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A simple and sensitive method for visual detection of heparin using positively-charged gold nanoparticles as colorimetric probes†

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The electrostatic interaction between the negatively-charged heparin and the positively-charged gold nanoparticles leads to aggregation of gold nanoparticles and a color change from red to blue in solution, thus providing a simple and visual approach to colorimetric detection of heparin.

Heparin, a naturally occurring sulfated polysaccharide, is widely used as an anticoagulant/antithrombotic agent during clinical procedures such as cardiac/vascular surgery and kidney dialysis. Heparin overdose can induce some adverse effects (such as hemorrhages and thrombocytopenia).[1,2] Beyond the need for simple and ideally real-time continuous measurements of heparin levels in serum during such surgery and postoperative therapy period, there is also a desire for detection methods that can monitor the levels of heparin within infusion solutions to avoid dangerous human errors in dosing, especially for pediatric patients. Traditional clinical procedures for heparin detection rely on the measurements of the activated clotting time or activated partial thromboplastin time.[2,3] These methods are not sufficiently reliable and accurate for clinical settings because of their lack of specificity and potential interference from other factors.[4] Thus, many researchers have attempted to develop new methods for the detection of heparin, including fluorimetry,[5–8] colorimetry,[9,10] and electrochemical methods.[11–14]

Gold nanoparticles (AuNPs) exhibit several interesting physical and chemical properties. The well-dispersed AuNPs solution is red, whereas aggregated AuNPs appear as a blue color. The color change induced by aggregation of AuNPs provides the basis for the colorimetric analysis.[15,16] The major advantage of the AuNPs-based colorimetric assay is that the molecular recognition event can be transformed into color changes, which can be easily observed by the naked eye.[16] Importantly, owing to the extremely high extinction coefficients of AuNPs,[17] the AuNPs-based colorimetric assay has extremely high sensitivity, and has been used for the detection of many substances including DNA and proteins.[16–19]

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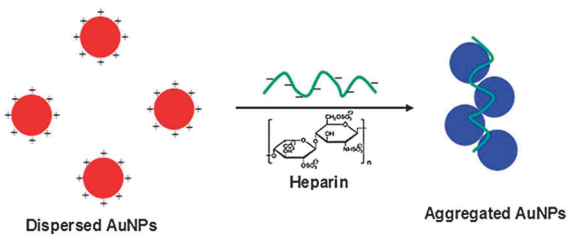
Recently, Jena and Raj proposed an AuNPs-based colorimetric assay for protamine, and they also used this method to detect heparin.[20] In their system, the polycationic protamine firstly induces the aggregation of AuNPs, resulting in a color change of the AuNPs from red to blue. Then, addition of heparin dissipates the aggregated AuNPs due to its strong affinity to protamine and the blue color changes to the native color (red). It is obvious that the method is rather complex.

In this work, we observed the interesting phenomenon that the AuNPs solution showed a color change from red-to-blue when heparin was directly added into the cysteamine-stabilized AuNPs solution. The cysteamine-stabilized AuNPs are positively-charged, and the electrostatic attraction between the positively-charged AuNPs ((+)AuNPs) and polyanionic heparin leads to the aggregation of AuNPs. Based on this experimental phenomenon, the (+)AuNPs are used as colorimetric probes, and a simple colorimetric detection of heparin is proposed. Scheme 1 depicts the mechanism for detecting heparin. The (+)AuNPs were synthesized by sodium borohydride reduction of hydrogen tetrachloroaurate(III) in the presence of cysteamine[21,22] (see ESI†). Because of the $-NH_3^+$ group of cysteamine, the cysteamine-capped AuNPs are positively-charged. The AuNPs solution is stabilized against aggregation due to the positive capping agent's electrostatic repulsion between AuNPs.[23] The dispersed AuNPs have a surface plasma resonance peak at about 520 nm, and appear red. Heparin contains functional groups of sulfate and carboxylate, and has an average charge of −75.[24] The electrostatic attraction between the (+)AuNPs and polyanionic heparin leads to the aggregation of AuNPs. This causes a rapid red-to-blue color change. We just make use of this property to design our assay.



Scheme 1 Visual detection of heparin utilizing electrostatic attraction between positively-charged AuNPs and negatively-charged heparin.

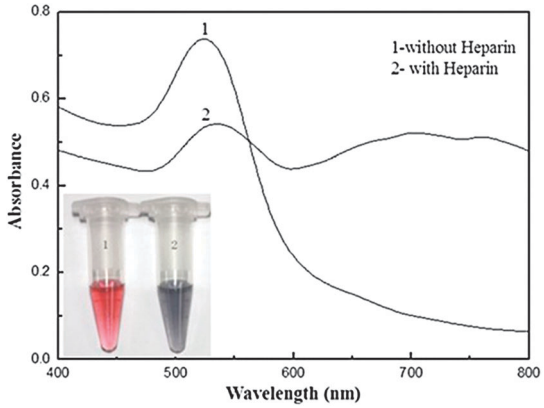


Fig. 1 Absorption spectra of AuNPs in the absence and presence of 5 μg mL^{-1} heparin. Inset shows the corresponding photographic images.

In order to confirm the principle of this assay, the ζ-potential of the system was measured. The ζ-potential of the cysteamine-capped AuNPs (*ca.* 34 nm) was +26.3 mV. After polyanionic heparin (5 μg mL^{-1}) was added into the AuNPs solution, the ζ-potential of the system became −0.5 mV. So, the low surface charge density would cause the aggregation of AuNPs. On the other hand, UV-Vis spectroscopy (Fig. 1) was used to explore the change of AuNPs induced by heparin. As expected from the original design, the AuNPs remain stable and display a maximal adsorption at 520 nm. In the presence of heparin, the visible spectrum displays the characteristic red shift and broadening of the surface plasmon band. This red shift in the plasmon resonance is ascribed to the near-field coupling that occurs when the interparticle distance decreases.[25] As heparin is polyanionic, its electrostatic interaction with the (+)AuNPs decreases the interparticle distance because of the charge screening effect. Note that the spectral response obtained upon the addition of heparin is not due to the dilution; the negative control experiment with the Britton–Robinson (BR) buffer solution (0.04 M H_3PO_4–0.04 M HAc–0.04 M H_3BO_3) does not show such a spectral response. The photographs in Fig. 1 show that the AuNPs solution is red in the absence of heparin, while the solution is blue in the presence of heparin. In order to know the microstructure of the AuNPs with and without heparin, the TEM images of the system were obtained. The TEM images (see Fig. S1, ESI†) showed that the native AuNPs were spherical in shape and were nearly monodispersed with an average diameter of 34 nm; however, after incubation with heparin for 15 min, irregular AuNP aggregates were observed. The TEM results gave the direct evidence for heparin-induced aggregation of AuNPs.

Furthermore, we prepared the citrate-capped AuNPs (*ca.* 13 nm) for the comparison study, and the ζ-potential of the citrate-capped AuNPs was negative at pH 3.6. Experiments showed that heparin could not lead to the aggregation of the negatively-charged AuNPs. On the other hand, protamine was firstly added into heparin solution. A color change from red-to-blue was not observed upon the addition of the mixture of protamine and heparin to the (+)AuNPs. It is well known that the polycationic protamine can bind the polyanionic heparin specifically *via* an electrostatic interaction.[26] Due to the neutralization by protamine, heparin cannot bind negatively-charged AuNPs *via* electrostatic attraction. Consequently, the nanoparticles solution shows its native color (red). These results further confirmed that electrostatic attraction between the (+)AuNPs and heparin resulted in the aggregation of AuNPs.

To optimize the conditions for the heparin assay, various factors, such as amount of AuNPs, media pH, binding time and binding temperature, were then examined in detail. The effect of the amount of AuNPs was examined in the range of 50–300 μL. The experimental results showed that the highest sensitivity was obtained when using 180 μL AuNPs (10.5 nM). The BR buffer solution was used as reaction media, and we examined the influence of pH. At pH 3.6, the highest absorption ratio (A_{670}/A_{520}) was obtained. Thus, the pH of media was chosen as 3.6. In this work, cysteamine was used as the capping agent of AuNPs. Cysteamine has one amino group with pK_a of 10.75.[27] Amino of cysteamine is protonated at pH 3.6, so the cysteamine-capped AuNPs are positively-charged, which leads to the efficient electrostatic interaction with the negatively-charged heparin. A change in temperature from 20 to 35 °C scarcely affected the colour reaction, and thus the detection of heparin can be carried out at room temperature. The results showed that the suitable binding time was 15 min. We used two different sized (+)AuNPs (36 nm and 48 nm) to study the effect of Au particle size on the relative optical absorbance change. The experiment showed that smaller AuNPs (36 nm) were more sensitive to heparin.

To quantitatively detect heparin using the developed method, UV-Visible spectra of the (+)AuNPs in the presence of heparin with different concentrations were recorded under the optimized conditions. As shown in Fig. 2, the absorption of AuNPs at 520 nm gradually decreases, and the absorption at 670 nm gradually increases with gradually increasing the heparin concentration. The absorption ratio (A_{670}/A_{520}) increased with increasing heparin concentration (see Fig. S2, ESI†). The absorption ratio (A_{670}/A_{520}) exhibited a linear correlation to heparin concentration in the range from 0.09 μg mL^{-1} to 3.12 μg mL^{-1}. The present limit of detection for heparin in water was 0.03 μg mL^{-1}, which is lower than

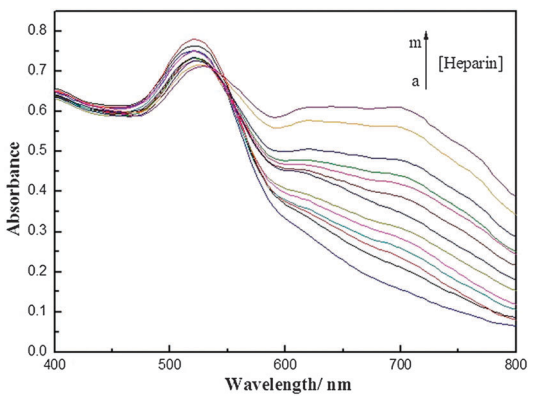


Fig. 2 Absorption spectra of AuNPs in the presence of heparin with different concentrations (a–m: 0, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, and 2.4 μg mL^{-1}).

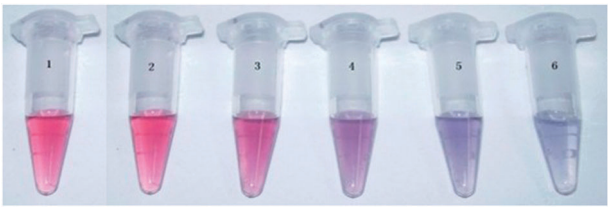


Fig. 3 Photographs of a solution of (1) AuNPs, (2) AuNPs + 0.087 μg mL^{-1} heparin, (3) AuNPs + 0.5 μg mL^{-1} heparin, (4) AuNPs + 1.0 μg mL^{-1} heparin, (5) AuNPs + 1.62 μg mL^{-1} heparin, and (6) AuNPs + 2.0 μg mL^{-1} heparin.

that of the reported colorimetry for heparin.[9] The relative standard deviation (RSD) was about 2% for the determination of 1.0 μg mL^{-1}. In addition, the heparin-induced aggregation of AuNPs would result in a color change from red to blue. As shown in Fig. 3, the naked eye alone can judge the presence of or absence of 0.5 μg mL^{-1} heparin without the aid of any advanced instruments. To demonstrate the potential application of (+)AuNPs probes for heparin detection in biological media, 1% serum was used as the sample matrix for further studies. In the presence of serum, (+)AuNPs gave a linear response for heparin in 0.3–7.0 μg mL^{-1} (Fig. S3, ESI†), and the limit of detection in serum was 0.1 μg mL^{-1}.

The selectivity of this method for heparin was evaluated by testing the response of the assay to other compounds. The results showed that 5 μg mL^{-1} bovine serum albumin could not interfere with an assay of 1.0 μg mL^{-1} heparin. Serum albumin (pI = 4.6–5.3[28]) is positively charged at pH 3.6. So, the binding of bovine serum albumin to the positively-charged AuNPs does not occur through an electrostatic mechanism. Some cationic biopolymers such as chitosan, polylysine and protamine also didn't induce the color change of (+)AuNPs (see Fig. S4, ESI†). In addition, the presence of physiological levels of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, CO_3^{2-}, PO_4^{3-}, glucose, lactic acid and cysteine could not interfere with the detection of 1.0 μg mL^{-1} heparin. To study (+)AuNPs response towards other polysaccharides, similar experiments were conducted with hyaluronic acid (HA), an analogue of heparin. Upon addition of 3 μg mL^{-1} HA, the AuNPs almost remain red color in the presence of HA (see Fig. S5 and S6, ESI†). Heparin has four negatively-charged side groups per repeat unit, while HA possesses only one negatively-charged group per repeat unit,[6] implying a weaker electrostatic attraction between HA and AuNPs than that between heparin and AuNPs. So, HA with low charge density cannot lead to the significant aggregation of AuNPs under the experimental condition (15 min binding time and pH 3.6 media). Calf thymus DNA was used as a model to investigate the effect of other polyanionic substances. The experimental result (Fig. S7, ESI†) showed that 50 ng mL^{-1} DNA (plasma DNA normal level[29]) did not interfere with the detection of heparin. These results implied that this method showed a highly selective response to heparin. Of course, it was necessary to bear in mind that the cysteamine-capped AuNPs have colloidal stability problem, and high temperature (>45 °C) or high salt (>0.2 M NaCl) or high pH (>5.5) could induce the self-aggregation of (+)AuNPs.

To further test the possibility of applying the method to real samples, recovery experiments were carried out by analyzing the spiked human serum samples. 1.0 μg mL^{-1} or 2.0 μg mL^{-1} heparin was added to a 100-fold diluted serum samples, and the measured recoveries were between 109% and 90% with less than 3% RSD. The results showed that the recovery and precision of the proposed method were satisfactory.

In summary, we describe a simple and sensitive colorimetric assay for detecting heparin. This assay is based on the distance-dependent optical property of AuNPs and the efficient electrostatic interaction between the (+)AuNPs and heparin. The use of AuNPs offers a convenient "mix-and-detect" approach for detecting heparin. In addition, we are planning to develop a visual method for studying oxidative damage of polyanionic DNA with (+)AuNPs probes.

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Notes and references

1 T. E. Warkentin, M. N. Levine, J. Hirsh, P. Horsewood, R. S. Roberts, M. Gent and J. G. Kelton, *New Engl. J. Med.*, 1995, **332**, 1330.
2 P. D. Raymond, M. J. Ray, S. N. Callen and N. A. Marsh, *Perfusion*, 2003, **18**, 269.
3 D. J. Murray, W. J. Brosnahan, B. Pennell, D. Kapalanski, J. M. Weiler and J. Olson, *J. Cardiothorac. Vasc. Anesth.*, 1997, **11**, 24.
4 M. N. Levine, J. Hirsh, M. Gent, A. G. Turpie, M. Cruickshank, J. Weitz, D. Anderson and M. Johnson, *Arch. Intern. Med.*, 1994, **154**, 49.
5 K.-Y. Pu and B. Liu, *Adv. Funct. Mater.*, 2009, **19**, 277.
6 K.-Y. Pu and B. Liu, *Macromolecules*, 2008, **41**, 6636.
7 L. Zeng, P. Wang, H. Zhang, X. Zhuang, Q. Dai and W. Liu, *Org. Lett.*, 2009, **11**, 4294.
8 E. Climent, P. Calero, M. D. Marcos, R. Martínez-Máñez, F. Sancenón and J. Soto, *Chem.–Eur. J.*, 2009, **15**, 1816.
9 R. Zhan, Z. Fang and B. Liu, *Anal. Chem.*, 2010, **82**, 1326.
10 T. Bříza, Z. Kejík, I. Císařová, J. Králová, P. Martásek and V. Král, *Chem. Commun.*, 2008, 1901.
11 K. L. Gemene and M. E. Meyerhoff, *Anal. Chem.*, 2010, **82**, 1612.
12 R. Yu, M. Ma, L. Wang, Q. Xie, Z. Cao, X. Jiang and S. Yao, *Biosens. Bioelectron.*, 2009, **24**, 1771.
13 J. Guo and S. Amemiya, *Anal. Chem.*, 2006, **78**, 6893.
14 J. Langmaier, J. Olsak, E. Samcova, Z. Samec and A. Trojanek, *Electroanalysis*, 2006, **18**, 1329.
15 Y. Takeuchi, T. Ida and K. Kimura, *Surf. Rev. Lett.*, 1996, **3**, 1205.
16 W. Zhao, M. A. Brook and Y. Li, *ChemBioChem*, 2008, **9**, 2363.
17 S. K. Ghosh and T. Pal, *Chem. Rev.*, 2007, **107**, 4797.
18 N. Wangoo, J. Kaushal, K. K. Bhasin, S. K. Mehta and C. R. Suri, *Chem. Commun.*, 2010, **46**, 5755.
19 L. Li, B. Li, D. Cheng and L. Mao, *Food Chem.*, 2010, **122**, 895.
20 B. K. Jena and C. R. Raj, *Biosens. Bioelectron.*, 2008, **23**, 1285.
21 T. Niidome, K. Nakashima, H. Takahashi and Y. Niidome, *Chem. Commun.*, 2004, 1978.
22 J.-W. Kim, J. H. Kim, S. J. Chung and B. H. Chung, *Analyst*, 2009, **134**, 1291.
23 H. Li and L. Rothberg, *J. Am. Chem. Soc.*, 2004, **126**, 10958.
24 A. Shvarev and E. Bakker, *J. Am. Chem. Soc.*, 2003, **125**, 11192.
25 K.-H. Su, Q.-H. Wei, X. Zhang, J. J. Mock, D. R. Smith and S. Schultz, *Nano Lett.*, 2003, **3**, 1087.
26 Y. Okajima, S. Kanayama, Y. Maeda, S. Urano, T. Kitani, M. Watada, M. Nakagawa and H. Ijichi, *Thromb. Res.*, 1981, **24**, 21.
27 S. P. Mezyk, *J. Phys. Chem.*, 1995, **99**, 13970.
28 S. H. Brewer, W. R. Glomm, M. C. Johnson, M. K. Knag and S. Franzen, *Langmuir*, 2005, **21**, 9303.
29 Z. H. Huang, L. H. Li and D. Hua, *Cancer Lett.*, 2006, **243**, 64.