan, the particles grew bigger and more round-shaped nanoparticles were obtained, and the obtained chitosan-gold nanoshell (CGN) was 200 nm in size. When 1-10 mM of dopamine reacted with chitosan-gold nanoshell, there was a change in the intensity of Raman shift near 1,382 cm^{-1} field, which was used to draw a calibration curve and derive a linear equation. Based on this equation, changes in the sensitivity of CGN can be expressed in a linear equation, depending upon the concentration of dopamine administered to Parkinson's disease patients. Conclusively, as a result, it is found that chitosan-gold nanoshell can be utilized as a biosensor that senses the concentration of dopamine secreted from human brain.

Acknowledgments. This research was supported by the GRRC program of Gyeonggi province [GRRC Gachon 2012-B06, Development of nanosensor for detecting biomarker].

References

1. Dash, M.; Chiellini, F.; Ottenbrite, R. M.; Chiellini, E. *Progress in*

Polymer Science **2011**, *36*(8), 981.
2. Casettari, L.; Vllasaliu D.; Castagnino E. *Progress in Polymer Science* **2012**, *37*(5), 659.
3. Sinha, V. R.; Singla, A. K.; Wadhawan, S.; Kaushik, R. *International Journal of Pharmaceutics* **2004**, *274*(1), 1.
4. Berger, J.; Reist, M.; Mayer, J. M.; Felt, O.; Gurny, R. *European Journal of Pharmaceutics and Biopharmaceutics* **2004**, *57*(1), 32.
5. Guibal, E. *Progress in Polymer Science* **2005**, *30*(1), 71.
6. Pillai, C. K.; Paul, W.; Sharma, C. P. *Progress in Polymer Science* **2009**, *34*(7), 641.
7. Mao, S.; Sun, W.; Kissel, T. *Advanced Drug Delivery Reviews* **2010**, *62*(1), 12.
8. Kumar, K. P.; Paul, W.; Sharma, C. P. *Process Biochemistry* **2011**, *46*(10), 2007.
9. Rad, A. G.; Abbasi, H.; Afzali, M. H. *Physics Procedia* **2011**, *22*, 203.
10. Song, J. Y.; Jang, H.; Kim, B. S. *Process Biochemistry* **2009**, *44*(10), 1133.
11. Streszewski, B.; Jaworski, W.; Pacławski, K. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2012**, *397*, 62.
12. Fayaz, A. M.; Girilal, M.; Rahman, M. *Process Biochemistry* **2011**, *46*(10), 1958.
13. Tanaka, K.; Miyake, Y.; Fukushima, W. *Parkinsonism & Related Disorders* **2011**, *17*(6), 446.
14. Kiyohara, C.; Miyake, Y.; Koyanagi, M.; Fujimoto, T.; Shirasawa, S. *Parkinsonism & Related Disorders* **2010**, *16*(7), 447.
15. Lewitt, P.; Shiez, L.; Auinger, P. *Brain Research* **2011**, *1408*, 88.
16. Pankratz, N.; Pauciulo, M. W. *Neuroscience Letters* **2006**, *408*(3), 209.
17. Qin, Z.; Zhang, L.; Sun, F.; Fang, X. *Parkinsonism & Related Disorders* **2009**, *15*(10), 767.
18. Murakami, K.; Miyake, Y.; Sasaki, S. *Nutrition* **2010**, *26*(5), 515.
19. Miyake, Y.; Tsuboi, Y.; Koyanagi, M. *Journal of the Neurological Sciences* **2010**, *297*, 15.
20. Zhang, Z. Y.; Sun, F.; Liu, H. *Clinical Neurology and Neurosurgery* **2009**, *111*(9), 733.
21. Wang, Y. Q.; Ma, S.; Yang, Q. Q. *Applied Surface Science* **2012**, *258*(15), 5881.
22. Zhu, Y.; Dluhy, R. A.; Zhao, Y. *Sensors and Actuators B: Chemical* **2011**, *157*(1), 42.
23. Sanci, R.; Volkan, M. *Sensors and Actuators B: Chemical* **2009**, *139*(1), 150.
24. Tan, R. Z.; Agarwal, A.; Balasubramanian, N. *Sensors and Actuators A: Physical* **2007**, *139*(1), 36.
25. Cozar, I. B.; Szabó, L.; Mare, D.; Leopold, N. *Journal of Molecular Structure* **2011**, *99*(1), 243.
26. Lucotti, A.; Zerbi, G. *Sensors and Actuators B: Chemical* **2007**, *121*(2), 356.
27. Panarin, A. Y.; Terekhov, S. N.; Kholostov, K. I. *Applied Surface Science* **2010**, *256*(23), 6969.
28. Kim, B. G.; Kang, I. J. *Ultramicroscopy* **2008**, *108*, 1168.
29. Lee, S. C.; Lee, S. W.; Kang, I. J. *J. Surface Review and Letters* **2010**, *17*, 165.