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A simple and sensitive AuNPs-based colorimetric aptasensor for specific detection of azlocillin

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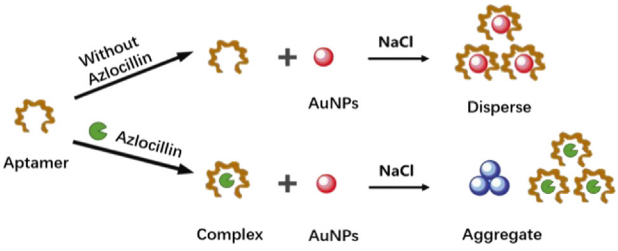
HIGHLIGHTS

- A novel label-free colorimetric aptasensor was developed for azlocillin detection.
- The color change of the solution is relevant to the concentration of azlocillin, and this can be easily observed by naked eye and quantitatively detected by an ultraviolet–visible spectrometer.
- The approaches displayed a detection limit of 11.6 nM towards azlocillin.
- The colorimetric aptasensor was successfully applied in the determination of azlocillin in real samples.

GRAPHICAL ABSTRACT



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ABSTRACT

A new colorimetric biosensor for specific detection of azlocillin was developed by using DNA aptamer as recognition element and unmodified gold nanoparticles (AuNPs) as colorimetric indicator. In the absence of azlocillin, the AuNPs were protected by the aptamer and stabilized at high NaCl concentrations, displaying a red solution. In the presence of azlocillin, the aptamer reacts specifically with azlocillin, resulting in the aggregation of AuNPs and an apparent red to blue color change. The characteristic change can be easily observed by the naked eye and quantitatively detected by an ultraviolet–visible (UV–Vis) spectrometer. Under the optimal conditions, the absorbance variation at 522 nm (ΔA_{522}) of AuNPs changed proportionally with increasing concentration of azlocillin, which exhibited a linear relationship in the concentration range of 50 nM to 500 nM, with a detection limit of 11.6 nM. Furthermore, the aptasensor was successfully used to detect azlocillin in milk and tap water samples, with recoveries ranging from 97.64% to 102.21% and a relative standard deviation (RSD) less than 3.81%.

1. Introduction

Antibiotics are chemical compounds used to prevent and treat bacterial infections in people and animals. They interfere with metabolic processes that inhibit the growth and replication of the bacterium or kill it outright [1,2]. Penicillin was first discovered in 1928 and was the first antibiotic used successfully in treating bacterial infections [3,4]. The penicillin class of antibiotics contains 21 chemically related drugs, such as penicillin V, penicillin G, azlocillin, amoxicillin, ticarcillin, ampicillin, piperacillin, oxacillin, flucloxacillin, nafcillin, dicloxacillin, carbenicillin, et al [5]. All of these medicines contain a β-lactam ring in their structure, so they

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belongs to a member of a wide class of antibiotic family named β-lactam, each type of penicillin has additional side chains that determine its activity.

Azlocillin is a member of β-lactam antibiotics, this class of antibiotics are widely used as growth promoters and anti-infective drugs in animal husbandry [6]. In dairy production, β-lactam antibiotics are the most used drugs to treat mastitis, the inappropriate and uncontrolled use of these antibiotics may leave residues in animal derived food such as milk and meat [6]. Furthermore, a large part of the antibiotics may excrete into the environment through urine and feces without metabolism. The undesirable residues in environment or in animal derived food may cause serious irreversible side effects on human health [7].

The traditional methods for detection of azlocillin include spectrophotometric method [8], back iodometric titration method [9] and high performance liquid chromatography (HPLC) method [10]. Generally, spectrophotometric method and back iodometric titration method are complicated and time-consuming, the HPLC method requires expensive instrumentation and professional experts [11,12]. Therefore, it is necessary to establish rapid, cost-effective and sensitive methods to detect azlocillin.

In recent years, biosensors have received extensive attention in food control, environmental monitoring and diagnostic applications [13,14]. An aptasensor is a particular class of biosensor where the biological recognition element is a DNA or RNA aptamer. Aptamers can recognize a broad range of specific targets ranging from small molecules to whole cells and viruses with high affinity and specificity [15]. The aptamer-target molecular interplay can be easily transduced to detectable signals, such as electrochemical, colorimetric, fluorescent and illuminant signals [16]. Furthermore, aptamers possess the notable advantages including small size, easy synthesis, low cost, high stability, high binding affinity and good thermal stability [17,18]. Recently, the first aptasensor for azlocillin based on competitive voltammetric assay was reported by Chinnappan et al., the proposed method exhibited high sensitivity and selectivity [19]. Therefore, there is a great potential for developing other aptasensors for azlocillin detection.

Nowadays, AuNPs are widely used in colorimetric assays for their extremely high extinction coefficients and unique distance-dependent optical propertites [15,20]. Surface plasmon resonance (SPR) is one of the most important characteristic of AuNPs, which refers to the collective oscillation of surface electrons of gold nanoparticles after photon excitation, resulting in a strong absorption band in the visible region [21]. The SPR band is influenced not only by the shape and size of AuNPs but also by the properties of the matrix surrounding the nanoparticles [22,23]. AuNPs colorimetric aptasensor is based on the sensitivity of the position and intensity of surface plasmon resonance band with the particle size and aggregation state, which produces a visible color change [24]. In general, the well-dispersed AuNPs display a red color, when AuNPs approach each other and aggregate, the color changes to blue or purple [24]. Colorimetric assays are simple and sensitive, thus various AuNPs-aptamer based colorimetric assays have been established to detect drugs, metal ions and toxins [25–27].

In this work, a simple and sensitive colorimetric detection method for azlocillin was developed using 58-mer single-stranded DNA as recognition element and AuNPs as a colorimetric indicator. In the prescence of azlocillin, aptamers specifically bind to azlocillin and lose the ability to protect AuNPs against the NaCl induced aggregation, correspondingly weaken the absorption peak of AuNPs and cause the color change of the solution, which can be used for the quantitative analysis of azlocillin. To the best of our knowledge, this is the first AuNPs-aptamer colorimetric biosensor for the detection of azlocillin.

2. Materials and methods

2.1. Materials

The 58-mer azlocillin aptamer with the sequence 5′-CAG GAA GAC AAC TCC GAC TAG AAT TGA TAA TCA AGA ATT CGT CTG GGG GGA ATG TGC G-3′ was developed by Chinnappan [19] and synthesized by Sangon Biotech Co., Ltd (Shanghai, China). Chloroauric acid ($HAuCl_4{\cdot}4H_2O$) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Sodium chloride was purchased from Tianjin Fengchuan Chemical Regant Technologies Co., Ltd (Tianjing, China). Azlocillin sodium salt was purchased from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Sodium citrate, amoxicillin, penicillin G sodium salt were purchased from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Chloramphenicol was purchased from Sangon Biotech Co., Ltd (Shanghai, China). Ampicillin sodium salt was purchased from Beijing Solarbio Science and Technology Co., Ltd (Beijing, China). All these chemicals were used without further purification.

2.2. AuNPs synthesis and characterization

AuNPs were synthesized by reduction of $HAuCl_4$ with sodium citrate according to the procedure reported by Liu et al [28]. All glasswares were cleaned in freshly prepared aqua regia (HCl: HNO_3 = 3:1), washed thoroughly in deionized water and fully dried before use. 100 mL of 1 mM $HAuCl_4$ was heated to boiling with vigorous stirring, 10 mL of 38.8 mM sodium citrate was quickly added to the solution. The color changed from pale yellow to wine red in 1 min. The mixture was boiled for another 20 min and then naturally cooled down to room temperature under continuously stirring. The prepared AuNPs solution was placed in a brown bottle and stored at 4℃ before use. The size of the AuNPs determined by a Tecnai G2 F20 transmission electron microscope was approximately 13 nm (Fig S1). The concentration was calculated to be 11.5 nM according to Beer-Lambert law based on the extinction measured at 522 nm, with the molar extinction coefficient of 2.7×10^8 $L{\cdot}mol^{-1}{\cdot}cm^{-1}$ [29].

2.3. General procedure

0.5 OD azlocillin aptamer was centrifuged at 8000 r/min for 5 min, then dissolved in 85 μL H_2O to obtain a stock solution of 10 μM. 10 μL azlocillin at final concentration of 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 nM were mixed with 10 μL of 0.8 μM azlocillin aptamer solution respectively and incubated for 6 min. Afterwards, 35 μL of 11.5 nM AuNPs was added into the mixture, incubated for 15 min. Subsequently, 145 μL of 62 mM NaCl was added into the above solution and the final volume was 200 μL. After equilibrated for another 12 min, the UV–Vis absorption spectra were recorded using a TU-1810 (Puxi, China) UV–Vis spectrophotometer over the range from 400 nm to 700 nm.

2.4. Determination of azlocillin in real samples

Tap water and milk samples were spiked with different concentrations of azlocillin for validating the effectiveness of the aptasensor in real samples. Tap water was collected in Baotou, China. Different concentration of azlocillin was spiked into the tap water samples and the detection was then carried out. Skim milk was purchased from supermarket and pretreated according to Wang's reference with slight modifications [30]. 200 μL of milk samples were spiked with azlocillin at concentration of 150 nM, 250 nM

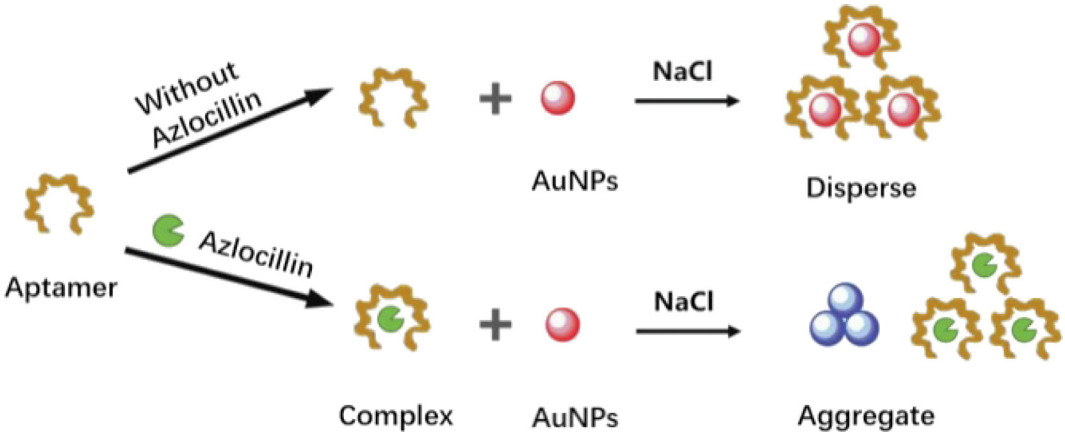

Fig. 1. Schematic description of colorimetric sensing of azlocillin based on the azlocillin aptamer and gold nanoparticels.

and 350 nM, respectively. Subsequently, 200 μL of ethyl acetate was added, the mixture was sonicated for 15 min and centrifuged at 3500 r/min for 12 min. The supernatant was collected and dried under nitrogen and then resuspended in 10 μL deionized water for future use.

3. Results and discussion

3.1. Principle of the colorimetric detection

The principle of the proposed method for quantitative detection of azlocillin was illustrated in Fig. 1. The synthesized citrate-stabilized AuNPs maintain a disperse state due to negative capping citrate generate an electrostatic repulsion force that prevents their aggregation. Upon the addition of NaCl, the negative charge of citrate neutralized, resulting in gold nanoparticles aggregation. Single-stranded DNA aptamer could be strongly adsorbed on the surface of AuNPs through coordination between the nitrogen atoms of aptamers and the AuNPs, thus enhanced the stability of AuNPs against salt induced aggregation [31,32]. In the presence of azlocillin, the aptamer specifically bind to azlocillin. As a result, AuNPs aggregated under certain NaCl concentration. Consequently, the color of the solution turns from red to blue.

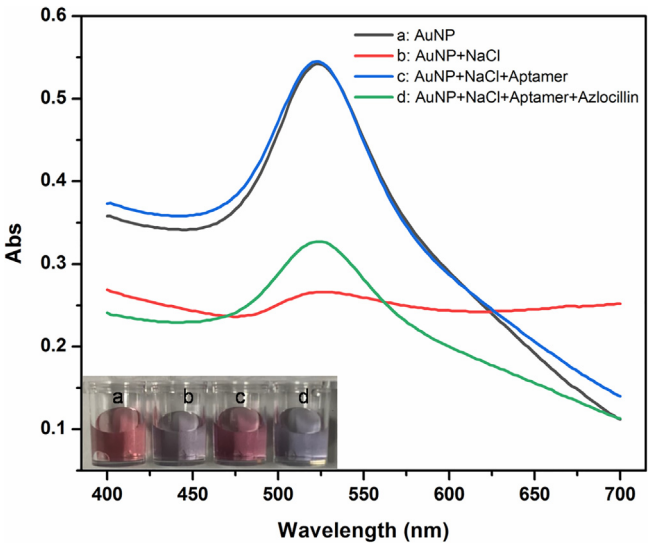

Fig. 2. UV–Vis spectra of AuNPs under different conditions (AuNPs: 2.01 nM; NaCl: 45 mM; aptamer: 40 nM; azlocillin: 500 nM).

The proposed azlocillin detection principle was further confirmed by UV–Vis absorption spectra. As shown in Fig. 2, the citrate-protected AuNPs were red in color and displays a strong absorption peak at 522 nm (a in Fig. 2). Addition of 45 mM NaCl would screen the repulsion between the gold nanoparticles, leading to the aggregation of AuNPs, meanwhile, the absorption peak significantly decreased and the color of the solution changed to blue (b in Fig. 2). When aptamer was introduced to the system, aptamer would protect AuNPs against NaCl induced aggregation, the solution remains in red color and the intensity of the absorption peak at 522 nm was restored, which indicated that the AuNPs get back to disperse status (c in Fig. 2). Once azlocillin was loaded, the aptamer specifically bind to azlocillin and expose AuNPs to get aggregated, the absorbance peaks decreased greatly and the solution turned blue (d in Fig. 2).

3.2. Optimization of experimental conditions

3.2.1. Effect of NaCl concentration

The concentration of NaCl is a critical factor for the aggregation degree of the AuNPs. Hence, minimum concentration of NaCl that is sufficient enough to induce the aggregation of AuNPs was determined. 165 μL different concentrations of NaCl were added to 35 μL AuNPs solution and incubated for 12 min, UV–Vis absorption spectra of AuNPs and color change of the solution were recorded. As shown in Fig. 3, the characteristic absorption peak at 522 nm decreases significantly with increased NaCl concentration, accompanied by a visible color change from red to blue. When the concentration of sodium chloride reached 50 mM, the UV–Vis absorption peak at 522 nm almost disappeared, indicating that AuNPs were seriously aggregated at this time. Therefore, 45 mM sodium chloride was selected for subsequent experiments.

3.2.2. Effect of reaction time

The reaction time is a key parameter in colorimetric assay. Thus the following reaction times were carefully optimized.

(A) Reaction time between AuNPs and NaCl

35 μL of 11.5 nM AuNPs and 165 μL of 54.5 mM NaCl was mixed and incubated for 0, 4, 8, 12, 16 min, respectively. As seen in Fig. 4A, the absorbance at 522 nm was greatly decreased with the increase of incubation time at the early stage and remains nearly constant after 12 min, which meant the reaction between AuNPs and NaCl reached a state of equilibrium. Therefore, 12 min was chosen as the optimal time between AuNPs and NaCl.

(B) Reaction time between AuNPs and aptamer

35 μL of 11.5 nM AuNPs and 20 μL of 0.4 μM aptamer was mixed adequately and then incubated for 0, 5, 10, 15, 20 min,

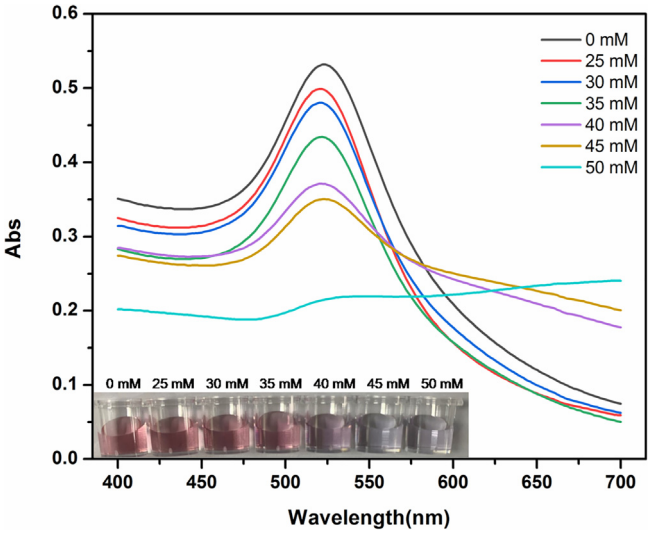

Fig. 3. Effect of NaCl concentration on the aggregation of AuNPs (AuNPs: 2.01 nM).

respectively. After that, 145 μL of 62 mM NaCl was added into the above solution and equilibrated for another 12 min. The UV–Vis absorption spectra were recorded. As shown in Fig. 4B, the absorbance at 522 nm increased with increasing reaction time before 15 min, a maximum absorbance was attained at 15 min. Therefore, 15 min was employed in the following experiments. This probably because the interaction between aptamer and AuNPs was slow and weak, the aptamers were gradually adsorbed to the surface of gold nanoparticles in the initial stage, the absorbance increased, as time goes on, a partial aptamer desorbed from the AuNPs, leading the aggregation of AuNPs in NaCl solution. As a consequence, the UV–Vis absorption spectra of AuNPs decreased slightly.

(C) Reaction time between azlocillin and aptamer

To optimize the binding efficiency between azlocillin and aptamer, 10 μL of 10 μM azlocillin was mixed with 10 μL of 0.8 μM azlocillin aptamer and incubated for 3, 6, 9, 12 min, respectively. Afterwards, 35 μL of 11.5 nM AuNPs was added into the mixture, incubated for 15 min. Subsequently, 145 μL of 62 mM NaCl was added into the above solution. After equilibrated for another 12 min, the UV–Vis absorption spectra were recorded. Aptamer binding to azlocillin induced a change in the absorbance at 522 nm of AuNPs, which decreased with the reaction time up to 6 min and increased thereafter (Fig. 4C), indicating that the maximum binding of azlocillin with aptamer was achieved at 6 min. This phenomenon was probably because at the early stage, the binding of aptamer and azlocillin was quite loose, which requires a large number of apatmer to bind to azlocillin. When the reaction time was more than 6 min, the binding of aptamer and azlocillin was getting tighter, a portion of aptamer was released from azlocillin-aptamer complex and adsorbed on the surface of AuNPs, aptamer protected AuNPs against NaCl induced aggregation, consequently, the intensity of the absorption peak was restored slightly. Thus, the optimal reaction time between azlocillin and aptamer was 6 min.

3.2.3. Effect of aptamer concentration

The concentration of the aptamer was an important factor to the sensitivity in colorimetric assays. Low concentration of the aptamer could not maintain AuNPs in disperse state completely because the aptamer was not enough to cover all the surface of the AuNPs. On the other hand, excessive aptamer with part of aptamer unbounded might reduce the sensitivity and the linear range of the assay. Hence, appropriate concentration of aptamer was

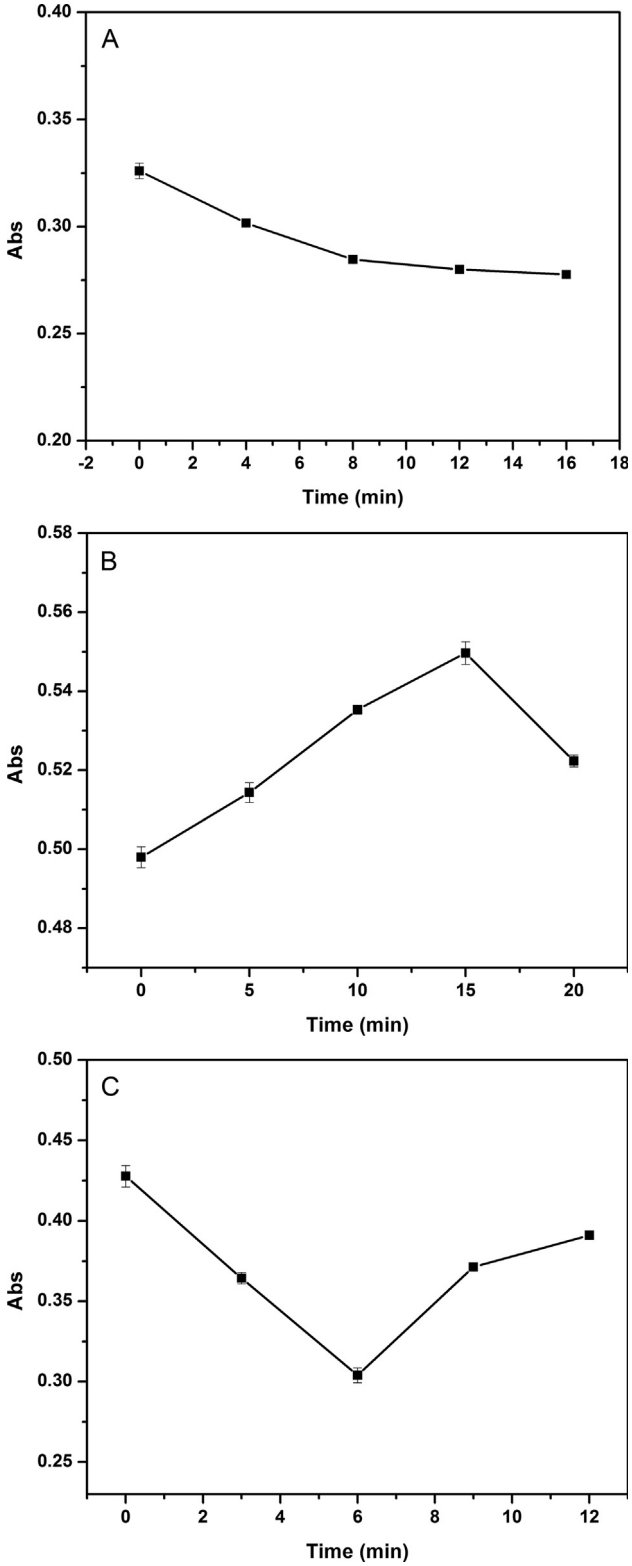

Fig. 4. Optimization of the reaction time between (A) AuNPs and NaCl (AuNPs: 2.01 nM; NaCl: 45 mM), (B) AuNPs and aptamer (AuNPs: 2.01 nM; NaCl: 45 mM; aptamer: 40 nM) and (C) azlocillin and aptamer (AuNPs: 2.01 nM; NaCl: 45 mM; aptamer: 40 nM; azlocillin: 500 nM).

optimized. 20 μL different concentration of aptamer (final aptamer concentration: 10, 20, 30, 40, 50, 60 nM) were mixed with 35 μL AuNPs solution respectively, the mixture was incubated for 15 min at room temperature. Subsequently, 145 μL of 62 mM NaCl

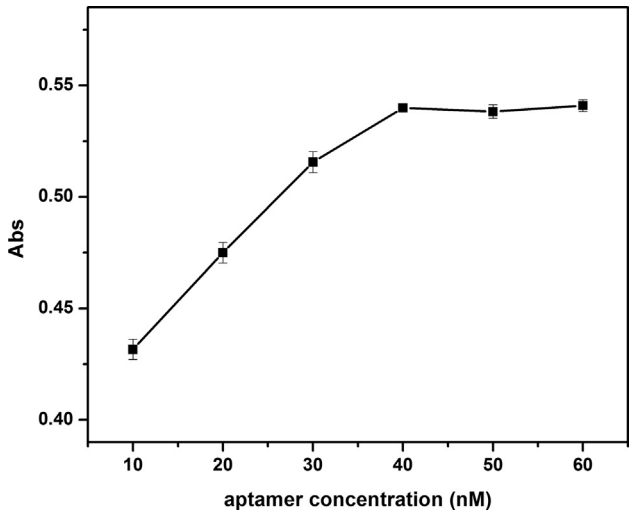

Fig. 5. Optimization of the concentration of the aptamer (AuNPs: 2.01 nM; NaCl: 45 mM).

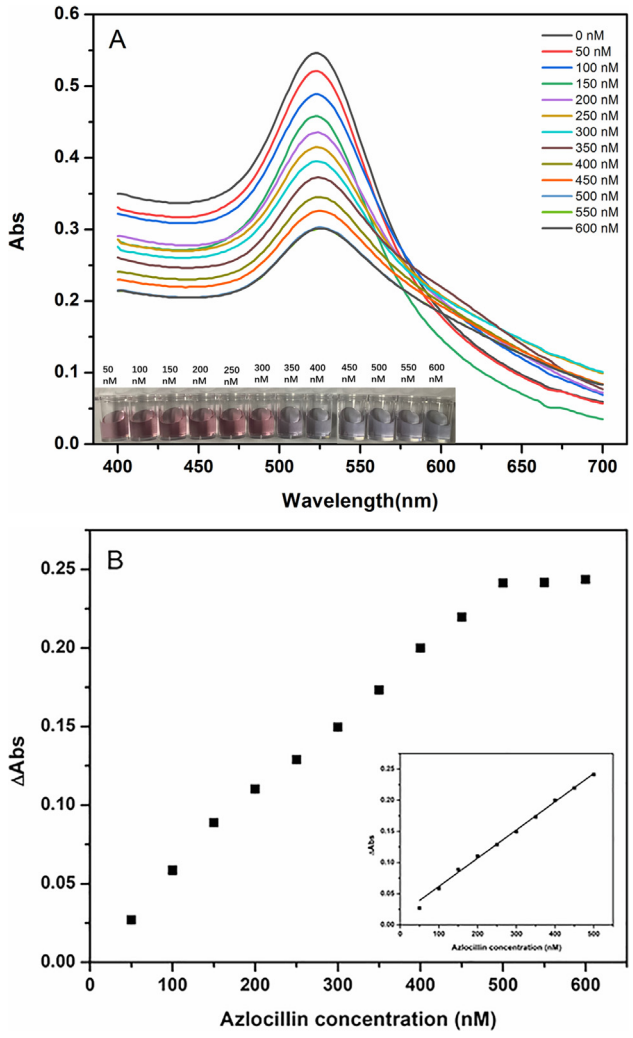

Fig. 6. Sensitivity of the aptasensor for azlocillin detection. (A) UV–Vis spectra and color change of AuNPs in the presence of different concentration of azlocillin, (B) The absorbance variations of ΔA_{522} versus the concentrations of azlocillin. Inset shows the linear calibration curve (AuNPs: 2.01 nM; NaCl: 45 mM; aptamer: 40 nM).

was added into the above mixture and incubated for another 12 min. As shown in Fig. 5, when the concentration of the aptamer was lower than 40 nM, AuNPs were not well protected by aptamer and could still partially aggregate under 45 mM NaCl, the absorbance increases with aptamer concentration. When the concentration of the aptamer was higher than 40 nM, AuNPs were stably protected by aptamer and their absorbance was stable. Therefore, 40 nM aptamer was selected for further studies.

3.3. Sensitivity and selectivity

Under the optimal conditions, the sensitivity of this colorimetric strategy was studied. The UV–Vis absorption spectra from 400 to 700 nm were recorded under different concentrations of azlocillin. The color change of the solution was photographed simultaneously. As presented in Fig. 6, the absorption peak at 522 nm decreased gradually with the increasing azlocillin concentration from 50 nM to 500 nM and the solution color changed from red to blue. When azlocillin concentration was more than 500 nM, the absorption spectra tended to be stable. The inset of Fig. 6B showed a good linear relationship between ΔA_{522} and azlocillin concentration ranging from 50 nM to 500 nM. The linear equation was y = 0.00045x + 0.01657, with a correlation coefficient of 0.9944. A detection limit of 11.6 nM was obtained according to the 3σ rule [33,34].

To test the selectivity of this azlocillin detection assay, different analogs of azlocillin, including amoxicillin, penicillin G, ampicillin and chloramphenicol, were tested using the sensing system. The absorption spectra of each sample were recorded and the change in the absorbance at 522 nm was compared. As shown in Fig. 7, azlocillin caused the most obvious absorbance change, while no obvious changes were observed for other analogs at the same concentration as azlocillin. These results indicated that this colorimetric sensor showed a good selection, other analogues would not affect the analysis of azlocillin.

3.4. Detection of azlocillin in real samples

The practical applicability of the proposed colorimetric assay was then assessed by detected azlocillin in milk and tap water samples. Various concentrations of azlocillin were spiked into the

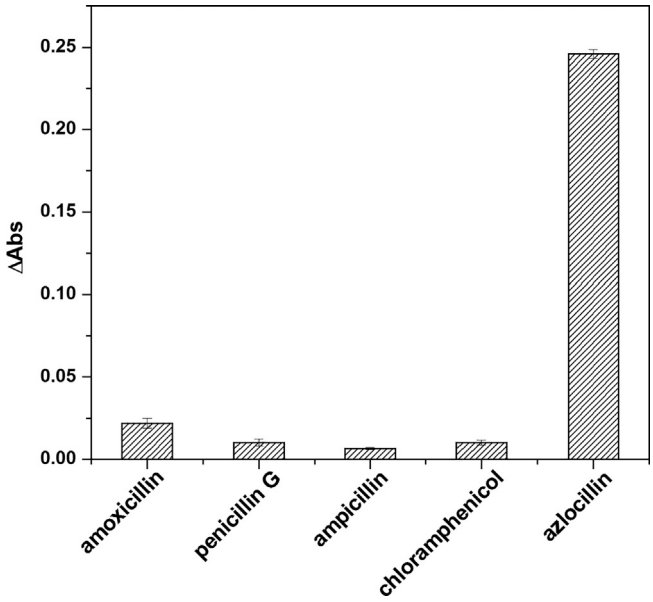

Fig. 7. Selectivity of the aptasensor for azlocillin detection. (AuNPs: 2.01 nM; NaCl: 45 mM; aptamer: 40 nM; antiobiotic: 500 nM).

Table 1
Recovery of azlocillin from spiked samples (n = 3).

Sample	Spiked (nM)	Detected (nM)	Recovery (%)	RSD (%)
Milk	150.00	153.31 ± 5.84	102.21 ± 3.90	3.81
	250.00	249.73 ± 3.37	99.89 ± 1.35	1.35
	350.00	341.73 ± 2.55	97.64 ± 0.73	0.75
Tap water	150.00	151.10 ± 4.42	100.73 ± 2.94	2.92
	250.00	247.52 ± 4.60	99.01 ± 1.84	1.86
	350.00	341.73 ± 3.37	97.64 ± 0.96	0.99

Table 2
Comparison with other reported methods for the detection of azlocillin.

Methods	Samples	LOD	Analytical ranges	Recoveries	Reference
HPLC	Serum and aqueous solutions	0.4 μg/ml	0 - 100 μg/ml	90.4%-110.5%	[10]
Voltammetric aptasensor	Tap water and waste water	1.2 pg/mL	1 pg/mL- 100 μg/mL	96.2%-113%	[19]
Colorimetric aptasensor	Milk and tap water	11.6 nM (5.6 ng/ml)	50–500 nM (24–240 ng/ml)	97.64%-102.21%	This method

milk and tap water and the recoveries of azlocillin were determined at optimal conditions. As shown in Table 1, the average recoveries of the azlocillin were 97.64%-102.21% and the relative standard deviations (RSDs) were 0.75%-3.81%. The results indicated that the developed aptasensor was feasible for rapid detection of azlocillin in real samples.

Different analytical methods for determination of azlocillin were summarized briefly in Table 2. The recovery of this method were superable to other method, limit of detection (LOD) was higher than the voltammetric aptasensor but lower than HPLC method.

4. Conclusions

In summary, a simple, rapid and cost-effective colorimetric method was developed for the detection of azlocillin by using DNA aptamer and unmodified AuNPs. The principle of the method is based on the aggregation of AuNPs that is regulated by specific interactions between azlocillin and aptamer. The results proved that the developed colorimetric assay exhibited good selectivity and high sensitivity. Furthermore, this method was successfully employed to detect azlocillin in milk and tap water samples, with a recovery of 97.64%-102.21% and relative standard deviations of 0.75%-3.81%. This strategy is convenient, reliable and easy to operate, without the requirements of large instruments and modifications of AuNPs and aptamers, which promoted the practical application in the on-site detection of azlocillin.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2022.120924.

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