Talanta 106 (2013) 255–260

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Highly selective colorimetric detection of spermine in biosamples on basis of the non-crosslinking aggregation of ssDNA-capped gold nanoparticles

Zhong De Liu [a,b], Hai Yan Zhu [c], Heng Xin Zhao [a], Cheng Zhi Huang [a,b,*]

[a] Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China
[b] College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, China
[c] College of Life Sciences, Southwest University, Chongqing 400715, China

ARTICLE INFO

Article history:
Received 22 June 2012
Received in revised form
25 October 2012
Accepted 25 October 2012
Available online 9 November 2012

Keywords:
Cationic polyamine
Gold nanoparticles
Colorimetric detection

ABSTRACT

The selective adsorption of single-stranded oligonucleotides (ssDNA) on gold nanoparticles (AuNPs) is well known for stabilizing the AuNPs against aggregation even at high salt concentrations. Our investigation shows that the non-crosslinking aggregation of arbitrary ssDNA-capped AuNPs occurs due to their interaction with the cationic polyamine, spermine (Spm), even without any addition of NaCl. The non-crosslinking aggregation mechanism is that the Spm, served as multivalent counterions, plays the dual roles of charge shielding and ion bridging among the ssDNA-capped AuNPs, which jointly result in the aggregation of the ssDNA-capped AuNPs. Therefore, a sensitive and highly selective colorimetric method for the detection of Spm was developed. To the best of our knowledge, it is the first successful case as to the efforts towards the development of optical assays for cationic polyamine, showing neither natural UV absorption nor fluorescence. Compared with the traditional methods of chromatography and capillary electrophoresis, the approach described here would provide a convenient alternative and new train of thought for the specific detection of Spm in both biological fluid and fermented products.

1. Introduction

The naturally occurring spermine (Spm), as one of many cationic polyamines, exists in all eukaryotic cells and plays important roles in cell growth and differentiation [1–3], including the regulation of gene expression [3], the stabilization of chromatin [4], the prevention of endonuclease-mediated DNA fragmentation [5], and the inhibition of DNA damage [6,7]. More meaningfully, because its concentrations in neoplastic cells were found to be higher than those in normal cells [8], the level of Spm, as a sign of tumorigenesis and indicator of effectiveness of chemotheraphy, have attracted much attention from clinical investigators [9]. Besides, in fermented products, especially in meat and fish, the level of Spm resulted from the decarboxylation of amino acids is also useful as a marker of spoilage during storage and, therefore, a quality index [10,11]. Thus, pursuing an efficient and reliable analytical approach for specific Spm determination is of particular importance and meantime it is a challenging undertaking.

Most of the polyamines (the molecular structure listed in Fig. 1), especially those of an aliphatic nature, show neither natural UV absorption nor fluorescence. Up to now, many analytical methods for polyamines have been described in the literature on the basis of gas chromatography [12,13], high performance liquid chromatography (HPLC) [14–17], and capillary electrophoresis [18]. However, the derivatization step is considered necessary in order to induce detectability and generally it also includes an extraction step with an organic solvent prior to chromatographic separation, which increases the risk of analyte loss, contamination, and prolonged analysis [14]. Although the mass spectrometric detection is sensitive and highly reliable, its apparatus and operating costs are too expensive for routine analysis [15]. To overcome the afore-mentioned obstacles, herein, we report a colorimetric method for specific Spm detection by using the ssDNA-capped gold nanoparticle as an optical probe.

Gold nanoparticles (AuNPs) based colorimetric assays are anything but novel, however, for its simplicity and practicality, ease of observation by naked eyes and without the requirements for complicated instrumentation, it has still wide-range use as a fertile ground for analytical purpose. In general, the colloidal solution of AuNPs with diameters of 5–20 nm have an optical absorption peak at around 520 nm caused by surface plasmon resonance [19]. Once the aggregation of AuNPs occurs, the absorption peak shifts to a longer wavelength and accordingly the

* Corresponding author at: Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, College of Chemistry and Chemical Engineering, and College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, China. Tel.: +86 23 68254659;
fax: +86 23 68254000.
E-mail address: chengzhi@swu.edu.cn (C.Z. Huang).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.10.079

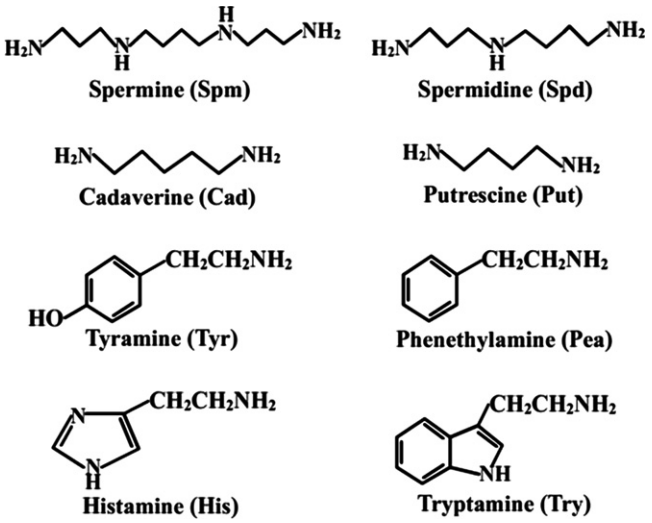

Fig. 1. The structure of several kinds of cationic polyamines.

color of the colloidal solution changes from red to purple or even blue [20,21]. Since the commencement of the first DNA sensor based on the hybridization between the mercaptoalkyloligonecleotide-modified AuNPs and the target ssDNA [22], similar platforms have been developed to detect various substances including DNA, proteins, heavy metal ions and small organic molecules [23–27]. A majority of these assays are based on the target-mediated crosslinking mechanism through the modification of AuNPs with specific ligand [28]. In particular, however, assay procedures based on non-crosslinking aggregation mechanism of AuNPs are more convenient and cost-effective due to the absence of elaborate and expensive synthesis of thio-containing ligand-modified AuNPs.

In this contribution, we document the selective adsorption of ssDNA on AuNPs, which can stabilize the AuNPs against aggregation even at high salt concentrations due to the strong electrostatic repulsion between gold particles [29]. However, when the Spm, a small organic molecule with high positive charge density, was introduced into the system, it play the dual roles of the charge shielding and ion bridging, which jointly results in the aggregation of the ssDNA-capped AuNPs without any addition of NaCl, along with color change from red to blue. Therefore, a sensitive and highly selective colorimetric method based on a non-crosslinking mechanism for the detection of Spm was developed. To the best of our knowledge, there is hardly any successful case as to the efforts towards the development of optical assays for cationic polyamine detection. The approach described here would provide a convenient new alternative for the specific detection of Spm in both biological fluid and fermented products.

2. Experimental

2.1. Apparatus

The UV–vis extinction spectra were recorded with a Shimadzu UV-3600 spectrophotometer (Shimadzu, Tokyo, Japan). The scanning electron microscope (SEM) images of AuNPs were performed with an S-4800 scanning electron microscope (Hitachi, Tokyo, Japan) operating at 30.0 kV. Solution-based ζ-potential and dynamic light scattering (DLS) measurements were completed on a Zetasizer Nano-ZS90 System (Malvern Instruments, UK). Besides, a LC system (Hitachi, Tokyo, Japan) with fluorescence detector, column oven L-2300, pump L-2130, and Waters Novapak C18 column (150 × 3.9 mm i.d., 4 μm) was applied for sample detection for comparison. Photographs were taken with an Olympus E-510 digital camera (Olympus, Tokyo, Japan). A vortex mixer QL-901 (Meditry Instruments, Haimen, China) was employed to mix the solutions.

2.2. Reagents

Colloidal AuNPs about 13 nm in diameter synthesized by means of citrate reduction of $HAuCl_4 \cdot 3H_2O$ [30], obtained from Sigma-Aldrich (St. Louis, MO, USA). The concentration of AuNPs was estimated by UV–vis spectroscopy based on the extinction coefficient of $2.7 \times 10^8\ M^{-1}\ cm^{-1}$ [31]. Oligonucleotide sequences S1 and S2 (S1, 5′–CGA CAA CCA CAA CAC ACA ATC TGA–3′; S2, 5′–GAA CGA CAA AAC CAT TAT ACG AT–3′), were purchased from Shanghai Shengong Genetech Co., Ltd. (Shengong Genetech Co., Shanghai, China) and used for comparison. The polyamines including Spermine (Spm), spermidine (Spd), putrescine (Put), cadaverine (Cad) and so on, *o*-phthaldialdehyde (OPA), and β-mercaptoethanol (β-ME) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Britton Robinson buffer (BR, pH 3.29) was used to control the acidity. All other reagents were of analytical reagent grade without further purification, and ultra pure water (18.2 MΩ) was used throughout.

2.3. General procedure

First, 100 μl of AuNPs suspension and 50 μl of 1.14 μM ssDNA sequences S1 was pipetted into a 1.5 ml vial. The mixture was vortex-mixed thoroughly and placed for 5 min. After that, 50 μl of BR buffer and an appropriate volume of Spm working solution were added. The mixture was diluted to 500 μl with ultra-pure water, vortex-mixed, and placed for another 25 min. Subsequently, the mixture was immediately transferred for absorption, ζ-potential, and SEM measurements.

2.4. Pretreatment of samples

The serum samples, which were sampled from the Hospital of Southwest University, were mixed with equal volume of acetonitrile and vortex-mixed thoroughly. Subsequently, centrifugal sedimentation was carried out for 10 min at 10000 rpm (6395 × *g*) to remove the protein. After that, the top liquid was filtered with 0.45 μm microporous membrane and the filtrate was used for the detection according to the general procedure using the proposed method and HPLC. Human urine samples have been obtained from a single donor, filtered with 0.45 μm microporous membrane and directly used for the analysis.

2.5. Derivatization procedure and chromatographic separation

The derivatization procedure and chromatographic detection of polyamines is performed according to the previously reported method [32] with a slight modification. Briefly, the formation of the OPA derivatives was performed automatically. The reaction solution consisted of 350 mg of OPA and 2.5 ml β-ME in 47.5 ml methanol. The OPA/β-ME reagent was left to stand for at least 2 h. For the derivatization reaction, 50 μl of the samples was automatically mixed with 20 μl of borate buffer (0.4 M, pH 10.5) and 40 μl of the reaction solution. Samples were filtered through Millipore filters (0.45 μm) and then directly injected into the HPLC system.

Eluent A contained $Na_2HPO_4 \cdot 12H_2O$ (3.6 mg/L, 10 mM). Eluent B consisted of 1% 2-octanol in acetonitrile and eluent A (70:30 v/v). The gradient elution program was as follows: 0–3 min (30% B, 0.8ml/min), 3–20 min (30–50%B, 0.8ml/min), 20–60 min (50–80% B, 0.8 ml/min). Derivatized amines were

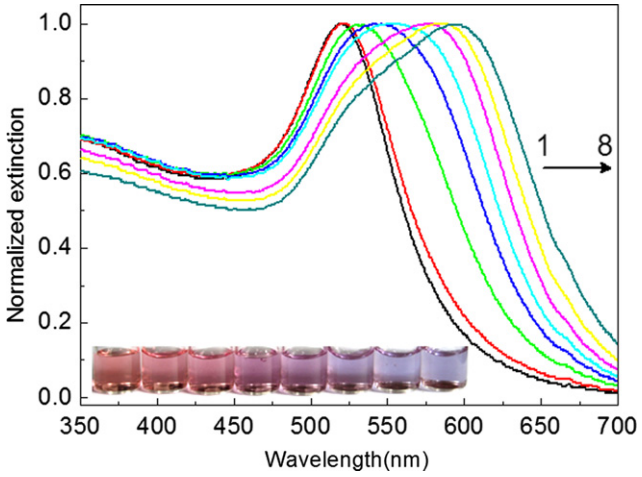

Fig. 2. UV–Vis spectra of ssDNA-capped AuNPs in the absence (Curve 1) and presence of Spm (Curves 2–8). Concentrations: AuNPs, 1.5 nM; ssDNA S1, 114 nM; Spm ($\times 10^{-6}$ M) from Curves 1–8, 0, 0.4, 0.6, 0.8, 1.2, 1.6, 2.0, 2.4; pH, 3.29. The inserted picture from left to right displays the color changes corresponding to Curves 1–8. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

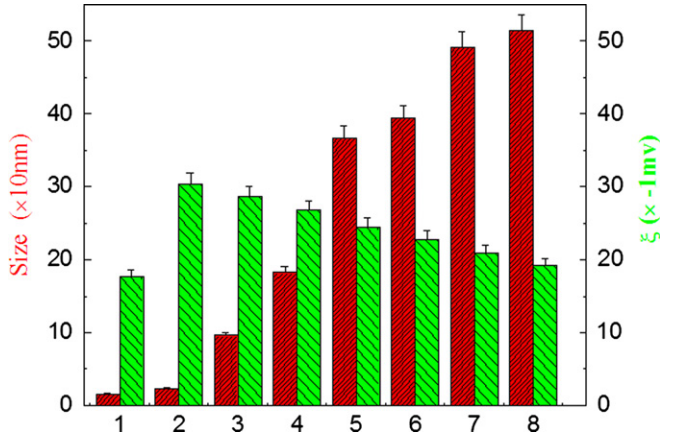

Fig. 3. Size information and zeta potential of the aggregation species of AuNPs with increasing Spm concentration. Concentration: AuNPs, 1.5 nM; ssDNA S1, 114 nM except that of column 1 in which no addition of ssDNA S1 was made; Spm from column 3 to 8, 0.4; 0.8; 1.2; 1.6; 2.0; 2.4 μM. pH, 3.29. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

detected using a fluorescence detector (excitation wavelength of 340 nm and emission wavelength of 425 nm).

3. Results and discussion

3.1. Spm-induced aggregation of ssDNA-AuNPs

It is well known that citrate anion capped AuNPs would get aggregated in a medium of high concentration salt for the screening effect, and ssDNA can be selectively adsorbed onto the surface of AuNPs, preventing the occurrence of aggregation process of AuNPs [29]. Indeed, experimental results verify the key role of ssDNA in keeping AuNPs against aggregation in salt medium (Fig. S1 in Supplementary Materials), and 1.5 nmol L^{-1} AuNPs could be well dispersed even if the concentration of NaCl reaches 175 mmol L^{-1}, when 114 nmol L^{-1} ssDNA sequences S1 was used. In the presence of Spm, however, the stability of ssDNA-adsorbed AuNPs disappears even without any addition of NaCl. As shown in Fig. 2, the alone AuNPs solution exhibits an absorbance maximum at 523.0 nm as a result of resonant excitation of surface plasma [19]. With the addition of trace Spm, the color of AuNPs solution gradually changes from red to blue (inset in Fig. 2). In line with this directly observable change in appearance, the extent of the plasma absorbance at 523.0 nm reduces and a new absorption band at longer wavelengths appears. With increasing Spm concentration, the extent of the red-shifted absorption peak gets increased (curve 2–8 in Fig. 2), corresponding to the surface plasmon resonance originated from strong coupling between neighboring nanoparticles [20,21]. In other words, the appearance of the new absorption band at longer wavelengths results from the close contact of AnNPs, demonstrating the occurrence of AuNPs aggregation in the presence of Spm. Similar phenomena also occurs to the ssDNA sequences S2 capped-AuNPs, illustrating that the aggregation of AuNPs is independent of the category of oligonucleotide.

Direct evidence for the aggregation of AuNPs could be provided by DLS and SEM measurements. As shown in Fig. 3, it was found that the particle size of ssDNA capped-AuNPs (the red column 2 in Fig. 3) almost has no obvious enhancement compared with the citrate anion capped AuNPs (the red column 1 in Fig. 3), however, the particle size get increased (the red columns 3–8 in Fig. 3) for a given amount of AuNPs with the increasing Spm concentration. Consistent with that of the DLS detection, as shown in Fig. 4, the SEM measurements exhibit that the ssDNA-capped AuNPs are monodispersed in the absence of Spm (Fig. 4a). On the contrary, the aggregation species of the AuNPs are formed in presence of Spm at the same conditions (b, c, d in Fig. 4), and it was observed that the extent of aggregation of AuNPs gets much more obviously with increasing Spm concentration.

3.2. Mechanism of aggregation of AuNPs

Generally, because the surface of ssDNA-capped AuNPs, carrying the same negative charges, are expected a priori to repel each other, the attractive force responsible for the non-crosslinking aggregation must be due to the presence of counterions. Thus, it seems reasonable to think that the introduction of tetravalent cationic Spm will be able to shield the negative charge and result in an efficient neutralization of the negative charges of ssDNA adsorbed on AuNPs, which is also confirmed by further ζ-potential measurements. As shown in Fig. 3, it was found that the zeta potential of ssDNA adsorbed AuNPs (the green column 2 in Fig. 3) decrease from −17.7 to −30.4 mV, compared with the citrate anion capped AuNPs (the red column 1 in Fig. 3), however, the zeta potential gradually restores (the red column 3–8 in Fig. 3) with the increasing Spm concentration and nearly reach to the original level (−19.2 mV).

It seems that the neutralization of the negative charges described above is not sufficient to cause the aggregation of ssDNA-capped AuNPs. However, the Spm, served as multivalent counterions, simultaneously may play the role of "ion bridging" among the ssDNA-capped AuNPs, according to the short-range electrostatic attractions between highly charged polyelectrolyte and multivalent counterions reported by Raspaud et al. [33]. In other words, the Spm play the dual roles, that is the charge shielding and ion bridging among the ssDNA-capped AuNPs, which jointly result in the aggregation of the ssDNA-capped AuNPs and this phenomenon is nearly independent on the monovalent salt concentration.

3.3. Optimal conditions

In order to verify the important roles of the Spm in the non-crosslinking aggregation of ssDNA-capped AuNPs, we could also

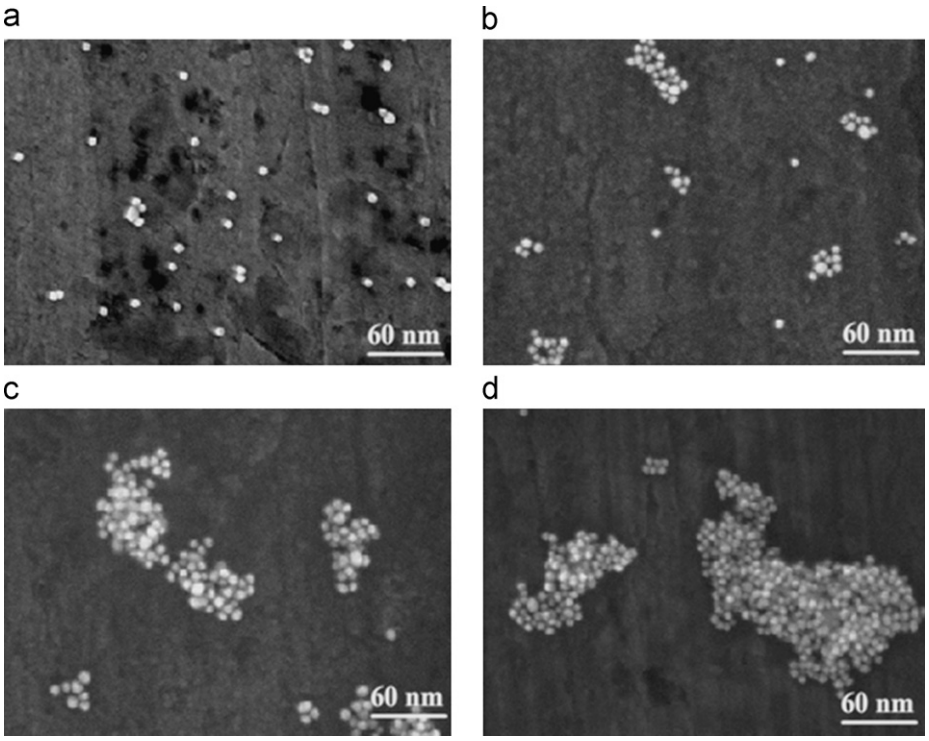

Fig. 4. SEM images of ssDNA-capped AuNPs in the absence (a) and presence of different concentrations of Spm (b, c, d). Concentration: AuNPs, 1.5 nM; ssDNA S1, 114 nM; Spm from a to d, 0; 0.4; 1.2; 2.0 μM. pH, 3.29.

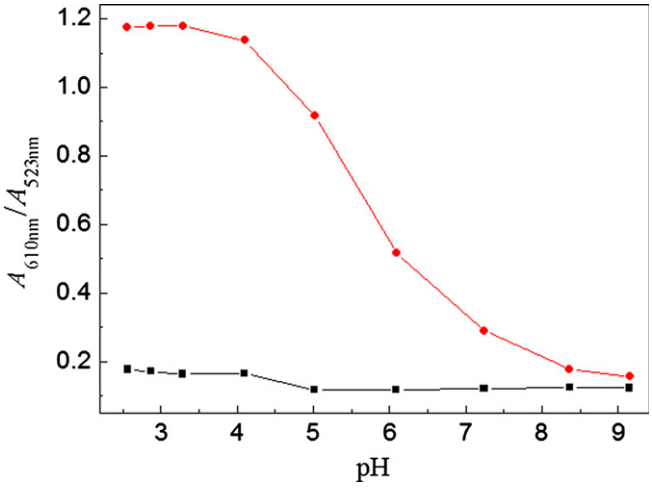

Fig. 5. Effect of pH on ssDNA capped-AuNPs aggregation in the presence (red curve) and absence (black curve) of Spm. Concentration: AuNPs, 1.5 nM; ssDNA S1, 114 nM; Spm, 1.2 μM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

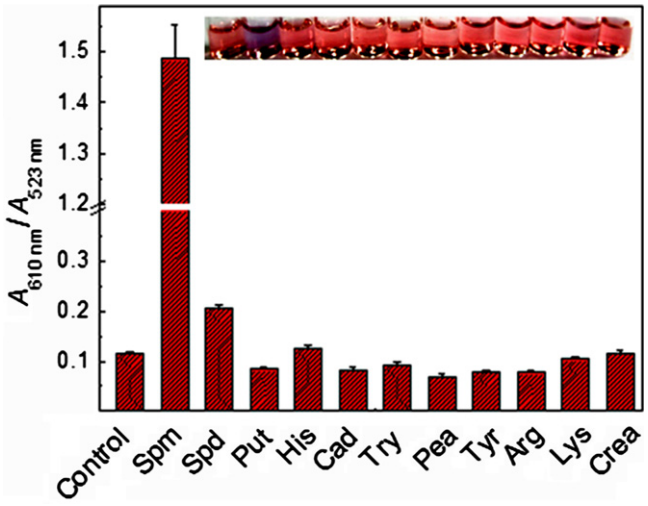

Fig. 6. The variation of the ratio values (A_{610nm}/A_{523nm}) upon the addition of various species. Concentration: AuNPs, 1.5 nM; ssDNA S1, 114 nM; Spm, 2.0 μM; Spd, 10 μM; the other species, 20 μM; pH, 3.29.

adjust the pH of the medium that decides the charge status of the Spm molecules and thereby affect the formation of ssDNA-capped AuNPs aggregates. As shown in Fig. 5, with the variation of pH, the ratio of absorption value, A_{610}/A_{523}, related to the quantities of aggregated and dispersed AuNPs, reach the highest level and keep stable in the pH range of 2.56–4.14 as a result of aggregate formation, and lessen distinctly with increasing pH so that they nearly approach the blank level of ssDNA-capped AuNPs when the medium is alkaline. The reason is that the deprotonation of Spm molecules in alkaline medium make it difficult to shield the negative charges and act the role of "ion bridging" among the ssDNA-capped AuNPs, which is indirectly in favor of the occurrence of charge neutralization and formation of ion bridging between ssDNA-capped AuNPs resulting in the aggregation of ssDNA-capped AuNPs.

In addition, the aggregation dynamics of ssDNA-capped AuNPs was elucidated by monitoring the ratio of absorption value, A_{610}/A_{523} time profiles in the presence of different concentrations of Spm (Fig. S2 in Supplementary Materials). The aggregation rate and extent of ssDNA-capped AuNPs is dynamic and dependent on the concentration of Spm. Obviously, the initial aggregation rate and extent gets increased with the increasing concentration of Spm, and the variation of A_{610}/A_{523} ratio tends to ease after 25.0 min, especially at high concentration of Spm. Therefore, reaction for 25.0 min at pH 3.29 after introduction of Spm is selected for the determination of Spm.

Table 1
Determination of the Spm in both serum and urine samples using the proposed method and HPLC ($n=3$, RSD, relative standard deviation).

Sample	Added ($\mu g\,L^{-1}$)	Intra-day variation of proposed method		Recovery (%)	Inter-day variation of proposed method		Recovery (%)	HPLC	
		Mean ($\mu g\,L^{-1}$)	RSD (%)		Mean ($\mu g\,L^{-1}$)	RSD (%)		Mean ($\mu g\,L^{-1}$)	RSD
Serum 1	208.9	198.4	3.8	91.4–98.6	196.8	4.2	90.2–98.2	202.7	3.1
Serum 2	766.0	728.7	2.6	92.5–95.4	731.0	3.1	93.0–98.8	746.8	2.9
Urine 1	139.3	135.3	4.6	92.9–101.8	136.6	5.4	92.2–102.5	136.8	3.7
Urine 2	835.7	811.5	3.5	94.3–100.9	815.5	3.8	94.1–101.4	822.4	4.2

3.4. Validation of analytical method

This methodology has been validated following the guidelines of the International Conference on Harmanisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines [34], which includes specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ).

The specificity of our analytical approach toward Spm against other cationic polyamines and common compounds, which may be expected to be present, such as spermidine (Spd), cadaverine (Cad), putrsescine (Put), histamine (His), tryptamine (Try) tyramine (Tyr), phenethylamine (Pea), arginine (Arg), lysine (Lys), creatinine (Crea) has been evaluated. As shown in Fig. 6, although the used concentration of most of species is 10 times than the one of Spm, the optical response of the probe toward Spm is about 12–21 times than those toward them. Only the Spd, served as trivalent counterions, could result in the aggregation of ssDNA-capped AuNPs under the condition of high Spd concentration. However, compared with Spm, the used concentration of Spd is 5 times than the one of Spm, the optical response toward Spm is 7 times than those toward Spd (Fig. S3 in Supplementary Materials), which indicate its negligible disturbance.

Under optimal conditions, the blanks was simultaneously spiked at 10 concentration levels of Spm in the range 0.07–0.8 $\mu g\,mL^{-1}$ (0.2–2.4 $\mu mol\,L^{-1}$), and the slope and intercept were obtained by least square linear regression between the value of A_{610}/A_{523} from the average of three determinations and the concentration of Spm (Fig. S4 in Supplementary Materials). The regression curve can be fitted as the equation of $A_{610}/A_{523}=0.09+2.2c$ (Spm, $\mu g\,mL^{-1}$). The LODs and LOQs, set as three and 10 times the standard deviation of the blank ($n=9$), were calculated to be 13.9 and 59.2 $ng\,mL^{-1}$, respectively.

The intra- and inter-day accuracy and precision of the proposed method were evaluated with serum and urine samples spiked at two concentration levels, respectively. The intra-day analysis was determined three times at different concentration levels on the same day, while the inter-day analysis corresponding to the average of three measurements of the intra-day values was taken on three consecutive days. The results, expressed as recovery for accuracy and relative standard error (RSD) for precision, are listed in Table 1. Besides good accuracy and adequate precision, the data show good agreement with the found values determined by HPLC, firmly indicating that the method is capable of detecting Spm in biological samples.

4. Conclusions

In this contribution, we present a simple and rapid colorimetric assay using an arbitrary ssDNA-capped AuNPs as an optical probe for the detection of Spm, on basis of the Spm, served as multivalent counterions, simultaneously acting the roles of charge shielding and ion bridging, and inducing the non-crosslinking aggregation of AuNPs. It is obvious that this method does not involve any modification or label procedure since the direct adsorption of arbitrary ssDNA on the surfaces of AuNPs is very easy. Well standing to reason, our approaches display the advantages of easily discerned with the naked eye, and therefore sophisticated instruments are not required for qualitative analysis. To the best of our knowledge, there is hardly any successful case as to the efforts towards the development of optical assays for cationic polyamine detection. Compared with the traditional methods of chromatography and capillary electrophoresis, the approach described here would provide a convenient new alternative and new train of thought for the specific detection of Spm in both biological fluid and fermented products.

Acknowledgments

All authors herein are grateful to the supports from the National Natural Science Foundation of China (Nos. 21035005, 21205096), and the Natural Science Foundation Project of China SWU (SWU208037).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.10.079.

References

[1] C.W. Tabor, H. Tabor, Annu. Rev. Biochem. 53 (1984) 749–790.
[2] B.G. Cipolla, J. Ziade, J.Y. Bansard, J.P. Moulinoux, F. Staerman, V. Quemener, B. Lobel, F. Guille, Cancer 78 (1996) 1055–1065.
[3] P. Celano, S.B. Baylin, R.A. Casero, J. Biol. Chem. 264 (1989) 8922–8927.
[4] B.G. Feuerstein, N. Pattabiraman, L.J. Marton, Nucleic Acids Res. 18 (1990) 1271–1282.
[5] B. Brune, P. Hartzell, P. Nicotera, S. Orrenius, Exp. Cell Res. 195 (1991) 323–329.
[6] A.U. Khan, Y.H. Mei, T. Wilson, Proc. Natl. Acad. Sci. USA 89 (1992) 11426–11427.
[7] U. Khan, P. Di Mascio, M.H.G. Medeiros, T. Wilson, Proc. Natl. Acad. Sci. USA 89 (1992) 11428–11430.
[8] E. Pegg, Cancer Res. 48 (1988) 759–774.
[9] M.F. Mitchell, G. Tortolero-Luna, J.J. Lee, W.K. Hittelman, R. Lotan, J.T. Wharton, W.K. Hong, K.J. Nishioka, J. Cell. Biochem. Suppl. 28/29 (1997) 125–132.
[10] M.H.Silla Santos, Int. J. Food Microbiol. 29 (1996) 213–231.
[11] G. Vinci, M.L. Antonelli, Food Control 13 (2002) 519–524.
[12] S. Yamamoto, H. Itano, H. Kataoka, M. Makita, J. Agric. Food Chem. 30 (1982) 435–439.
[13] M. Niitsu, K. Samejima, S. Matsuzaki, K. Hamana, J. Chromatogr. 641 (1993) 115–123.
[14] E.K. Paleologos, M.G. Kontominas, Anal. Chem. 76 (2004) 1289–1294.
[15] H. Nohta, H. Satozono, K. Koiso, H. Yoshida, J. Ishida, M. Yamaguchi, Anal. Chem. 72 (2000) 4199–4204.
[16] N.M. Tamim, L.W. Benett, T.A. Shellem, J.A. Doer, J. Agric. Food Chem. 50 (2002) 5012–5015.
[17] R. Romero, D. Gazquez, M.G. Bagur, M. Sanchez-Vinas, J. Chromatogr. A 871 (2000) 75–83.
[18] X. Liu, L.X. Yang, Y.T. Lu, J. Chromatogr. A 23 (2003) 213–219.
[19] S.K. Ghosh, T. Pal, Chem. Rev. 107 (2007) 4797–4862.
[20] U. Kreibig, L. Genzel, Surf. Sci. 156 (1985) 678–700.
[21] A. Mirkin, R.L. Letsinger, R.C. Mucic, J.J. Storhoff, Nature 382 (1996) 607–609.

[22] R. Elghanian, J.J. Storhoff, R.C. Mucic, R.L. Letsinger, C.A. Mirkin, Science 277 (1997) 1078–1081.
[23] M. Cho, M.S. Han, C. Ban, Chem. Commun. 46 (2008) 4573–4575.
[24] J.H. Lin, C.W. Chang, Z.H. Wu, W.L. Tseng, Anal. Chem. 82 (2010) 8775–8779.
[25] Y. Lin, C.J. Yu, Y.H. Lin, W.L. Tseng, Anal. Chem. 82 (2010) 6830–6837.
[26] Y.S. Kim, J.H. Kim, I.A. Kim, S.J. Lee, J.S. Jurng, M.B. Gu, Biosens. Bioelectron. 26 (2010) 1644–1649.
[27] K. Saha, S.S. Agasti, C. Kim, X.N. Li, V.M. Rotello, Chem. Rev. 112 (2012) 2739–2779.
[28] M.E. Stewart, C.R. Anderton, L.B. Thompson, J. Maria, S.K. Gray, J.A. Rogers, R.G. Nuzzo, Chem. Rev. 108 (2008) 494–521.
[29] H.X. Li, L. Rothberg, Proc. Natl. Acad. Sci. USA 101 (2004) 14036–14039.
[30] K.C. Grabar, R.G. Freeman, M.B. Hommer, M.J. Natan, Anal.Chem. 67 (1995) 735–743.
[31] L.M. Demers, C.A. Mirkin, R. Mucic, R.L. Reynolds, R. Letsinger, R. Elghanian, G. Viswanadham, Anal. Chem. 72 (2000) 5535–5541.
[32] A. Marcobal, M.C. Polo, P.J. Martin-Alvarez, M.V. Moreno-Arribas, Food Res. Int. 38 (2005) 387–394.
[33] E. Raspaud, M. Olvera de la Cruz, J.L. Sikorav, F. Livolant, Biophys. J. 74 (1998) 381–393.
[34] 〈http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html〉.