DNA-Templated Silver Nanoclusters as a Label-Free Optical Sensor for Cisplatin Detection

Kanyalak Kosai[1,2,3], Sirirat Ouiganon[1,2,3], Chongdee Thammakhet-Buranachai[1,2,3], Panote Thavarungkul[1,2,3,4], Proespichaya Kanatharana[1,2,4] and Chittanon Buranachai[1,2,3,*]

[1]Division of Physical Science, Faculty of Science, Prince of Songkla University, Songkhla, Thailand
[2]Thailand Center of Excellence in Physics, Commission on Higher Education, Bangkok, Thailand
[3]Center of Excellence for Trace Analysis and Biosensor, Prince of Songkla University, Songkhla, Thailand
[4]Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Songkhla, Thailand
* Corresponding author. E-mail address: chittanon.b@psu.ac.th

Abstract

Cisplatin or *cis*-diamminedichloroplatinum (II), is a potent anti-cancer drug widely applied to treat various types of cancer. Unfortunately, it also has various negative effects, such as neuro and renal toxicity. Therefore, it is of critical importance to avoid cisplatin overdosage in patients and to avoid cisplatin contamination in chemotherapy facilities. Conventionally, the detection of cisplatin in relevant samples of interest can be accomplished using various techniques, such as high-performance liquid chromatography (HPLC); however, these methods are often expensive and require skilled operators. To address the issue, this study proposes a simple fluorescent biosensor based on DNA-templated silver nanocluster (AgNC), with the specific binding between DNA and silver ions and acting as a reducing agent to convert silver ions into silver nanoclusters. Under the 570 nm excitation light, the nanocluster emits bright fluorescence (peaked at 620 nm) partially due to the enhancement from nearby guanine bases on the DNA-template. However, the fluorescence intensity is quenched by cisplatin likely because of cisplatin – guanine bonding. Therefore, cisplatin can be easily quantified by measuring the decrease in AgNC fluorescence emission. Under optimal conditions, the biosensor demonstrates a good linear range of detection from 5.0 to 40.0 μM, a low limit of detection (LOD) of 2.7 μM and a limit of quantitation (LOQ) of 9.0 μM. With further development, the developed biosensor should be able to measure cisplatin in samples of interest, such as biological fluids from patients undergoing chemotherapy or water samples from wastewater treatment plants in hospitals.

Keywords: *cisplatin, biosensor, DNA-templated AgNC, silver nanocluster*

1. Introduction

Cis-diamminedichloroplatinum (II), or cisplatin is a potent anti-cancer drug that is widely used to treat various types of cancer, including lymphomas, sarcomas, skin, ovarian, testicular, bladder and lung cancer (Christie & Tansey, 2007; Jung & Lippard, 2007; Dasari & Bernard Tchounwou, 2014). Once entering cancer cells, cisplatin molecules turn into a more reactive diaqua form that bonds to the N7 position of one or two purine bases, with high preference for consecutive guanines (Wang & Lippard, 2005; Wang et al., 2013). The resulting DNA-cisplatin adducts interfere with DNA replication and transcription, ultimately leading to cell death (Wang & Lippard, 2005). However, cisplatin does not distinguish between cancerous and normal cells. Therefore, it can cause severe negative side effects, such as neuro and renal toxicity (Cornelison & Reed, 1993; Smoorenburg et al., 1999; Dasari & Bernard Tchounwou, 2014). Ironically, cisplatin is classified as a type II carcinogenic substance (National Toxicology Program, 2021).

Unintentional cisplatin exposure may be prevented by closely monitoring cisplatin contamination in chemotherapy workplaces, in patient's body fluid, especially urine samples or in wastewater treatment facilities within hospitals. Conventional techniques for cisplatin detection include liquid chromatography mass spectrometry (LC-MS) (Shaik et al., 2017), inductively coupled plasma mass spectrometry (ICP-MS) (Brouwers

et al., 2008), and high-performance liquid chromatography (HPLC) (Toro-Córdova et al., 2016). Despite being highly specific and sensitive, these techniques are expensive and requires well-trained personnels to operate. To address the problems, several research groups developed cisplatin fluorescent biosensors as simpler and cheaper alternatives (Yang et al., 2014; Jantarat et al., 2021). However, with a few exceptions, they are still based on organic fluorescent dyes that are sensitive to ambient light and often require expensive labeling.

Fluorescent nanostructures, such as quantum dots, QDs offer intriguing alternatives due to their high robustness, high fluorescence quantum yields, and extended shelf life (Farzin & Abdoos, 2021). Despite tremendous efforts to apply greener approaches in recent years, many QDs syntheses still involve toxic reagents and tedious processes (Liu et al., 2009, Dhamo et al., 2022). On the other hand, metallic nanoclusters (NCs), such as silver NCs, are gaining widespread support due to their ease of synthesis and requires lesser or non-toxic substances required during synthesis. Even though AgNCs can be synthesized as free clusters under suitable reducing conditions, DNA containing certain sequence rich in cytosine offers a stabilizing template for AgNC formation (Petty et al., 2004, Song et al., 2019). The resulting DNA-templated AgNCs shows tunable fluorescence excitation and emission peaks and high photo-stability (Xu et al., 2020). In addition, they are sensitive to microenvironments, such as ions (Lee et al., 2014). Accordingly, they are utilized as biosensors for detecting small molecules, DNA, or proteins (Sharma et al., 2010, Sharma et al., 2011, Liu et al., 2013; Zheng et al., 2015).

What interests us is that the fluorescence emission of DNA-templated AgNCs can be enhanced by nearby guanine bases (Li et al., 2020; He et al., 2021; Fredrick et al., 2023). They proposed that this enhancement is due to electron transfer between the nearby guanine bases and AgNCs. As a result, we hypothesize that the AgNC emission enhancement will be suppressed in the presence of cisplatin-DNA adduct formation after cisplatin-guanine's N7 binding allowing the DNA-templated AgNCs to be used as a simple cisplatin biosensor.

2. Objectives

To develop a simple and economical optical biosensor for cisplatin detection.

3. Materials and Methods

3.1 DNA-templated AgNCs synthesis and the proposed sensing mechanism

The DNA sequence named Ag4 (shown in Step 1 of Figure 1) is derived directly from a prior work (Sharma et al., 2010). It contains a nucleation domain (bolded letters) for Ag^+ ions binding and AgNC formation and multiple guanines for AgNC fluorescence enhancement and cisplatin binding (underlined letters). The nanoclusters synthesis is done following a protocol modified from a prior work by Sharma and co-workers (Sharma et al., 2012). Briefly, 3.0 μM of the DNA template was mixed with 18.0 μM $AgNO_3$ in 10.0 mM HEPES buffer pH 8.2 (approximately the highest for HEPES) to stabilize $NaBH_4$, and kept in darkness for 10 minutes to allow Ag^+ and DNA binding. Then 18.0 μM $NaBH_4$ was added to reduce Ag^+ to Ag^0 and facilitate the AgNC formation on the DNA template. Next, the mixture was vigorously mixed by a vortex mixture for 1 minute and incubated in darkness for 4 hours at 4°C. The DNA-template AgNC was then kept at 4°C until use.

For the proposed cisplatin sensing as shown in Step 2 of Figure 1, in the absence of cisplatin, the DNA-templated AgNCs emits an intense fluorescence possibly due to electron transfer from nearby guanine bases, as proposed in a prior work (Zoughi et al., 2024). In the presence of cisplatin, however; the cisplatin-guanine complex formation greatly alters the efficiency of electron transfer causing the reduction of AgNC fluorescence intensity. Consequently, one may quantify cisplatin concentration simply by measuring the reduction in AgNC fluorescence.

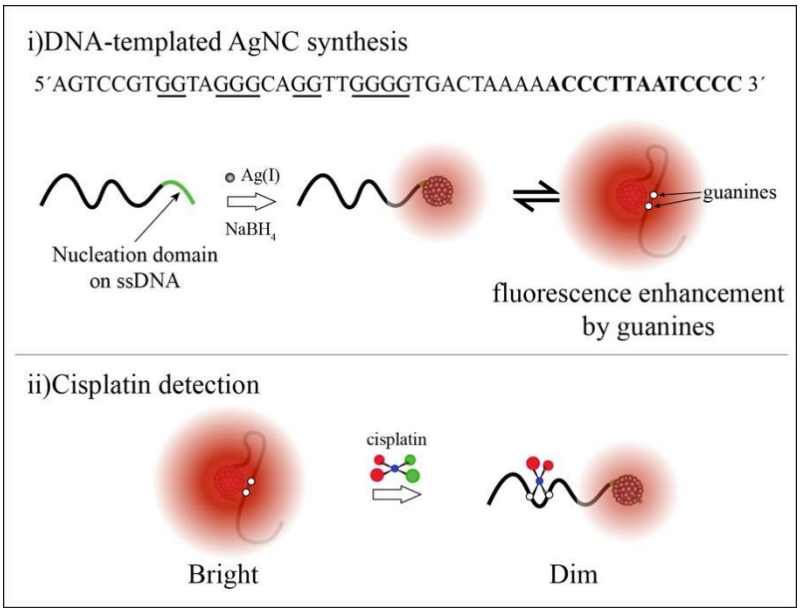

Figure 1 DNA-templated AgNC synthesis and the proposed sensing mechanism

3.2 Chemicals and DNA

HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), $AgNO_3$, and $NaBH_4$ were purchased from Sigma-Aldrich (Darmstadt, Germany), while NaOH and NaCl were sourced from Merck (either in (Darmstadt, Germany or Copenhagen, Denmark). The DI water having a resistivity of 18.2 MΩ.cm was from a Milli-Q system (Merck, Germany). The Ag4 DNA sequence (5′ AGTCCGTGGTAGGGCAGGTTGGGGTGACTAAAAACCCTTAATCCCC 3′) was purchased from Macrogen (Korea). After resuspending in DI water, the DNA sample was divided into aliquots and stored at -20 °C until use.

3.3 Standard cisplatin activation and preparation

The preparation of cisplatin in its diaqua form ($[Pt(NH_3)_2((H_2O)_2]^{2+}$) with the help of Ag^+ to remove extra chloride ions was done following the protocol reported earlier (Jantarat et al., 2021). Briefly, a 5.0 mM cisplatin was dissolved in deionized water and incubated in the dark at 25 °C overnight. Subsequently, 5.0 mM of $AgNO_3$ was added to provide Ag^+ that prevented the re-association of chloride ions with the diaqua cisplatin. The solution was then incubated in the dark at 25 °C for another 15 hours before centrifugation at 12,000 rpm and 4 °C to remove AgCl. The standard diaqua cisplatin was kept at 4 °C in the dark before use. For the preparation of diaqua cisplatin without Ag^+, the process was repeated without $AgNO_3$ added.

3.4 Fluorescence Measurements

For each fluorescence emission spectrum measurement, 3.0 µM of freshly prepared DNA-templated AgNC was first mixed with a cisplatin standard solution at the desired concentration in 10.0 mM HEPES buffer at pH 6.8 (approximately the minimum for HEPES) to maintain cisplatin in its diaqua form (Andersson et al., 1994). Subsequently, 120 µL of the mixture was transferred to a micro-cuvette (16.100-Q-10/Z8.5, Starna Cells Inc., USA) for measurement in a benchtop fluorometer (F-2700, Hitachi, Japan) having the excitation wavelength

of 570 nm, and emission wavelengths ranging from 585 nm to 800 nm. The excitation and emission slits were set at 5 nm and 10 nm, respectively.

4. Results and Discussion

4.1 Fluorescence Emission from DNA-Templated Silver Nanocluster

Under 570 nm excitation, the solution containing freshly prepared DNA-templated AgNC emits red fluorescence with a peak at 620 nm, as shown in Figure 1. This peak position of the fluorescence emission spectrum is somewhat different from 700 nm reported earlier for the Ag4 DNA template (Sharma et al., 2010), possibly due to the difference in the buffer composition and pH being used. Nevertheless, the fluorescence intensity of the DNA-templated AgNC in our work was still high hinting that it was successfully synthesized. It is worthwhile mentioning that only vigorous shaking over a brief period, as described in section 3.1 is critical because the solutions prepared differently did not fluoresce (data not shown). To eliminate any instrumental effects, the area under the fluorescence emission spectrum in the presence of cisplatin at a given concentration (F) was divided by that of the solution without cisplatin (F_0). This F/F_0 ratio was then used as the biosensor's signal in the key parameters optimization and evaluating the linear range and limit of detection.

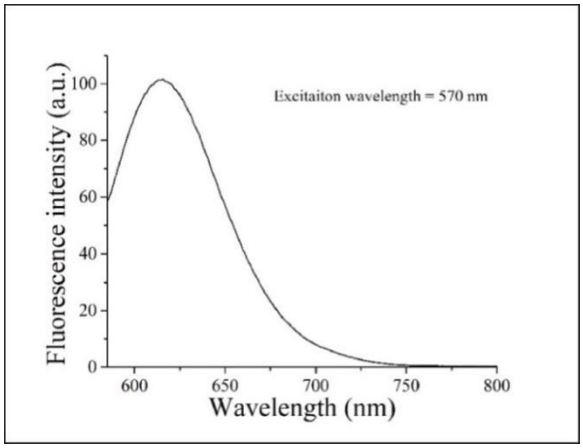

Figure 2 Fluorescence emission spectrum of silver nanoclusters formed on Ag4 ssDNA taken from prior work (Sharma et al., 2010)

4.2 Key Parameters Optimization

Next, key parameters affecting the performance of the developed DNA-templated AgNC were optimized. During the optimization, one parameter was varied while the rest was fixed, with the experiments were conducted 3 times for each parameter. The criteria for selecting the optimal values were the highest sensitivity, defined as the slope of the calibration graphs, and the shortest analysis time.

The concentrations of template DNA, $AgNO_3$ and $NaBH_4$ during AgNC synthesis

A preliminary test (data not shown) suggested that the $AgNO_3$:$NaBH_4$ ratio should be kept at 1:1 to avoid the problem of excess Ag^+ interfering with sensor performance. Therefore, during the step of optimizing DNA, $AgNO_3$, $NaBH_4$ concentrations, the concentrations of $AgNO_3$ and $NaBH_4$ were varied together while the concentration of DNA was kept at 3.0 µM. The results shown in Figure 3 suggested that biosensor sensitivity increases with rising $AgNO_3$ and $NaBH_4$ concentrations from 18.0 to 54.0 µM before reaching the plateau. The early increase is likely due to the fact that increasing $AgNO_3$ and $NaBH_4$ leads to more formation of AgNC-DNA

and a better response to cisplatin. However, at high concentration, such as 72.0 µM, the DNA template could be exhausted and no further increase in the sensitivity was observed. To ensure maximum sensitivity and stability, the 3.0:72.0:72.0 µM were chosen as the optimal concentrations.

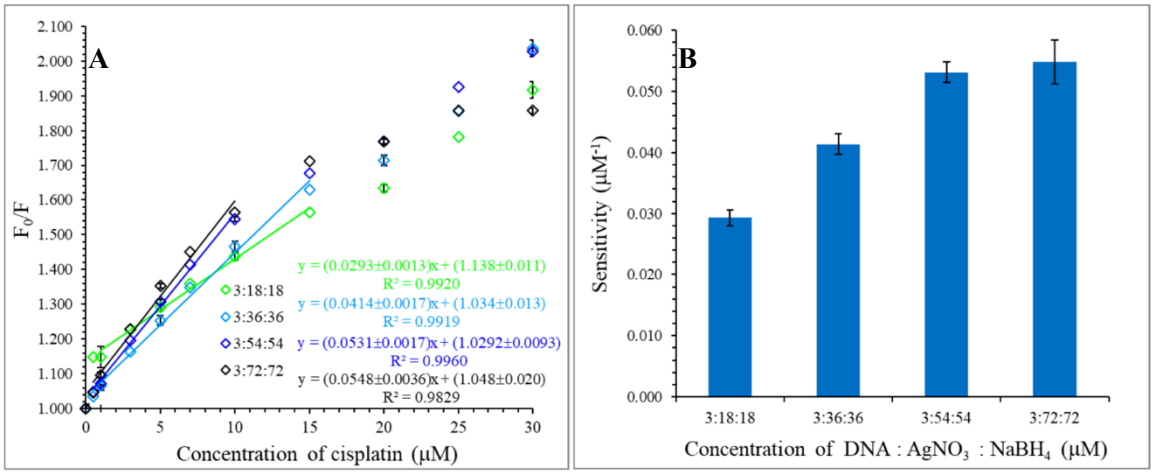

Figure 3 Calibration graphs (A) and bar graphs (B) comparing the sensitivities of the sensor at various concentrations of DNA: $AgNO_3$: $NaBH_4$

The incubation time for Ag^+ - DNA template binding

Next, the incubation time of DNA and $AgNO_3$ prior to mixing with $NaBH_4$ was optimized. The results presented in Figure 4 indicate that 10 minutes gave the highest sensitivity. We hypothesize that at 5 minutes, the incubation time is too short for Ag^+ to stably bind to the DNA template, while at 20 and 30 minutes (or longer), Ag^+ might start to crosslink two cytosines resulting in unwanted DNA structures. Both of these negative effects could reduce the sensitivity of the sensor. Therefore 10 minutes was chosen as the optimal duration for DNA-templated $AgNO_3$ incubation time.

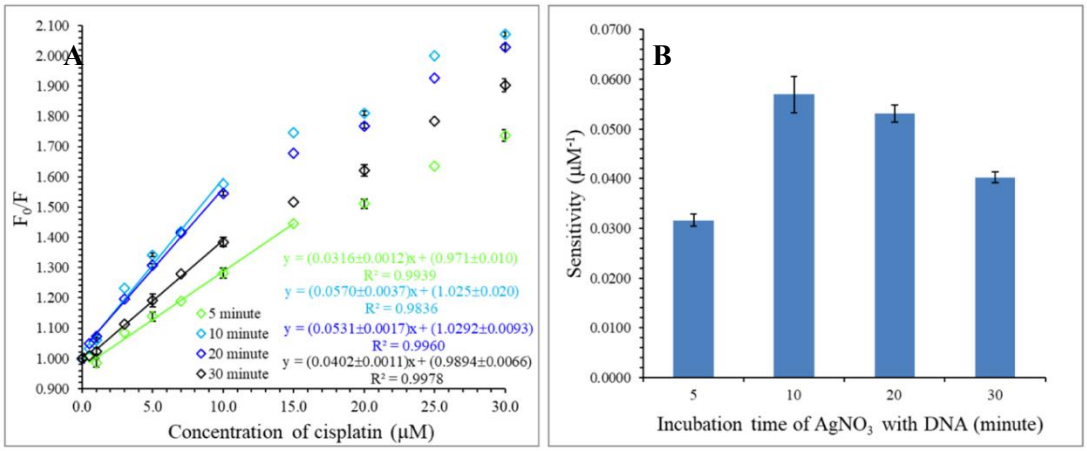

Figure 4 Calibration graphs (A) and bar graphs (B) comparing the sensitivities of the sensor at various incubation times for the DNA and $AgNO_3$ mixture

The incubation time for cisplatin – DNA-templated AgNC binding

The incubation time between cisplatin and AgNC-DNA was optimized. The results presented in Figure 5 shows that 5 minutes is the optimal value. We hypothesize that at 1minute incubation time, cisplatin still does not stably bind to the AgNC-DNA probe, while at 10 and 20 minutes (or longer), cisplatin may bind to more than one guanine resulting in a decrease in the inhibition of electron transfer from guanine to AgNC on the DNA. While this hypothesis awaits testing, 5 minutes has been selected as the optimal incubation time.

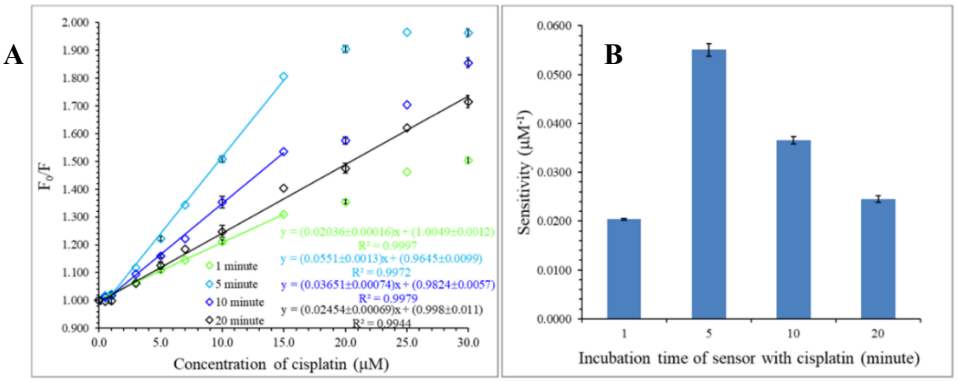

Figure 5 Calibration graphs (A) and bar graphs (B) comparing the sensitivities of the sensor at various incubation times for the DNA-templated silver nanocluster and $AgNO_3$

The DNA-templated AgNC synthesis time

It can be observed from Figure 6 that increasing the incubation time from 4 hours to 36 hours improved the fluorescence intensity emitted by the AgNC-DNA. This improvement is likely because the longer incubation time allows extra AgNCs to form on the DNA template. After 36 hours; however, the peak fluorescence intensity decreases, possibly due to the formation of larger silver nanoparticle since prolonged incubation may allow more silver ions to deposit on the silver nanocluster turning it into a bigger and non-fluorescent silver nanocluster. Therefore, 36 hours was chosen as the optimal incubation time for AgNC-DNA synthesis

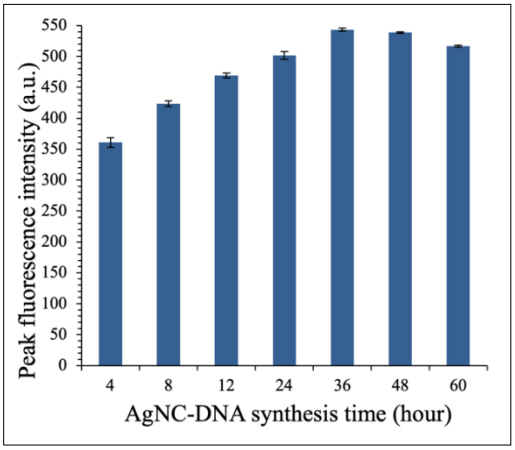

Figure 6 The effect of AgNC-DNA synthesis time on the peak fluorescence intensity

4.3 The Linear Range and Limits of Detection

After optimization, the linear range and limits of detection were obtained from a calibration graph plotted between F/F_0, measured under optimal conditions, and the concentration of standard cisplatin solutions. The results presented in Figure 7 indicate a linear range of detection from 5.0 to 40.0 µM from which the coefficient of determination (R^2) very close to 1.0000. The limit of detection (LOD = $3S_a/b$, where S_a is the standard deviation of the blank response and b is the slope of the calibration graph (Currie, 1999)) equals 2.7 µM µM and the limit of quantitation (LOQ = $10S_a/b$) equals 9.0 µM. Even though the LOD and LOQ are not lower than some of the previous works (Yang et al., 2014, Jantarat et al., 2021), the merits of the developed DNA-templated AgNC are still high because of its simplicity, low cost, and long excitation and emission wavelengths where interference from matrices tend to be minimal.

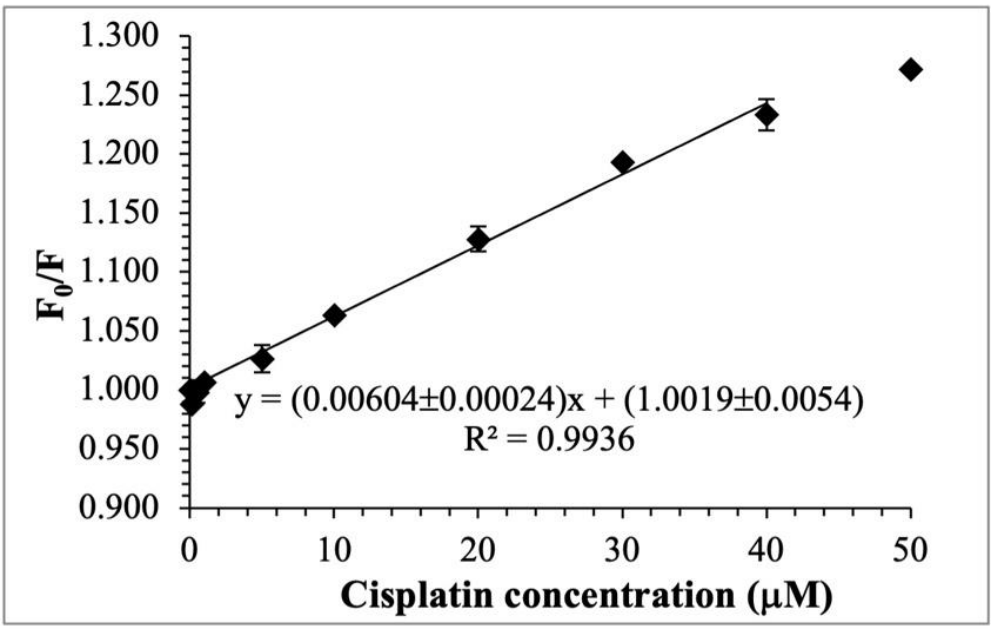

Figure 7 The calibration graph obtained under optimal conditions revealing the linear range of detection from 5.0 to 40.0 µM, with a limit of detection (LOD) of 2.7 µM and with limit of quantitation (LOQ) of 9.0 µM

5. Conclusion

In this study, a simple biosensor based on a DNA-templated silver nanocluster (AgNC) was successfully synthesized having a peak excitation wavelength of 570 nm and a peak fluorescence emission wavelength of 620 nm. The biosensor was sensitive to the presence of cisplatin possibly because cisplatin bonding to the DNA template alters the efficiency of electron transfer from guanine bases to the AgNC. Under the optimal sensing conditions, the developed biosensor was able to detect cisplatin in standard solutions with a linear range of detection from 5.0 to 40.0 µM, with a limit of detection (LOD) of 2.7 µM and a limit of quantitation of 9.0 µM. With further development, it is expected that the biosensor will find applications in the detection of cisplatin contamination in various samples, such as urine collected from patients receiving chemotherapy or wastewater treatment plant in hospitals.

6. Acknowledgements

The authors would like to express their gratitude for the financial support received from Thailand Science Research and Innovation (TSRI) (IRN62W0002). Ms. Kosai received financial supports from the Research Assistantship, Faculty of Science Research Fund, Prince of Songkla University (Contract no. 1-2563-02-007), as well as the Graduate School Dissertation Funding for Thesis Fiscal Year 2019. In addition, we gratefully acknowledge the partial support from the Thailand Center of Excellence in Physics (ThEP), Prince of Songkla University, and the Faculty of Science, Prince of Songkla University, Thailand (ThEP-61-PHM-PSU2), from the Center of Excellence for Trace Analysis and Biosensor (TAB-CoE) and from the Division of Physical Science, Faculty of Science, Prince of Songkla University. We would also like to recognize the support provided by the Talent Management Project of Prince of Songkla University.

7. References

Andersson, A., Hedenmalm, H., Elfsson, B., & Ehrsson, H. (1994). Determination of the acid dissociation constant for Cis–Diammineaquachloroplatinum (II) ion. A hydrolysis product of cisplatin. *Journal of pharmaceutical sciences*, *83*(6), 859-862.

Brouwers, E. E., Tibben, M., Rosing, H., Schellens, J. H., & Beijnen, J. H. (2008). The application of inductively coupled plasma mass spectrometry in clinical pharmacological oncology research. *Mass Spectrometry Reviews*, *27*(2), 67-100.

Christie, D. A., & Tansey, E. (2007). *The discovery, use and impact of platinum salts as chemotherapy agents for cancer*. Wellcome Trust Centre for the History of Medicine at UCL.

Cornelison, T. L., & Reed, E. (1993). Nephrotoxicity and hydration management for cisplatin, carboplatin, and ormaplatin. *Gynecologic oncology*, *50*(2), 147-158.

Currie, L. A. (1999). Nomenclature in evaluation of analytical methods including detection and quantification capabilities. *Pure and Applied Chemistry*, *67*, Article 1699.

Dasari, S., & Tchounwou, P. B. (2014). Cisplatin in cancer therapy: molecular mechanisms of action. *European journal of pharmacology*, *740*, 364-378.

Dhamo, L., Wegner, K. D., Würth, C., Häusler, I., Hodoroaba, V. D., & Resch-Genger, U. (2022). Assessing the influence of microwave-assisted synthesis parameters and stabilizing ligands on the optical properties of AIS/ZnS quantum dots. *Scientific reports*, *12*(1), Article 22000.

Farzin, M. A., & Abdoos, H. (2021). A critical review on quantum dots: From synthesis toward applications in electrochemical biosensors for determination of disease-related biomolecules. *Talanta*, *224*, 121828.

Fredrick, D., Yourston, L., & Krasnoslobodtsev, A. V. (2023). Detection of cancer-associated miRNA using a fluorescence switch of AgNC@ NA and guanine-rich overhang sequences. *Luminescence*, *38*(7), 1385-1392.

He, J. Y., Shang, X., Yang, C. L., Zuo, S. Y., Yuan, R., & Xu, W. J. (2021). Antibody-responsive ratiometric fluorescence biosensing of biemissive silver nanoclusters wrapped in switchable DNA tweezers. *Analytical Chemistry*, *93*(33), 11634-11640.

Jantarat, T., Chuaychob, S., Thammakhet-Buranachai, C., Thavarungkul, P., Kanatharana, P., Srisintorn, W., & Buranachai, C. (2021). A label-free DNA-based fluorescent sensor for cisplatin detection. *Sensors and Actuators B: Chemical*, *326*, 128764.

Jung, Y., & Lippard, S. J. (2007). Direct cellular responses to platinum-induced DNA damage. *Chemical reviews*, *107*(5), 1387-1407.

Lee, J., Park, J., Lee, H. H., Kim, H. I., & Kim, W. J. (2014). DNA-templated silver nanoclusters as label-free, sensitive detection probes for potassium ions and nitric oxide. *Journal of Materials Chemistry B*, *2*(17), 2616-2621.

Li, C., Chen, Z., Zhang, Y., He, J., Yuan, R., & Xu, W. (2020). Guanine-lighting-up fluorescence biosensing of silver nanoclusters populated in functional DNA constructs by a pH-triggered switch. *Analytical Chemistry*, *92*(19), 13369-13377.

Liu, L., Zhuang, Z., Xie, T., Wang, Y. G., Li, J., Peng, Q., & Li, Y. (2009). Shape control of CdSe nanocrystals with zinc blende structure. *Journal of the American Chemical Society*, *131*(45), 16423-16429.

Liu, X., Wang, F., Aizen, R., Yehezkeli, O., & Willner, I. (2013). Graphene oxide/nucleic-acid-stabilized silver nanoclusters: functional hybrid materials for optical aptamer sensing and multiplexed analysis of pathogenic DNAs. *Journal of the American Chemical Society*, *135*(32), 11832-11839.

National Toxicology Program. (2021). 15th report on carcinogens. *Report on carcinogens: carcinogen profiles*, *15*, roc15.

Petty, J. T., Zheng, J., Hud, N. V., & Dickson, R. M. (2004). DNA-templated Ag nanocluster formation. *Journal of the American Chemical Society*, *126*(16), 5207-5212.

Shaik, A. N., Altomare, D. A., Lesko, L. J., & Trame, M. N. (2017). Development and validation of a LC–MS/MS assay for quantification of cisplatin in rat plasma and urine. *Journal of Chromatography B*, *1046*, 243-249.

Sharma, J., Rocha, R. C., Phipps, M. L., Yeh, H. C., Balatsky, K. A., Vu, D. M., ... & Martinez, J. S. (2012). A DNA-templated fluorescent silver nanocluster with enhanced stability. *Nanoscale*, *4*(14), 4107-4110.

Sharma, J., Yeh, H. C., Yoo, H., Werner, J. H., & Martinez, J. S. (2010). A complementary palette of fluorescent silver nanoclusters. *Chemical Communications*, *46*(19), 3280-3282.

Sharma, J., Yeh, H. C., Yoo, H., Werner, J. H., & Martinez, J. S. (2011). Silver nanocluster aptamers: in situ generation of intrinsically fluorescent recognition ligands for protein detection. *Chemical Communications*, *47*(8), 2294-2296.

Smoorenburg, G. F., de Groot, J. C., HAMERS, F. P., & Klis, S. F. (1999). Protection and Spontaneous Recovery from Cisplatin-Induced Hearing Loss. *Annals of the New York Academy of Sciences*, *884*(1), 192-210.

Song, C., Xu, J., Chen, Y., Zhang, L., Lu, Y., & Qing, Z. (2019). DNA-templated fluorescent nanoclusters for metal ions detection. *Molecules*, *24*(22), Article 4189.

Toro-Córdova, A., Ledezma-Gallegos, F., Mondragon-Fuentes, L., Jurado, R., Medina, L. A., Pérez-Rojas, J. M., & Garcia-Lopez, P. (2016). Determination of liposomal cisplatin by high-performance liquid chromatography and its application in pharmacokinetic studies. *Journal of chromatographic science*, *54*(6), 1016-1021.

Wang, D., & Lippard, S. J. (2005). Cellular processing of platinum anticancer drugs. *Nature reviews Drug discovery*, *4*(4), 307-320.

Wang, G., He, X., Chen, L., Zhu, Y., Zhang, X., & Wang, L. (2013). Conformational switch for cisplatin with hemin/G-quadruplex DNAzyme supersandwich structure. *Biosensors and Bioelectronics*, *50*, 210-216.

Xu, J., Zhu, X., Zhou, X., Khusbu, F. Y., & Ma, C. (2020). Recent advances in the bioanalytical and biomedical applications of DNA-templated silver nanoclusters. *TrAC Trends in Analytical Chemistry*, *124*, Article 115786.

Yang, H., Cui, H., Wang, L., Yan, L., Qian, Y., Zheng, X. E., ... & Zhao, J. (2014). A label-free G-quadruplex DNA-based fluorescence method for highly sensitive, direct detection of cisplatin. *Sensors and Actuators B: Chemical*, *202*, 714-720.

Yeh, H. C., Sharma, J., Han, J. J., Martinez, J. S., & Werner, J. H. (2010). A DNA− silver nanocluster probe that fluoresces upon hybridization. *Nano letters*, *10*(8), 3106-3110.

Zheng, X., Yao, T., Zhu, Y., & Shi, S. (2015). Cu2+ modulated silver nanoclusters as an on–off–on fluorescence probe for the selective detection of l-histidine. *Biosensors and Bioelectronics*, *66*, 103-108.

Zoughi, S., Faridbod, F., & Moradi, S. (2024). Rapid enzyme-free detection of miRNA-21 in human ovarian cancerous cells using a fluorescent nanobiosensor designed based on hairpin DNA-templated silver nanoclusters. *Analytica Chimica Acta*, *1320*, 342968.