



Research article

DNA based biosensing of *Acinetobacter baumannii* using nanoparticles aggregation methodFarnaz Bahavarnia^{a,b,c,d}, Paria Pashazadeh-Panahi^{e,f,g}, Mohammad Hasanzadeh^{h,*}, Nasrin Razmiⁱ^a Food and Drug Safety Research Center, Tabriz University of Medical Sciences, Tabriz, Iran^b Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran^c Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran^d Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran^e Hematology-Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, 51664, Iran^f Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran^g Endocrinology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran^h Pharmaceutical Analysis Research Center, Tabriz University of Medical Sciences, Tabriz, Iranⁱ Department of Science and Technology, Physics, Electronics and Mathematics, Linköping University, Sweden

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ABSTRACT

Acinetobacter baumannii is the main cause of nosocomial infections in blood, urinary tract, wounds and in lungs leading to pneumonia. Apart from its strong predilection to be the cause of serious illnesses in intensive care units. Herein, we present a specific and sensitive approach for the monitoring of *Acinetobacter baumannii* genome based on citrate capped silver nanoparticles (Cit-AgNPs) using spectroscopic methods. In this study, (5' SH-TTG TGA ACT ATT TAC GTC AGC ATG C3') sequence was used as a probe DNA (pDNA) of *Acinetobacter baumannii*. Then, complementary DNA (cDNA) was used for hybridization. After the hybridization of pDNA with cDNA, target DNA (5' GCA TGC TGA CGT AAA TAGTTC ACA A 3') was recognized and detected using turn-on fluorescence bioassay. After the hybridization of pDNA with cDNA, the target DNA was successfully measured in optimum time of 2 min by spectrophotometric techniques. Moreover, the selectivity of designed bioassay was evaluated in the presence of two mismatch sequences and excellent differentiation was obtained. 1 Zepto-molar (zM) of low limit of quantification (LLOQ) was achieved by this genosensor. The present study paved the way for quick (2 min) and accurate detection of *Acinetobacter baumannii*, which can be a good alternative to the traditional methods. Current study proposed a novel and significant diagnostic test towards *Acinetobacter baumannii* detection based on silver nanoparticles aggregation which has the capability of being a good alternative to the traditional methods. Moreover, the proposed genosensor successfully could be applied for the detection of other pathogens.

1. Introduction

Acinetobacter is a genus of Gram-negative bacteria that is found in environment, soil and water ubiquitously. Among different types, *Acinetobacter baumannii* (*A. baumannii*) strain accounts for most of the infections in human. This bacterium is Gram-negative, oxidase-negative, catalase-positive and non-motile coccobacilli. There are more than 20 genomic *Acinetobacter* species, but only few of them are considered potentially pathogenic. *A. baumannii* was found to be the most clinically relevant species which is resistance to decolorization during Gram staining. Therefore, *A. baumannii* can be identified incorrectly as a Gram-

positive bacterium and affects adversely. Moreover, *A. baumannii* has a potential ability to reveal resistance mechanisms against several antibiotic classes including third generation cephalosporins, carbapenems, fluoroquinolones and aminoglycosides [1]. Also, *A. baumannii* is an opportunistic pathogenic bacteria which has a significant role in hospital-acquired infections [1].

The worldwide prevalence of *Acinetobacter* has been reported about 6.8% in North America, 7% in Europe, 18.6% in Latin America and 17.5% in Asia, in 2006–2009 [2]. According to its prevalence and important microbial criteria, phenotypic identification of *Acinetobacter* is significantly important [3]. Therefore, rapid identification of this

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pathogen is critical for appropriate therapy and preventing the spread of infection. Most common *Acinetobacter* species have been successfully identified using conventional methods such as phenotypic systems and molecular methods. However, not only these methods are unable to detect genome 3 and genome 13TU of *Acinetobacter* but also require specific methods (such as biosensing) and several days of incubation [4].

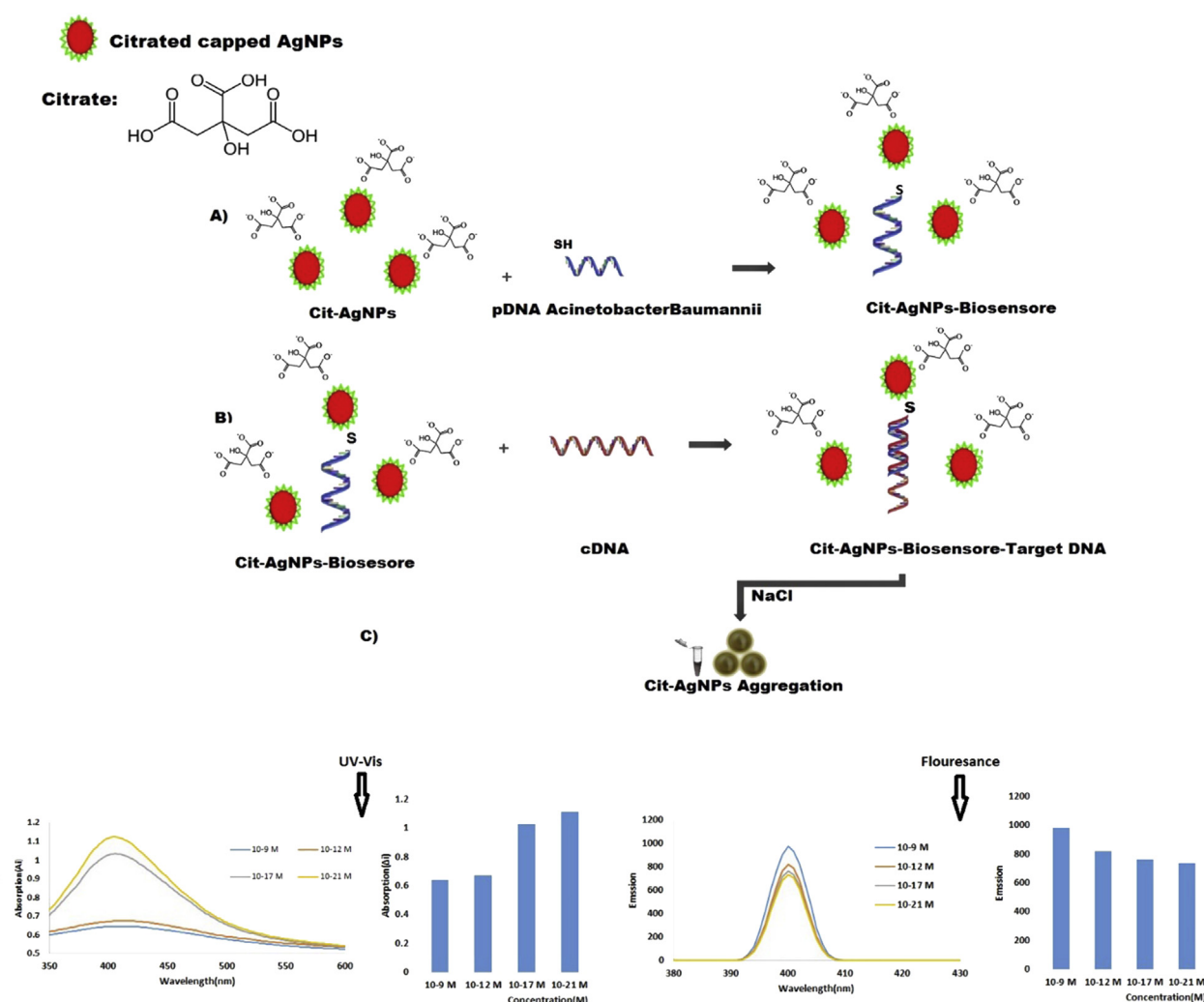
Other conventional methods such as bacteria culture [5], polymerase chain reaction (PCR) [6], and enzyme-linked immunosorbent assay (ELISA) are time-consuming and laborious for detection of this bacteria [7, 8]. Moreover, surface-enhanced Raman scattering have been used increasingly in recent years which requires hard pretreatment and a comprehensive database for distinguishing of this bacteria accurately [9, 10, 11].

Although many methods have been applied for detection of *A. baumannii*, they are time-consuming and not affordable methods [4]. To overcome the drawbacks of conventional existing molecular methods, there is an urgent demand for a sensitive, selective and cost effective tool to detect the low level of *A. baumannii*. Spectrophotometric methods are widely used analytical methods in laboratories and industries due to their easy to use and low operational costs [5]. This method is widely used to detect and measure organic and inorganic compounds and wide range of products such as food products, fertilizers, petrochemicals, proteins and nucleic acids.

In this study, spectroscopic determination of *A. baumannii* based on citrate capped silver nano particles that functions via specific sequence of *A. baumannii* genome is presented. The important advantages of these

nanoparticles used in sensors are unique electronic, optical and chemical properties [6]. In addition, high extinction coefficient with sharp extinction bands make these materials superior candidates for use in sensors [7]. Due to the unique properties of nanoparticles, AuNPs is used to increase the sensitivity of diagnosis. The fundamental idea in the present work is based on DNA hybridization and target sequence detection via the spectrophotometric method. For this purpose, ssDNA can uncoil to expose its bases, whereas dsDNA has a stable double-helix geometry that always presents the negatively charged phosphate backbone [12, 13, 14, 15]. Conferring to the results of present study, the engineered genosensor show simple structure with high sensitivity, stability, and high selectivity. It seems that this biodevice can be developed in conjunction with most pathogens and detection of microorganism due to the specific features, especially the simple structure of the biosensor.

In this study, citrate capped silver nanoparticles were used to spectrophotometric detection of *Acinetobacter*. In the present work, we report a highly specific and sensitive approach for *A. baumannii* genome detection based on innovative citrate capped silver nanoparticles (Cit-AgNPs) using spectrophotometric method. In this study, probe DNA of *Acinetobacter baumannii* (5' SH-TTG TGA ACT ATT TAC GTC AGC ATG C3') was designed and after hybridization with cDNA, target DNA (5' GCA TGC TGA CGT AAA TAG TTC ACA A 3') was detected using optical methodology. The purpose of proposed methodology was presenting a novel, simple, low-cost, sensitive and selective system as a diagnostic test for recognizing of *Acinetobacter baumannii* based on silver nanoparticles and its capability as a good alternative to conventional methods. General



Scheme 1. Illustration of engineered DNA based biosensor for detection of *Acinetobacter baumannii*.

procedure for the preparation of optical probe and detection mechanism of *Acinetobacter* was shown in Scheme 1. To the best of our knowledge, this is the first report on the detection of *A. baumannii* based on DNA targeting using Citrat-Ag NPs. This is the main novelty of this study. Also, 1 zM of achieved low limit of quantification with sensitivity and selectivity is another advantage of this report.

2. Methods and materials

2.1. Chemicals and reagents

All solutions were prepared with doubly distilled deionized water purchased from Shahid Ghazi Pharmaceutical Company (Tabriz, Iran). DNA oligonucleotide sequences were acquired from Takapouzist Co. (Iran). Tris buffer, NaCl and Sodium acetate were obtained from Merck (Darmstadt, Germany). Ethyl acetate obtained from Sharlo Company (Spanish). Source of *Acinetobacter baumannii* is OMPA gene (IX87-RS045). *Acinetobacter baumannii* complete oligonucleotide sequences (5' SH-TTG TGA ACT ATT TAC GTC AGC ATG C 3'), GC ratio: 40%, basecount: 25, Tm: 59.4. Complementary target sequence (5' GCA TGC TGA CGT AAA TAG TTC ACA A 3'), basecount: 25, Tm: 59.4, GC ratio: 40%. Single nucleotide mismatch target sequence (5' GTA TGC TGA CGT AAA TAG TTC ACA A3'), GC ratio: 36%, Tm: 56.7, basecount: 25. Two nucleotide mismatch target sequence (5' GTA TGA TGA CGT AAA TAG TTC ACA A 3'), basecount: 25, GC ratio: 32%, Tm: 54.6 [16]. The oligonucleotide stock solutions were diluted with 0.1 M Tris-HCl buffer, pH 7.4 solution (Tris). Dithiothreitol (DTT) (Sigma-Aldrich company united-states) solved in Tris-HCl and employed as a redox indicator for revealing DNA hybridization. All the above solutions were kept at 4 °C before use. DTT solution containing 10 mM sodium acetate and 500 mM DTT, pH (5.2) was prepared and kept at 4 °C.

2.2. Instruments

UV-VIS spectrophotometer analysis achieved by shimadzu UV-1800 with a resolution of 1 nm. Fluorescence spectrometry analysis achieved by Jasco FP-750 spectrofluorometer (Jasco, Kyoto, Japan) equipped with a 150 W xenon lamp using a micro-volume cell with 1.0 cm path length. The centrifugation was performed on a KUBOTA 6800 centrifuge (KUBOTA Corporation, Japan).

2.3. Synthesis of citrate capped silver nanoparticles (Cit-AgNPs)

Chemical reductions for synthesized Cit-AgNPs occurred in glass wares rinsed thoroughly with deionized water. In which AgNO₃ was source of Ag⁺, NaBH₄ was reducing agent and citrate ions (Na₃C₆H₅O₇) were used as stabilizing capping agents. Briefly, 400 mL of 1.06mM Na₃C₆H₅O₇ solution was thoroughly mixed with 25 mL of 5mM AgNO₃ solution while stirring in an ice/water bath at around 0 °C. Next, 2500 µL of a freshly prepared aqueous solution of NaBH₄ (100 mM) was added dropwise to it over 5 min. The color of the solution immediately changed from colorless to light yellow. The mixture was then stirred vigorously under dark conditions for about 95 min until a shiny yellow hue appears which marks the end of the reaction and confirms the successful synthesis of the Cit-AgNPs. The ice-bath was then removed and temperature of the suspension was allowed to reach room temperature by storing it in a dark place overnight [8]. All characterization data and supporting information are indicated in (Figs. S1 to S3 (see supporting information)).

3. Results and discussion

3.1. Activation of *Acinetobacter baumannii* primer (pDNA) and its conjugation with Cit-AgNPs as an optical probe

Dithiotriitol (DTT) was utilized for the activation of *Acinetobacter* primer. DTT has been used, typically as a reducing or "deprotecting"

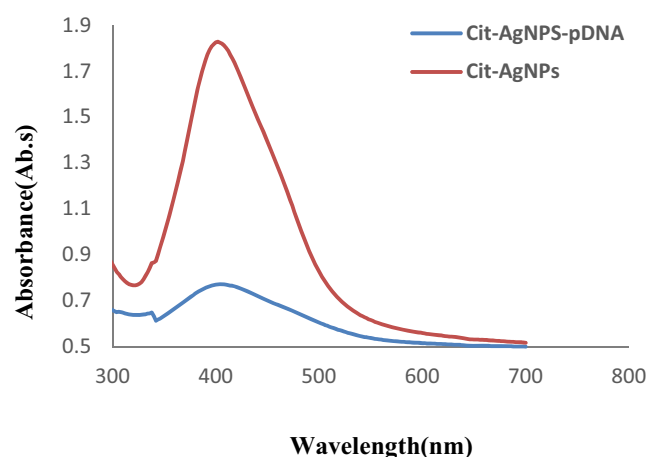


Figure 1. UV/Vis absorbance spectrum of Cit-AgNPs and Cit AgNPs after conjugation with pDNA.

agent for thiolated *Acinetobacter* primer [9]. The terminal sulfur atoms of thiolated pDNA have an affinity to form dimers in solution, mainly in the presence of oxygen. Moreover, DTT prevents oxidation of thiol groups and can be used as protecting agent [10]. In this study, 0.01 M of DTT and 0.01M of sodium acetate (10 ml) was dissolved in deionized water (DW). Then, 10 µl of the prepared solution mixed with 15 µl of pDNA and incubated for 15 min. After incubation time, 200 µl of ethyl acetate was added to solution and vortexed for 5 min. Prepared solution centrifuged in 8000 rpm for 10 min. After removing of supernatant, 200 µl of ethyl acetate was added to the solution and centrifuged in 8000 rpm for 10 min. After removing of supernatant, 200 µl of Cit-AgNPs was added properly and the solution incubated in 45 °C for 2 h. At the end of the incubation time, 400 µl of Cit-AgNPs and mixed Cit-AgNPs-pDNA pipetted in the cuvettes and optical analysis via fluorescence and UV/Vis were performed. As it is shown in Figures 1 and 2, after adding pDNA, a significant change occurred in UV-Vis and fluorescence spectra that indicate covalent bonding of Cit-AgNPs to the thiol groups of probe oligonucleotides. The UV-Vis spectrum peak of pure Cit-AgNPs appeared at wavelength of 400 nm with intensity of 1.8. However, the same analysis for Cit-Ag NPs in the present of pDNA showed a peak at wavelength of 400 nm with intensity of 0.77. While the fluorescence spectra of solutions were opposite. The UV-Vis spectrum peak of pure Citrate capped Ag NPs appeared at wavelength of 400 nm with intensity of 225, however the same analysis for Cit-AgNPs in the present of pDNA showed a peak at wavelength of 400 nm with intensity of 1000. Therefore, results indicate that covalent bonding of Cit-AgNPs to thiol groups of *Acinetobacter* primer will increase the intensity of fluorescence spectra peak.

3.2. Optimization of hybridization time

Hybridization of the probe DNA with *Acinetobacter* complementary sequences was developed according to Strelau et al., protocol [10]. Based on previous studies, adsorption of ssDNA on silver nanoparticles is selective. It is considered that the mentioned feature stabilizes the silver nanoparticles against aggregation by concentrations of salt that would typically screen the repulsive interactions of the citrate ions in the lack of a complementary target sequence [17, 18]. For this purpose, after removal of supernatant, 200µl of Cit-AgNPs were added to solutions and incubated in 45 °C for 2h. Subsequently, 15 µl of cDNA was added to 15 µl of *Acinetobacter baumannii* pDNA. So, NaCl was added to the solution. Finally, UV/Vis and fluorescence spectra of the prepared solutions were recorded at different successive times (2,5,10, and 15 min). As displayed in the Figure 3, the fluorescence spectrum peak of Citrate capped Ag NPs conjugated with pDNA appeared at wavelength of approximately 400 nm with intensity of 932, 996, 790 and 741 in 2, 5, 10, and 15 min

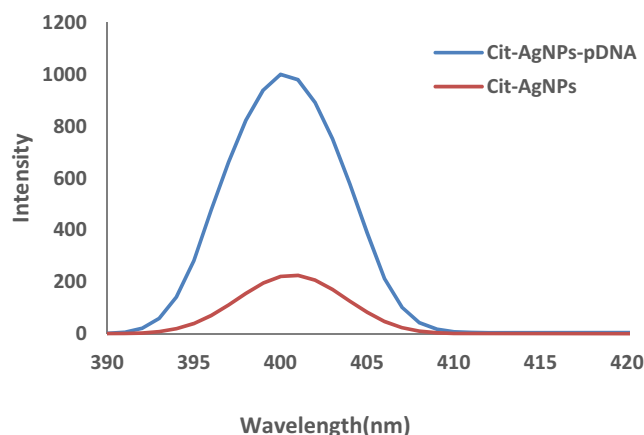


Figure 2. Fluorescence and absorbance spectrum of Cit-AgNPs and Cit-AgNPs-pDNA after conjugation with pDNA.

respectively. According to the obtained result, the optimization time for the hybridization of *Acinetobacter* DNA with pDNA was 2 min.

4. Analytical study

The sensitivity analysis of the fabricated genosensors is one of the requirements of DNA-based bioassay. For the hybridization detection and recording the readout signals, different concentrations of cDNA (10^{-9} , 10^{-12} , 10^{-17} , and 10^{-21} M) were prepared. In accordance with the previous step, 0.01 M of DTT and 0.01M of sodium acetate (10 ml) was

dissolved in deionized water (DW). Then, 10 μ l of the prepared solution mixed with 15 μ l of pDNA and incubated for 15 min. After the incubation time, 200 μ l of ethyl acetate was added to solution and vortexed for 5 min. The prepared solution centrifuged in 8000 rpm for 10 min. After removing of supernatant, 200 μ l of ethyl acetate was added to the solution and centrifuged in 8000 rpm for 10 min. After removing the supernatant, 200 μ l of Cit-AgNPs were added properly and incubated in 45 $^{\circ}$ C for 2 h. Then, 1 mM of NaCl was added to enhance the stability of nanoparticles. Finally 15 μ L of this solution was added to the prepared solution and incubated for 2 min and UV/Vis and fluorescence spectrum data were recorded. As displayed in Figure 4, The fluorescence spectrum peak of Cit-AgNPs-pDNA appeared at wavelength of approximately 400 nm with intensity of 980, 822, 764, 737 in concentrations of 10^{-9} , 10^{-12} , 10^{-17} , 10^{-21} M respectively. Similar to UV/Vis results, the designed genosensor can be used to detection of target sequence (cDNA) on the concentration of 1ZM. Accordingly, dynamic range was obtained as $1\text{nM-}1\text{ZM}$ and regression equation recorded was $y = -78.603 C_{\text{Acinetobacter baumannii}} + 1022.5$ ($R^2 = 0.8724$). Obtained results by proposed biosensor were compared with the previously reported method [Table 1]. Analytical result show that the proposed biosensor has the capability of detecting *A. baumannii* with sensitivity and selectivity compared with previously reported studies [19, 20, 21, 22, 23, 24, 25, 26, 27].

5. Selectivity

Considering the fact that the selectivity is one of the important aspects of any representative biosensor, selectivity assessment of the fabricated *Acinetobacter* genosensor was done by applying two mismatch sequences of (5'GTA TGC TGA CGT AAA TAG TTC ACA A3') basecount: 25, GC

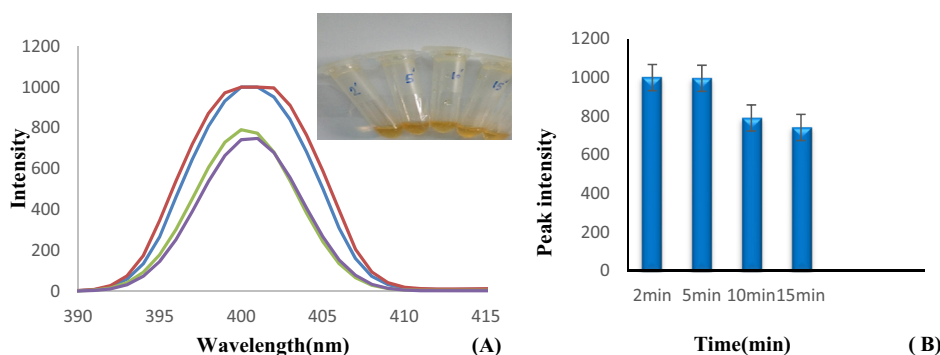


Figure 3. A: Fluorescence and absorbance spectrum of Citrate-AgNPs after conjugation with pDNA in different incubation time (2,5,10, and 15 min). B: Histogram of peak intensity in different incubation time (2,5,10, and 15 min) ($n = 3$, $SD = 1.26$).

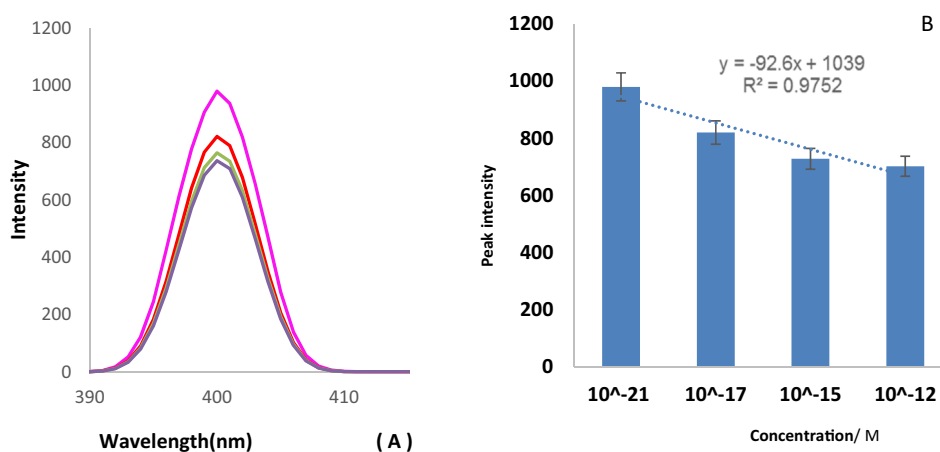


Figure 4. A: Fluorescence and absorbance spectrum of hybridization in various concentrations (10^{-9} , 10^{-12} , 10^{-17} , and 10^{-21} M) of *Acinetobacter* DNA with pDNA. B: Calibration curve ($n = 3$, $SD = 2.06$).

Table 1. Recently developed biosensors for detection of *Acinetobacter baumannii*.

Pathogen	Method	Nano Particles	Detection range	Detection Limit	Year and Ref
<i>Acinetobacter baumannii</i>	Spectrophotometric	Gold Nano particles	0.11–0.166 $\mu\text{mol/l}$	0.8125 ng/ μl	2014 [19],
	fluorescence	Au Ag nanoclusters	1×10^4 – 5×10^7 cfu/ml	2.3×10^3 cfu/m	2018 [20],
	UV-Vis Spectroscopy	TMCN-Ag Nano particles	0–12.25 $\mu\text{g/ml}$	≤ 6.13 $\mu\text{g/ml}$	2017 [21],
	fluorescence	Magnetic Nano particles	-	1×10^4 , 1×10^5 cells/ml	2019 [22],
	Electro-microchip DNA biosensor	Au NPs and Ag supported estereptoavidin	-	0.825 ng/ml (1.2 fM)	2010 [23],
	Electrochemical genosensor	Au NPs supported beta cyclodextrin	0.3nM–0.24 μM	0/14 nM	2018 [24],
	fluorescence microscopy	Megnetic Nano particles	1×10^3 – 1×10^8 cfu/ml	1×10^5 cfu/ml	2013 [25],
	*Localized surface plasmon resonance	Gold Nano Particles	-	4×10^2 to 4×10^6 cfu mL ⁻¹	2019 [26],
	Fluorescence	Cit-Ag NPs	1 μM –1 ZM	1 ZM (LLOQ)	This Work

* LSPR: Localized Surface Plasmon Resonance.

ratio: 36%, Tm: 56.7 and (5' GTA TGC TGA CGT AAA TAG TTC ACA A3'), GC ratio: 32% Tm: 54.6, basecount: 25. Similar to previous step, the *Acinetobacter baumannii* pDNA was incubated with 15 μl of mismatch primers. Spectrophotometric evaluations were conducted and UV/Vis and fluorescence spectrum were carried out to record the spectral absorbance. As it is shown in Figure 5 the fluorescence spectrum peak of Cit-rate capped Ag NPs with p DNA and two different mismatched sequences appeared at wavelength of 400 nm with intensity of 956 for mismatch 1 and 1000 for mismatch 2 and positive samples respectively. According to the obtained results, proposed bioassay is able to differentiate this sequences selectively.

6. Stability

One of the most important advantages of an ideal biosensor is its high stability. To increase the stability of the fabricated genosensor, Cit-AgNPs have been used in this study. Silver nanoparticles have been widely used in different applications owing to their distinct chemical, physical and biological properties. These properties of silver nanoparticles are highly influenced by their shape and size. The stability of the designed genosensor was evaluated within 24 h. As shown in Figure 6. The proposed platform is completely stable and usable for 24 h. Result shows that the reported methodology despite of its simplicity has acceptable stability.

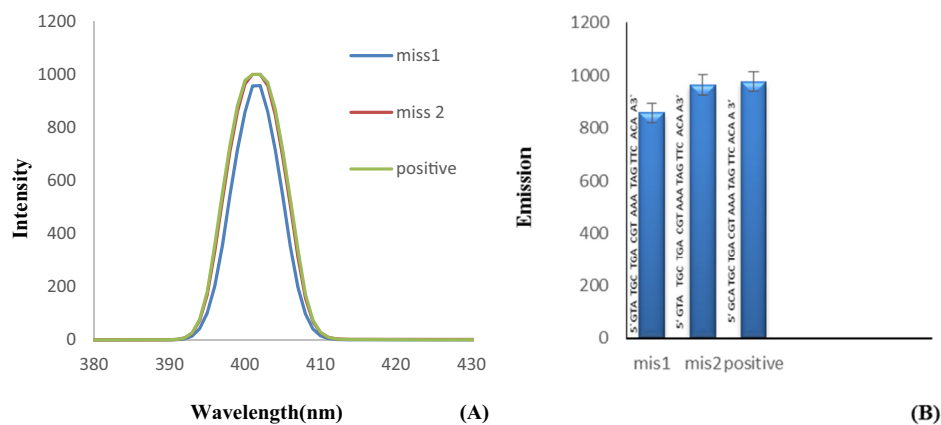


Figure 5. A: Fluorescence and absorbance spectrum of cDNA hybridization with mismatch 1 and mismatch 2. B: Histogram of peak intensity for the cDNA hybridization with Mismatch 1 and Mismatch 2 (n = 3, SD = 2.24).

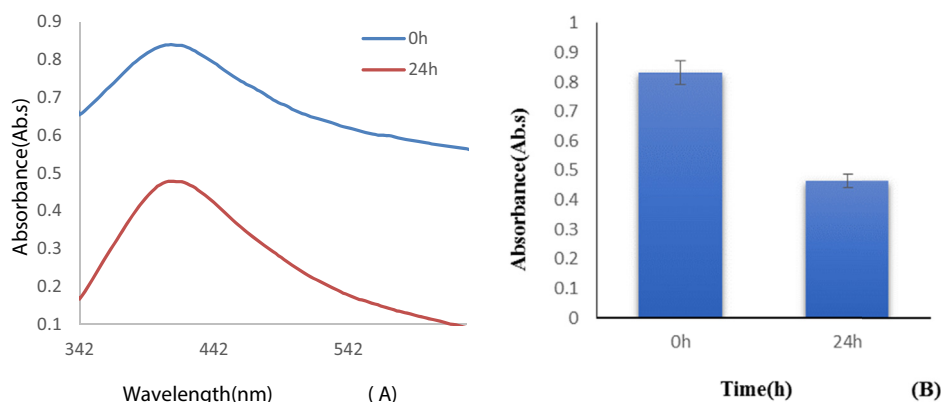


Figure 6. A: UV/Vis absorbance spectrum of designed biosensor after 24h of storage. B: Histogram of peak intensity versus time of test (n = 3, SD = 1.98).

7. Conclusion

In current study, optical biosensing of *Acinetobacter* based on citrate capped silver nanoparticles was performed for the first time. The unique electronic, optical and chemical properties of silver nanoparticles in comparison to their bulk material have made them suitable candidate to be applicable in spectrophotometric fluorescence detection of *Acinetobacter* in the current study together with citrate enhancing the selectivity, sensitivity and stability of the assay. After the hybridization of pDNA with cDNA, the target DNA was successfully measured in optimum time of 2 min by spectrophotometric techniques. Moreover, the selectivity of designed bioassay was evaluated in the presence of two mismatch sequences and excellent differentiation was obtained. 1 zM of low limit of quantification (LLOQ) was achieved by this genosensor. The present study paved the way for quick (2 min) and accurate detection of *Acinetobacter baumannii*, which can be a good alternative to the traditional methods. Also, proposed biosensor is capable be used in clinical studies after final analytical validation.

Declarations

Author contribution statement

Farnaz Bahavarnia: Performed the experiments; Wrote the paper.
 Paria Pashazadeh-Panahi: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
 Mohammad Hasanzadeh: Conceived and designed the experiments; Analyzed and interpreted the data.
 Nasrin Razmi: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflicts of interest.

Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2020.e04474>.

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