Sensors & Actuators: B. Chemical 302 (2020) 127145

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A fluorescent assay for alkaline phosphatase activity based on inner filter effect by in-situ formation of fluorescent azamonardine

Pengjuan Ni[a], Chuanxia Chen[a], Yuanyuan Jiang[a], Chenghui Zhang[a], Bo Wang[a], Yizhong Lu[a,*], Hua Wang[b,*]

[a] School of Materials Science and Engineering, University of Jinan, Jinan 250022, China
[b] Institute of Medicine and Materials Applied Technologies, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu 273165, China

ARTICLE INFO

Keywords:
Dopamine
Resorcinol
p-Nitrophenol
Inner filter effect
Fluorescent assay

ABSTRACT

A simple and sensitive fluorescent assay for determination of alkaline phosphatase (ALP) activity has been developed based on the inner filter effect (IFE) of *p*-nitrophenol (PNP) through the in-situ formation of azamonardine. Herein, *p*-nitrophenylphosphate (PNPP) was used as ALP substrate, which could be hydrolyzed to PNP with an absorption band centered at 405 nm. Under the alkaline condition, the introduction of resorcinol into the oxygen-containing dopamine solution would trigger the formation of fluorescent azamonardine with an intense emission band centering at 460 nm when excited at 415 nm. As a result, the fluorescence of azamonardine could remarkably decrease in the presence of PNP via IFE due to the absorption spectrum of PNP overlaps with the excitation spectrum of azamoardine. An IFE-based fluorescent assay has been thereby proposed for the ALP activity detection showing a linear range from 0.1 to 6.0 mU mL^{-1} with the detection limit down to 0.07 mU mL^{-1}. Moreover, the developed fluorescent assay was successfully applied to probe ALP in human serum samples with satisfactory results. This IFE-based detection strategy exhibits several merits such as high sensitivity, good anti-interference ability, time-saving and easy operation simply by mixing the commercial reagents together. Therefore, it may promote the development of simple and sensitive fluorescent assays for the enzyme activity detections.

1. Introduction

Alkaline phosphatase (ALP) that is widely distributed in mammal tissues can catalyze the dephosphorylation of many phosphorized proteins, nucleic acids and small molecules [1]. As a result, ALP plays a key role in the signal transduction and regulation of intracellular processes [2]. In the clinic diagnostics filed, ALP is usually used as an important biomarker since its abnormal level is closely associated with several diseases, such as liver tumor, leukemia, bone and kidney disease [3]. Thus, developing a facile, sensitive and selective method for the ALP activity detection is of great importance.

To date, several kinds of analysis methods have been developed for the ALP activity detection, which are mainly based on the utilization of techniques such as colorimetry [4], fluorimetry [5], electrochemistry [6] and surface-enhance Raman scattering (SERS) [7]. Among them, fluorimetry has attracted great attentions due to its simplicity, short detection time and high sensitivity. Currently, various organic probes have been employed for probing ALP activity [8–10]. However, there are some disadvantages like high toxicity, poor water-solubility, laborious and time-consuming probe preparation and especially the low sensitivity affected by the auto-fluorescence from the complex matrices, which have undoubtedly limited their large-scale applications in real sample detections. With the development of nanotechnology, nanostructures show great potentials in bioanalysis [11,12]. A variety of nanostructures, such as carbon dots [13,14], gold nanoclusters [15] and semiconducting polymer dots [16], have been utilized for the fluorescent detections of ALP. Though the preparation procedures for C-dots are usually simple, they may encounter with the complex surface modification or the introduction of other nanostructures. Moreover, the high cost and poor stability of gold nanoclusters and the complex synthesis procedure for semiconducting polymer dots may have also limited their further practical applications. Therefore, it is still urgent to develop simple, cost-effective and sensitive fluorescent assays for ALP activity detection.

Recently, several fluorescent assays for ALP detection have been proposed based on the in situ formation of fluorescent materials by simply mixing commercial reagents together, which do not need to prepare fluorescent probes in advance [17–19]. Besides, most of these

* Corresponding authors.
E-mail addresses: mse_luyz@ujn.edu.cn (Y. Lu), huawang@qfnu.edu.cn (H. Wang).

https://doi.org/10.1016/j.snb.2019.127145
Received 15 August 2019; Received in revised form 11 September 2019; Accepted 13 September 2019
Available online 16 September 2019
0925-4005/

assays have been developed by utilization of L-ascorbic acid-2-phosphate as ALP substrate since it can be hydrolyzed to ascorbic acid that can takes place in various kinds of reactions. For example, Li et al has reported a fluorescent assay for the ALP activity detection based on the reaction between ascorbic acid and Cu^{2+} in the presence of DNA template to obtain fluorescent copper nanoclusters [20]. Though it shows high sensitivity, it can be largely limited by the high cost and low stability of DNA. Similarly, Zhao et al has developed an ALP-based fluorescent assay by making use of the interaction between *o*-phenylenediamine and ascorbic acid to obtain quinoxaline fluorophore [21]. This assay shows the distinctive advantages of simplicity by firstly incubating L-ascorbic acid-2-phosphate and ALP for a certain time and then mixing with commercially available *o*-phenylenediamine together to produce fluorescent signals. However, the long interaction time between ascorbic acid and *o*-phenylenediamine may have severely impeded its application in real samples detections. In addition, instead of use L-ascorbic acid-2-phosphate as ALP substrate, Zhao et al has reported an enzyme cascade-triggered fluorogenic reaction applied in ALP activity assay by using *p*-aminoethyl-phenyl phosphate disodium salt (PAPP) as ALP substrate [22]. Under the aid of ALP and tyrosinase, PAPP is hydrolyzed to dopamine that can interact with resorcinol to obtain fluorescent azamonardine. Despite its high sensitivity and simplicity in operation, it suffers from the complex preparation procedure of PAPP and high cost and instability of tyrosinase. Therefore, it is still challenging to develop fluorescent assay based on the in situ formation of fluorescent materials with simplicity, low cost and high sensitivity.

In this work, we report a fluorescent assays for ALP activity detection based on the in situ synthesis of azamonardine to detect ALP via inner filter effect (IFE). IFE, a vital non-irradiation energy conversion model in fluorimetry, describes the absorption of the excitation and/or emission light by absorbers in the detection system [23]. IFE can result from the overlapping between the absorption spectra of absorbers and the fluorescence excitation and/or emission spectra of fluorophores. Due to its high sensitivity, considerable flexibility and simplicity, IFE shows great potentials in construction of fluorescent assays for ALP detection. So far, several kinds of fluorescent probes, such as carbon dots [24], gold nanoclusters [25], titanium carbide MXene quantum dots [26], polymer dots [27], CdTe/CdS quantum dots [28] and MoS_2 quantum dots [29] have been used as for ALP detection based on IFE between them and the *p*-nitrophenol (PNP) generating from ALP-catalyzed *p*-nitrophenylphosphate (PNPP). Though these methods can show a high sensitivity, the preparation procedures for these fluorescent probes are either complex or time-consuming. Consequently, it irradiates us to develop a fluorescent method for ALP detection using in situ formation of fluorescent materials.

In this paper, azamonardine obtained from the reaction between dopamine and resorcinol under alkaline condition was used as the fluorophores in the IFE-based assay. By utilization of PNPP as the ALP substrate, PNPP could be catalytically hydrolyzed to PNP, whose absorption spectrum would largely overlap with the excitation spectrum of azamonardine, resulting in the fluorescence quenching of azamonardine. Therefore, an IFE-based fluorescent assay for ALP activity detection has been established with a detection limit down to 0.07 mU mL^{-1}. This assay has also been successfully applied to detect ALP in human serum samples with satisfactory results. As far as we know, this is the first report on an IFE-based fluorescent assay for ALP activity based on the in-situ synthesis of azamonardine rather than pre-synthesized fluorescent materials. Moreover, this highly sensitive and selective assay is simple to operate by mixing the commercially available reagents together, showing great potential in practical applications.

2. Experimental

2.1. Instrumentation

UV–vis absorption spectra were recorded by a UV-8000 spectrophotometer (Shanghia Metash Instruments Co., Ltd., China). Fluorescence excitation and emission spectra were recorded on a RF-6000 spectrofluorometer (Shimadzu, Japan). Time-resolved fluorescence decay tests were obtained from a FLS-920 fluorescence spectrophotometer (Edinburgh, England)

2.2. Chemicals

Lysozyme, pepsin and pancreatin were supplied by Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Resorcinol, PNPP, PNP, bovine serum albumin (BSA), trypsin, and magnesium chloride were purchased from Aladdin Reagent Company (Shanghai, China). Trizma base, dopamine and ALP were acquired form Sigma-Aldrich (St. Louis, USA). All the reagents were used as received without any further purification.

2.3. Fluorescent assay for ALP activity detection

Firstly, 70 μL of Tris-HCl buffer solution (10 mM, pH = 9.0), 10 μL of 1 mM $MgCl_2$, 10 μL of 40 mM PNPP and 10 μL of various concentrations of ALP were added sequentially into a 1.5 mL calibrated test tube, mixed thoroughly and incubated at 37 °C for 30 min. Then, 780 μL of ultrapure water, 100 μL of 15 mM Na_2CO_3, 10 μL of 10 mM dopamine and 10 μL of 5 mM resorcinol were introduced and incubated at 37 °C for another 5 min. Finally, the obtained solution was transferred for fluorescence spectral measurements.

For detection ALP in human serum samples by standard addition method, human serum samples were firstly diluted 100 times and spiked with various concentrations of ALP. Then, the detection procedure is similar to the above mentioned method for ALP detection except that 10 μL of various concentrations of ALP prepared by distilled water was replaced by 10 μL of spiked serum samples.

For direct detection ALP in human serum samples, 20 μL of serum were added into the detection system including 70 μL of Tris-HCl (10 mM, pH = 9.0), 10 μL of 1 mM $MgCl_2$ and 10 μL of 40 mM PNPP, which were incubated at 37 °C for 30 min. Then, 770 μL of distilled water, 100 μL of Na_2CO_3 (15 mM), 10 μL of 10 mM dopamine and 10 μL of 5 mM resorcinol were introduced and incubated at 37 °C for another 5 min. Finally, the fluorescent spectra were recorded under the excitation of 415 nm.

3. Results and discussions

3.1. Mechanism of ALP activity detection based on IFE

The fluorescent assay for ALP activity was developed based on the IFE of PNP through the in-situ formation of fluorescent azamonardine from the reaction of dopamine and resorcinol under alkaline condition. Previous reports have proven that dopamine can react with resorcinol to form fluorescent azamonardine [22,30]. The obtained azamonardine shows an intense absorption band centered at 420 nm (Fig. S1A) and maximum emission intensity at 460 nm when excited at 415 nm (Fig. S1B). Moreover, this reaction can be finished in short time (Fig. S1C and Fig. SD), making the obtained azamonardine more attractive to be used for fluorescent assay. PNPP was chosen as the ALP substrate due to its low cost, easily available and good water solubility [24]. As shown in Fig. 1A, when either ALP or PNPP was introduced, the fluorescence of azamonardine stayed unchanged. However, when ALP and PNPP were simultaneously added, PNPP could be catalytically hydrolyzed to PNP, resulting in the fluorescence quenching. When increasing concentrations of PNP, the fluorescence intensity at 460 nm could decrease gradually (Fig. 1B), suggesting the fluorescence quenching of azamoardine might be triggered by the PNP yielded from ALP-catalyzed PNPP.

In order to verify the mechanism of the fluorescence quenching of azamonardine by PNP, the UV–vis absorption spectra by incubation PNPP with various concentrations of ALP were measured. After the

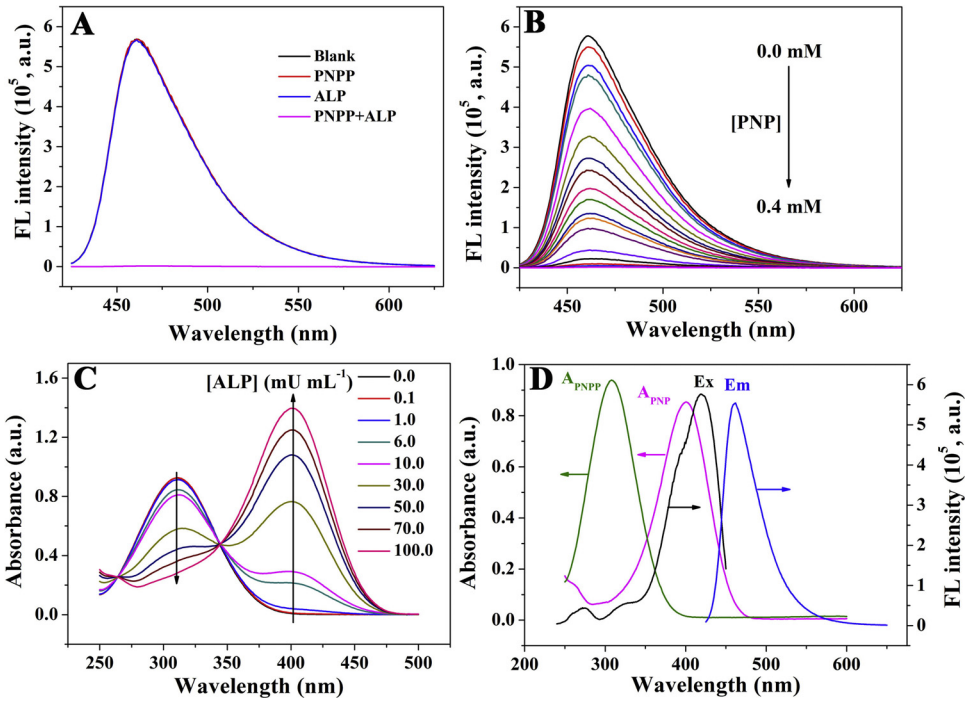


Fig. 1. (A) Fluorescence spectra of the in situ formation of azamonardine in the absence (blank) and presence of PNPP, ALP, PNP and PNPP-ALP. The concentrations of PNPP, ALP, PNP are 0.4 mM, 100 mU mL^{-1} and 0.4 mM. (B) Fluorescence spectra of the in situ synthesis of fluorescent azamonardine in the absence and presence of various concentrations of PNPP. From top to down the concentrations of PNPP are 0.0, 0.001, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4 mM. (C) UV–vis absorption spectra of the enzymatic reaction solution after the introduction of ALP with various activities from 0.0 mU mL^{-1} to 100.0 mU mL^{-1} with 0.1 mM PNPP and 10.0 μM $MgSO_4$ incubated at 37 °C for 30 min. (D) UV–vis absorption spectra of 0.1 mM PNPP and 0.05 mM PNP, and the excitation and emission spectra of the obtained azamonardine.

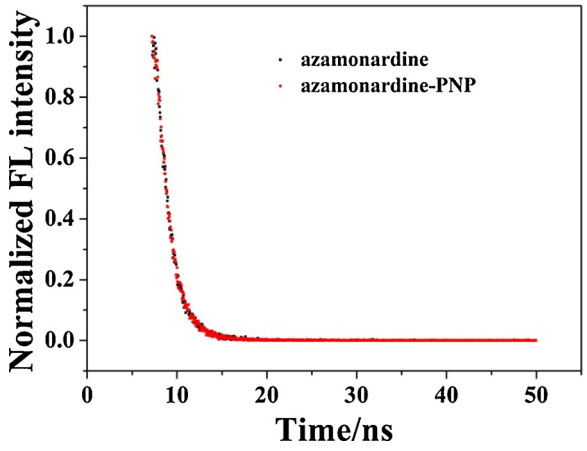


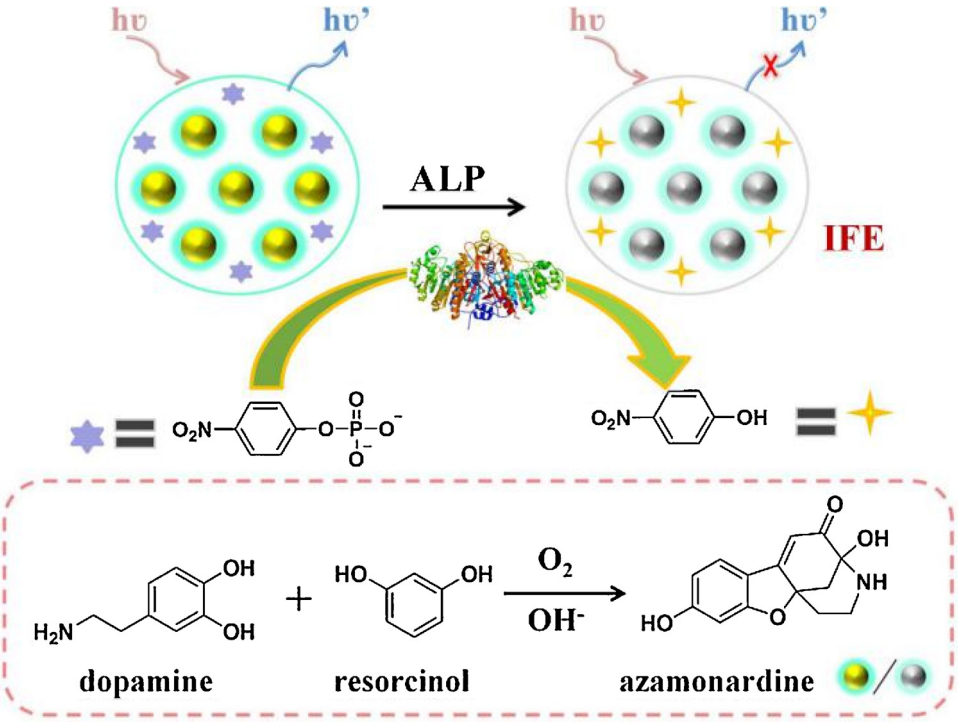
Fig. 2. Fluorescence decay curves of azamonardine as a function of time in the absence and presence of 0.4 mM PNP.

addition of ALP into PNPP solution, the absorbance at 310 nm could decrease gradually accompanied with the increased absorbance at 405 nm (Fig. 1C), suggesting the transformation from PNPP to PNP. One can note that the UV–vis absorption peak of PNPP can be centered at 310 nm (Fig. 1D), showing a negligible effect on the fluorescence excitation or emission of azamonardine. While, PNP displayed a strong absorption peak at 405 nm, which showed a large overlap with the fluorescence excitation spectrum of the azamonardine. As a consequence, PNP might induce the fluorescence quenching of azamonardine through IFE. Then, time-resolved fluorescence spectra of the azamonardine in the absence and presence of PNP was used to study the exact mechanism of the fluorescence quenching azamonardine caused by PNP. It can be clearly seen from Fig. 2 that the fluorescence decay curves of azamonardine before and after the addition of 0.4 mM PNP could maintain a high degree of consistency, and the fluorescent lifetime remained almost unchanged after the introduction of PNP. Therefore, the fluorescence quenching of azamonardine by PNP might be mainly due to IFE since IFE is usually considered to show no impact on the fluorescence lifetime of fluorophore. Finally, the IFE-based fluorescence quench can also be proved by excitation wavelength-dependent fluorescence quenching of azamonardine [31,32]. As displayed in Fig. S2, the excitation wavelength-dependent fluorescence quenching spectrum is almost similar to the UV–vis absorption spectrum of PNP, confirming IFE-based fluorescence quench of azamonardine by PNP. Based on the above facts, a novel fluorescent assay for ALP activity is proposed by the IFE between PNP yielded from ALP-catalyzed PNPP and the is-situ formation of azamonardine (Scheme 1).

3.2. Optimization of the detection conditions

To achieve higher sensitivity, several detection conditions including the concentrations of Na_2CO_3, PNPP, dopamine and resorcinol and incubation time were optimized before its application in ALP activity detection. We utilize ΔF as a criterion to obtain the optimal detection conditions. ΔF is F_0-F, where F_0 and F represent the fluorescence intensity at 460 nm of the detection assay before and after the introduction of ALP.

Since the reactions between dopamine and resorcinol can undergo under alkaline condition, we used Na_2CO_3 solution instead of buffer solutions for simplicity. In the absence of Na_2CO_3, both the F and F_0 are too low that can be ignored (Fig. S3A). In contrast, the F_0 increases gradually with the increasing concentration of Na_2CO_3 ranging from 0.10 to 1.5 mM and reaches a plateau when Na_2CO_3 concentration is higher than 1.5 mM (a, Fig. S3A). While the F increases gradually with the increasing concentration of Na_2CO_3 (b, Fig. S3A). The largest ΔF is obtained when the concentration of Na_2CO_3 is 1.5 mM (Fig. S3B). Next, as ALP substrate, PNPP concentrations ranging from 0 to 1.2 mM were used to study its effects. The F_0 keeps almost unchanged with increasing concentrations of PNPP, suggesting that PNPP shows negligible impact on the fluorescence of azamonardine (a. Fig. S4A). The F decreases gradually with increasing concentrations of PNPP and stays stable when the concentration is higher than 0.40 mM which was ascribed to that more PNP was obtained in certain concentration range resulting in fluorescence quenching of azamonardine (b. Fig. S4A). Therefore, ΔF reaches maximum and keeps constant when PNPP concentration were 0.40 mM (Fig. S4B). Then, the influences of dopamine in the concentration ranges from 0 to 0.2 mM were investigated. F_0 increases sharply when its concentration if below 0.1 mM and decreases step-by-



Scheme 1. Schematic illustration of the detection principle for ALP based on the in-situ formation of azamonardine.

step when the concentration increases further (a. Fig. S5A), which may be due to high concentrations of dopamine can be oxidized to dopamine quinine molecules under the alkaline condition [33], causing the fluorescence quenching of azamonardine. The F increases and keeps constant when its concentration is higher than 0.05 mM (b. Fig. S5A). The maximum ΔF is obtained when dopamine concentration is 0.1 mM (Fig. S5B). After that, the effects of resorcinol concentration ranging from 0 to 0.2 mM were further explored. Both F_0 and F increases firstly and stays constant when its concentration is higher than 0.05 mM (Fig. S6A). Similar results are also found for ΔF (Fig. S6B). Finally, the impact of incubation time ranging from 0 to 15 min is studied (Fig. 7). F_0 increases dramatically in the first 1 min and reaches the maximum at 5 min, which further indicates the fast reaction between dopamine and resorcinol. Moreover, similar result is also found for F, indicating the fast response of IFE. Consequently, the optimal concentrations of Na_2CO_3, PNPP, dopamine and resorcinol are 0.5 mM, 0.4 mM, 0.1 mM and 0.05 mM, the incubation time is 5 min.

3.3. Analytical performances for ALP activity detection

Under the optimized detection conditions, a fluorescent assay for ALP activity detection is proposed by utilization of PNPP as the enzyme substrate. In the absence of ALP, the in situ cyclization between dopamine and resorcinol results in remarkable fluorescence intensity (Fig. 3A). Nevertheless, due to the increasing concentrations of PNP generated by ALP-catalyzed hydrolysis of PNPP, the fluorescence intensity decreases gradually with the increasing concentrations of ALP, while the ΔF increases gradually with the increasing concentrations of ALP and reaches a plateau when its concentration is higher than 30 mU mL^{-1} (Fig. 3B). There is a good linear relationship between ΔF and ALP concentration in the range from 0.1 to 6.0 mU mL^{-1}. The linear regression equation is $\Delta F = 11707.1 + 59584.3c_{ALP}$ (mU mL^{-1}, $R^2 = 0.990$). The detection limit is calculated to be 0.07 mU mL^{-1} based on a signal-to-noise of 3. Furthermore, we compare the analytical performance of this assay for ALP activity detection with previously reported method and the corresponding results are shown in Table 1. It is clearly found that the sensitivity of this assay is comparable or even better than most of these methods. Moreover, the detection time is shorter and this assay is free of complex preparation of fluorescent probe by mixing the commercial reagents together, making it much simpler. The high sensitivity, short detection time and easy operation make this assay more attractive.

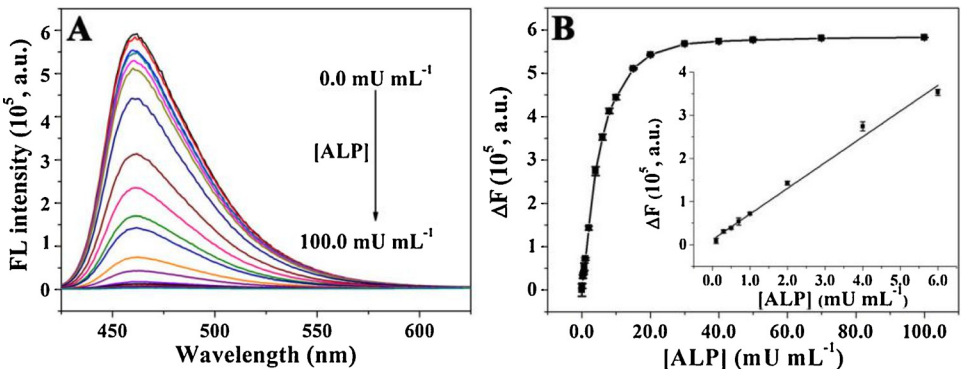


Fig. 3. (A) Fluorescence spectra of the detection assay in the absence and presence of different concentrations of ALP. From top to down, the ALP concentrations are 0, 0.1, 0.3, 0.5, 0.7, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0 70.0 100.0 mU mL^{-1}. (B) The plot of the ΔF versus various concentrations of ALP, where ΔF is F_0-F (F_0 and F are the fluorescence intensity of the detection assay in the absence and presence of ALP). Inset shows the corresponding linear curve.

Table 1
Comparison of the analytical performance of different methods for ALP detection.

Methods	Materials	Linear range (mU mL^{-1})	Detection limit (mU mL^{-1})	Reference
Electrochemistry	DNAzyme	0.1-5	0.03	[34]
Chemiluminescence	MSN[a]@RhB[b]@β-CD[c]@AMPPD[d]	0-400	0.0748	[35]
SERS	Alkyne-tagged Au NPs-Ag +	0.78-32.61	0.01	[36]
Colorimetry	Nanoceria	0.5-10	0.32	[37]
Colorimetry	Cu-MOFs	1-34	0.19	[38]
Colorimetry	Ce4 + -TMB	0-50 50-250	2.3	[39]
Phosphorimetry	Mn-doped ZnS QDs	0.001-0.1	4 × 10-4	[40]
Phosphorimetry	Mn-ZnS QDs/Eu3 +	0.15-18	0.065	[41]
Fluorimetry	Ti3C2 MXene quantum dots	0.1-2	0.02	[26]
Fluorimetry	CdTe/CdS QDs	2.2-220	0.34	[28]
Fluorimetry	NGQDse	0.1-5	0.07	[42]
Fluorimetry	Dopamine-resorcinol	0.1-6.0	0.07	This work

[a] mesoporous silica nanoparticles.
[b] rhodamine B.
[c] beta-cyclodextrin.
[d] (3-[2-Spiroadamatane]-4-methoxy-4-[3-phosphoryloxy]-phenyl-1,2-dioxetane)dioxetane, [e] nitrogen-doped graphene quantum dots.

3.4. Selectivity of IFE-based assay

To study the specificity of this assay, the effects of BSA, trypsin, pancreatin, pepsin and lysozyme were studied. Under the same analytical conditions, these proteins or enzymes were separately introduced into the detection system both in the absence and presence of ALP. As shown in Fig. 4, when they were added individually, none of them could cause obvious fluorescence changes compared with blank solution. While, the fluorescence decreased drastically after the introduction of ALP. In addition, when they were simultaneously added with ALP, dramatic fluorescence decrease was observed. These results demonstrate that these possible interfering substances show negligible influence on ALP activity detection and indicate that this assay shows good selectivity.

3.5. Detection of ALP in human serum samples

To evaluate its potential in practical application, this assay was firstly employed to detect ALP in human serum samples using standard addition approach. Human serum samples were obtained from Hospital of University of Jinan. The accuracy of this method was assessed by the recoveries of ALP in serum samples. As shown in Table S1, recoveries ranging from 90.6% to 110% with relative standard deviation (RSD) varying from 4.69% to 7.00% is obtained. The satisfactory recoveries and acceptable RSD suggest that this fluorescent assay is highly feasible for ALP detection in real samples. Then this method is directly used to detect ALP in human serum samples without treatment. These samples were respectively analyzed by our method and clinic method. As depicted in Fig. 5, the results obtained by using our method are consistent with those obtained by clinic method, suggesting the high potential of our assay in real sample detections.

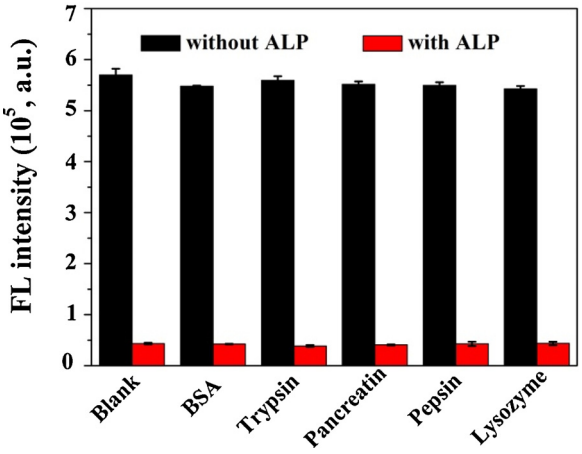


Fig. 4. Fluorescence responses of the detection assay against the control protein or enzymes in the absence and presence of ALP. All of their concentrations are 2.0 μg/mL.

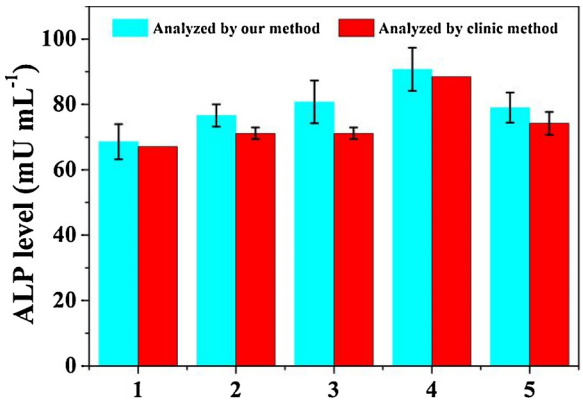


Fig. 5. Detection of ALP in five volunteers serums by our method and clinic method.

4. Conclusion

In summary, we have proposed a simple, time-saving and sensitive fluorescent assay for the ALP detection using the in-situ formation of azamonardine as the fluorescent probe by IFE. The azamonardine with high fluorescence can be simply obtained by mixing dopamine and resorcinol together under alkaline condition in short time, making it a simper and time-saving operation. PNP generating from ALP-catalyzed hydrolysis of PNPP results in the fluorescence quench of azamonardine due to IFE. This IFE-based assay can achieve sensitively and selectively detect ALP with a detection limit down to 0.07 mU mL^{-1}. More importantly, this assay has been successfully applied to detect ALP in human serum samples with the accurate results. This work may not only pave a new way for the ALP detection, but also broaden the application of the in-situ materials for biosensing analysis.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (21705056, 21902061, 21902062 and 21904048), the Young Taishan Scholars Program (tsqn201812080), the Natural Science Foundation of Shandong Province (ZR2019YQ10,

ZR2018BB057, ZR2017MB022 and ZR2018PB009) and the Open Funds of the State Key Laboratory of Electroanalytical Chemistry (SKLEAC201901), the start-up funding from University of Jinan (511-1009408, 511-1009424) and the Doctoral Fund of University of Jinan (160100410).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.snb.2019.127145.

References

[1] Z. Tang, H. Chen, H. He, C. Ma, Assays for alkaline phosphatase activity: progress and prospects, TrAC-Trends Anal. Chem. 113 (2019) 32–43.
[2] T. Xiao, J. Sun, J. Zhao, S. Wang, G. Liu, X. Yang, FRET effect between fluorescent polydopamine nanoparticles and MnO_2 nanosheets and its application for sensitive sensing of alkaline phosphatase, ACS Appl. Mater. Interfaces 10 (2018) 6560–6569.
[3] J. Sun, B. Wang, X. Zhao, Z.-J. Li, X. Yang, Fluorescent and colorimetric dual-readout assay for inorganic pyrophosphatase with Cu^{2+}-triggered oxidation of *o*-phenylenediamine, Anal. Chem. 88 (2016) 1355–1361.
[4] D.E. Wang, X.H. Gao, G.B. Li, T. Xue, H. Yang, H.Y. Xu, Facile colorimetric assay of alkaline phosphatase activity using polydiacetylene liposomes with calcium ions and pyrophosphate, Sens. Actuators B Chem. 289 (2019) 85–92.
[5] M. Cai, C. Ding, F. Wang, M. Ye, C. Zhang, Y. Xian, A ratiometric fluorescent assay for the detection and bioimaging of alkaline phosphatase based on near infrared Ag_2S quantum dots and calcein, Biosens. Bioelectron. (2019).
[6] L. Sappia, B. Felice, M.A. Sanchez, M. Martí, R. Madrid, M.I. Pividori, Electrochemical sensor for alkaline phosphatase as biomarker for clinical and in vitro applications, Sens. Actuators B Chem. 281 (2019) 221–228.
[7] Y. Chen, H. Cheng, K. Tram, S. Zhang, Y. Zhao, L. Han, Z. Chen, S. Huan, A paper-based surface-enhanced resonance Raman spectroscopic (SERRS) immunoassay using magnetic separation and enzyme-catalyzed reaction, Analyst 138 (2013) 2624–2631.
[8] L. Xu, X. He, Y. Huang, P. Ma, Y. Jiang, X. Liu, S. Tao, Y. Sun, D. Song, X. Wang, A novel near-infrared fluorescent probe for detecting intracellular alkaline phosphatase and imaging of living cells, J. Mater. Chem. B 7 (2019) 1284–1291.
[9] C. Gao, S. Zang, L. Nie, Y. Tian, R. Zhang, J. Jing, X. Zhang, A sensitive ratiometric fluorescent probe for quantitive detection and imaging of alkaline phosphatase in living cells, Anal. Chim. Acta 1066 (2019) 131–135.
[10] Q. Zhang, S. Li, C. Fu, Y. Xiao, P. Zhang, C. Ding, Near-infrared mito-specific fluorescent probe for ratiometric detection and imaging of alkaline phosphatase activity with high sensitivity, J. Mater. Chem. B 7 (2019) 443–450.
[11] G. Ma, M. Yang, C. Li, H. Tan, L. Deng, S. Xie, F. Xu, L. Wang, Y. Song, Preparation of spinel nickel-cobalt oxide nanowrinkles/reduced graphene oxide hybrid for nonenzymatic glucose detection at physiological level, Electrochim. Acta 220 (2016) 545–553.
[12] G. Ma, H. Xu, F. Xu, L. Wang, Growth of worm-like and flower-like molybdenum disulfide on graphene nanosheets for sensitive determination of dopamine, Int. J. Electrochem. Sci. 12 (2017) 7365–7376.
[13] C. Tang, Z. Qian, Y. Huang, J. Xu, H. Ao, M. Zhao, J. Zhou, J. Chen, H. Feng, A fluorometric assay for alkaline phosphatase activity based on β-cyclodextrin-modified carbon quantum dots through host-guest recognition, Biosens. Bioelectron. 83 (2016) 274–280.
[14] B. Dong, H. Li, J. Sun, G.M. Mari, X. Yu, Y. Ke, J. Li, Z. Wang, W. Yu, K. Wen, J. Shen, Development of a fluorescence immunoassay for highly sensitive detection of amantadine using the nanoassembly of carbon dots and MnO_2 nanosheets as the signal probe, Sens. Actuators B Chem. 286 (2019) 214–221.
[15] J.-G. You, C.-Y. Lu, A.S.K. Kumar, W.-L. Tseng, Cerium(III)-directed assembly of glutathione-capped gold nanoclusters for sensing and imaging of alkaline phosphatase-mediated hydrolysis of adenosine triphosphate, Nanoscale 10 (2018) 17691–17698.
[16] J. Sun, H. Mei, F. Gao, Ratiometric detection of copper ions and alkaline phosphatase activity based on semiconducting polymer dots assembled with rhodamine B hydrazide, Biosens. Bioelectron. 91 (2017) 70–75.
[17] J. Sun, T. Hu, C. Chen, D. Zhao, F. Yang, X. Yang, Fluorescence immunoassay system via enzyme-enabled in situ synthesis of fluorescent silicon nanoparticles, Anal. Chem. 88 (2016) 9789–9795.
[18] J. Sun, T. Hu, X. Xu, L. Wang, X. Yang, A fluorescent ELISA based on the enzyme-triggered synthesis of poly (thymine)-templated copper nanoparticles, Nanoscale 8 (2016) 16846–16850.
[19] J. Sun, J. Zhao, X. Bao, Q. Wang, X. Yang, Alkaline phosphatase assay based on the chromogenic interaction of diethanolamine with 4-Aminophenol, Anal. Chem. 90 (2018) 6339–6345.
[20] R. Li, Q. Liu, Y. Jin, B. Li, Fluorescent enzyme-linked immunoassay strategy based on enzyme-triggered in-situ synthesis of fluorescent copper nanoclusters, Sens. Actuators B Chem. 281 (2019) 28–33.
[21] D. Zhao, J. Li, C. Peng, S. Zhu, J. Sun, X. Yang, Fluorescence immunoassay based on the alkaline phosphatase triggered in situ fluorogenic reaction of *o*-phenylenediamine and ascorbic acid, Anal. Chem. 91 (2019) 2978–2984.
[22] J. Zhao, S. Wang, S. Lu, X. Bao, J. Sun, X. Yang, An enzyme cascade-triggered fluorogenic and chromogenic reaction applied in enzyme activity assay and immunoassay, Anal. Chem. 90 (2018) 7754–7760.
[23] S. Chen, Y.-L. Yu, J.-H. Wang, Inner filter effect-based fluorescent sensing systems: a review, Anal. Chim. Acta 999 (2018) 13–26.
[24] G. Li, H. Fu, X. Chen, P. Gong, G. Chen, L. Xia, H. Wang, J. You, Y. Wu, Facile and sensitive fluorescence sensing of alkaline phosphatase activity with photo-luminescent carbon dots based on inner filter effect, Anal. Chem. 88 (2016) 2720–2726.
[25] H. Liu, M. Li, Y. Xia, X. Ren, A turn-on fluorescent sensor for selective and sensitive detection of alkaline phosphatase activity with gold nanoclusters based on inner filter effect, ACS Appl. Mater. Interfaces 9 (2017) 120–126.
[26] Z. Guo, X. Zhu, S. Wang, C. Lei, Y. Huang, Z. Nie, S. Yao, Fluorescent Ti_3C_2 MXene quantum dots for an alkaline phosphatase assay and embryonic stem cell identification based on the inner filter effect, Nanoscale 10 (2018) 19579–19585.
[27] J. Ma, X. Cheng, F. Peng, N. Zhang, R. Li, L. Sun, Z.-L. Li, H. Jiang, A polymer dots fluorescent sensor for detection of alkaline phosphatase activity and inhibitor evaluation, J. Mater. Sci. 54 (2019) 10055–10064.
[28] G. Mao, Q. Zhang, Y. Yang, X. Ji, Z. He, Facile synthesis of stable CdTe/CdS QDs using dithiol as surface ligand for alkaline phosphatase detection based on inner filter effect, Anal. Chim. Acta 1047 (2019) 208–213.
[29] Y. Zhong, F. Xue, P. Wei, R. Li, C. Cao, T. Yi, Water-soluble MoS_2 quantum dots for facile and sensitive fluorescence sensing of alkaline phosphatase activity in serum and live cells based on the inner filter effect, Nanoscale 10 (2018) 21298–21306.
[30] A. Ulises Acuña, M. Álvarez-Pérez, M. Liras, P.B. Coto, F. Amat-Guerri, Synthesis and photophysics of novel biocompatible fluorescent oxocines and azocines in aqueous solution, Phys. Chem. Chem. Phys. 15 (2013) 16704–16712.
[31] J. Zhang, R. Zhou, D. Tang, X. Hou, P. Wu, Optically-active nanocrystals for inner filter effect-based fluorescence sensing: achieving better spectral overlap, Trac-Trends Anal. Chem. 110 (2019) 183–190.
[32] J. Zhang, X. Lu, Y. Lei, X. Hou, P. Wu, Exploring the tunable excitation of QDs to maximize the overlap with the absorber for inner filter effect-based phosphorescence sensing of alkaline phosphatase, Nanoscale 9 (2017) 15606–15611.
[33] X. Guo, F. Wu, Y. Ni, S. Kokot, Synthesizing a nano-composite of BSA-capped Au nanoclusters/graphitic carbon nitride nanosheets as a new fluorescent probe for dopamine detection, Anal. Chim. Acta 942 (2016) 112–120.
[34] Y. Liu, E. Xiong, X. Li, J. Li, X. Zhang, J. Chen, Sensitive electrochemical assay of alkaline phosphatase activity based on TdT-mediated hemin/G-quadruplex DNAzyme nanowires for signal amplification, Biosens. Bioelectron. 87 (2017) 970–975.
[35] X. Liu, N. Fan, L. Wu, C. Wu, Y. Zhou, P. Li, B. Tang, Lighting up alkaline phosphatase in drug-induced liver injury using a new chemiluminescence resonance energy transfer nanoprobe, Chem. Commun. 54 (2018) 12479–12482.
[36] Y. Zeng, J.-Q. Ren, S.-K. Wang, J.-M. Mai, B. Qu, Y. Zhang, A.-G. Shen, J.-M. Hu, Rapid and reliable detection of alkaline phosphatase by a hot spots amplification strategy based on well-controlled assembly on single nanoparticle, ACS Appl. Mater. Interfaces 9 (2017) 29547–29553.
[37] P. Ni, J. Xie, C. Chen, Y. Jiang, Z. Zhao, Y. Zhang, Y. Lu, J. Yu, Spectrophotometric determination of the activity of alkaline phosphatase and detection of its inhibitors by exploiting the pyrophosphate-accelerated oxidase-like activity of nanoceria, Microchim. Acta 186 (2019) 320.
[38] C.H. Wang, J. Gao, Y.L. Cao, H.L. Tan, Colorimetric logic gate for alkaline phosphatase based on copper (II)-based metal-organic frameworks with peroxidase-like activity, Anal. Chim. Acta 1004 (2018) 74–81.
[39] H. Song, H. Wang, X. Li, Y. Peng, J. Pan, X. Niu, Sensitive and selective colorimetric detection of alkaline phosphatase activity based on phosphate anion-quenched oxidase-mimicking activity of Ce(IV) ions, Anal. Chim. Acta 1044 (2018) 154–161.
[40] J. Zhang, M. Lu, Y. Lei, X. Hou, P. Wu, Exploring tunable excitation of QDs to maximize the overlap with absorber for inner filter effect-based phosphorescent sensing of alkaline phosphatase, Nanoscale 9 (2017) 15606–15611.
[41] D. Li, J. Qin, Q. Xu, G. Yan, A room-temperature phosphorescence sensor for the detection of alkaline phosphatase activity based on Mn-doped ZnS quantum dots, Sens. Actuators B Chem. 274 (2018) 78–84.
[42] J.J. Liu, D.S. Tang, Z.T. Chen, X.M. Yan, Z. Zhong, L.T. Kang, J.N. Yao, Chemical redox modulated fluorescence of nitrogen-doped graphene quantum dots for probing the activity of alkaline phosphatase, Biosens. Bioelectron. 94 (2017) 271–277.

Dr. Pengjuan Ni received her bachelor's degree from Hebei University in 2011. She obtained the PhD degree from Changchun Institute of Applied Chemistry, Chinese Academy of Sciences in 2017. Currently, she works as a lecture in the School of Materials Science and Engineering, University of Jinan. Her scientific interests are focused on biochemical analysis and nanobiosensing.

Dr. Chuanxia Chen received her PhD degree in 2017 from Changchun Institute of Applied Chemistry, Chinese Academy of Sciences. Currently, she works as a lecturer in School of Materials Science and Engineering, University of Jinan, China. Her research interests focus mainly on the the synthesis of fluorescent nanomaterials and their chemosensors application.

Dr. Yuanyuan Jiang received her bachelor's degree from Jilin University in 2009. She then obtained the PhD degree from the Changchun Institute of Applied Chemistry, Chinese Academy of Sciences in 2015. Then she worked as a postdoctoral fellow in Nanyang Technological University. In 2017, she joined the School of Materials Science and Engineering, University of Jinan. Her scientific interests focused on the development of nanomaterials for energy conversion applications and electroanalysis-relevant applications.

Dr. Chenghui Zhang received her Ph.D. in 2018 from Jilin University. Currently, she works as a lecurer in School of Materials Science and Engineering, University of Jinan. Her research is focused on the synthesis of metal-organic frameworks for sensing and electrochemistry-related applications.

Dr. Bo Wang received his BS degree from Department of Chemistry & Chemical Engineering, Yantai University in 2013 and his Ph.D. from State Key Laboratory of Photocatalysis on Energy and Environment, Fuzhou University in 2019. Currently, he joined the School of Materials Science and Engineering, University of Jinan as a lecturer. His research interests are focused on development of novel photocatalysts for energy and evironment applications.

Prof. Yizhong Lu received his Ph.D. from Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, in 2015. He then worked as a research fellow at Nanyang Technological University & Cambridge Center for Advanced Research in Energy Efficiency in Singapore. Currently, he is a full proferssor in the School of Materials Science and Engineering, University of Jinan, Shandong. His research interests focused on size-controlled synthesis, characterization, and size-dependent property study of metal nanocusters, nanobiosensing, and electrochemisty.

Prof. Hua Wang received his Ph.D from Hunan University in 2004, and now is working at Qufu Normal University as a distinguished professor in Chemistry. His search interests mainly include chemical and biological sensors, advanced materials and organic synthesis.