Biosensors and Bioelectronics 85 (2016) 445–449

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Ultrasensitive aptamer biosensor for malathion detection based on cationic polymer and gold nanoparticles

Rajni Bala [a], Munish Kumar [a], Kavita Bansal [c], Rohit K. Sharma [a,*], Nishima Wangoo [b,c,**]

[a] Department of Chemistry & Centre of Advanced Studies in Chemistry, Panjab University, Sector-14, Chandigarh 160014, India
[b] Department of Applied Sciences, University Institute of Engineering & Technology (U.I.E.T.), Panjab University, Sector-25, Chandigarh 160014, India
[c] Centre for Nanoscience and Nanotechnology (U.I.E.A.S.T), Panjab University, Chandigarh 160014, India

ARTICLE INFO

Article history:
Received 16 February 2016
Received in revised form
21 April 2016
Accepted 12 May 2016
Available online 13 May 2016

Keywords:
Colorimetric detection
Aptasensor
Gold nanoparticles
Malathion

ABSTRACT

In this work, we have demonstrated a novel sensing strategy for an organophosphorus pesticide namely, malathion, employing unmodified gold nanoparticles, aptamer and a positively charged, water-soluble polyelectrolyte Polydiallyldimethylammonium chloride (PDDA). The PDDA when associated with the aptamer prevents the aggregation of the gold-nanoparticles while no such inhibition is observed when the aptamer specific pesticide is added to the solution, thereby changing the color of the solution from red to blue. This type of biosensor is quite simple and straightforward and can be completed in a few minutes without the need of any expensive equipment or trained personnel. The proposed method was linear in the concentration range of 0.5–1000 pM with 0.06 pM as the limit of detection. Moreover, the proposed assay selectively recognized malathion in the presence of other interfering substances and thus, can be applied to real samples for the rapid screening of malathion.

1. Introduction

Malathion (Diethyl 2-[(dimethoxyphosphorothioyl)sulfanyl] butanedioate), is a broad-spectrum insecticide used to control a variety of outdoor insects in both agricultural and residential settings. Malathion is registered for use on food, feed, and ornamental crops and in mosquito, boll weevil and fruit fly eradication programs. The widespread application of this pesticide has resulted in the serious contamination of drinking water due to its presence in soil and water at concentrations far exceeding its permissible limits, causing major health based concerns (Mionettol et al., 1994; Bouchard et al., 2010). Therefore, in order to control such a hazardous pesticide, a rapid yet sensitive method of detection is required urgently. For the detection of trace levels of pesticides, various analytical methods such as high-performance liquid chromatography, gas chromatography, thin-layer chromatography and gas chromatography-mass spectrometry are already being used (Brito et al., 2002; Berijani et al., 2006; Boyd-Boland et al., 1996; Liu et al., 2011). However, due to various problems associated with these methods such as complexity, long hours, cost and instrumentation, researchers are now shifting to low cost and rapid sensing strategies (Weerathunge et al., 2014). The last decade saw the rise of immunoassay based detection techniques as an alternative for the detection of pesticides at high sensitivity levels (Qian et al., 2009; Gabaldon et al., 2007; Jiang et al., 2011; Suri et al., 2009). However, the use of antibodies in such immunoassays still remains the major hurdle. This inadequacy of the above mentioned methods highlights the need for new, high-speed and sensitive techniques for the detection of pesticides. Aptamers have received huge attention in the past few years due to their applicability in various range of analytical applications. Aptamers are single stranded nucleic acid or peptide molecules of size less than 25 kDa which can be natural or synthetic by origin (Lau and Li, 2011; Lau et al., 2011). Aptamers are highly specific and selective towards their target compounds, namely, ions, proteins, toxins, microbes and viruses, due to their precise and defined three-dimensional structures (Wei et al., 2007; Kuang et al., 2011; Chen et al., 2013; Wu et al., 2012). In comparison with antibodies, aptamers based biosensors have many advantages such as – no use of animals, better stability, and enhanced specificity in various kinds of assays such as electrochemical (Liu et al., 2012; Ho et al., 2012), fluorescence (Zhang et al., 2014), chemiluminescence (Freeman et al., 2011), or colorimetric (Chen et al., 2013; Shi et al., 2013). Aptamers possess additional advantages such as conformational change on analyte-binding and high specificity for single target analyte. In conjunction with gold nanoparticles, aptamers serve as promising molecules for application as sensitive

* Corresponding author.
** Corresponding author at: Department of Applied Sciences, University Institute of Engineering & Technology (U.I.E.T.), Panjab University, Sector-25, Chandigarh 160014, India.
E-mail addresses: rohitksg@pu.ac.in (R.K. Sharma), nishima@pu.ac.in (N. Wangoo).

http://dx.doi.org/10.1016/j.bios.2016.05.042
0956-5663/

and specific bioprobes for the detection of pesticides. In such type of assays, AuNPs serve as colorimetric labels due to their Surface plasmon resonance (SPR) phenomenon (Lin et al., 2006). Although there have been reports regarding the detection of Arsenic (Wu et al., 2012) and thrombin protein (Chen et al., 2014) using AuNPs and polymer but, until now, colorimetric aptasensor for the detection of malathion using electrolyte has never been reported. Keeping these points in mind, in this paper, a sensitive and selective aptamer biosensor using electrolyte for the detection of malathion is presented. The aptamer sequence was derived from the literature where the detection of malathion was carried out using SERS (Barahona et al., 2013). On addition of positively charged electrolyte, the aptamer binds to it owing to its negatively charged backbone. However, when the pesticide, specific to the aptamer is present, the aptamer binds to the pesticide, and the electrolyte remains free to lead to the aggregation of the gold nanoparticles, imparting a color change from red to purple-blue.

2. Experimental

2.1. Materials and instrumentation

Hydrogen tetrachloroaurate (III) trihydrate, trisodium citrate dihydrate, Polydiallyldimethylammonium chloride (PDDA), aptamer and malathion were obtained from Sigma Aldrich (India). The oligonucleotide having the sequence 5′ATCCGTCACACCTGCTCTTATACACAATTGTTTTTCTCTTAACTTCTTGACTGCTGGTGTTGGCTCCCGTAT-3′ was desalted and HPLC purified. All the reagents used were of analytical grade and the experiments were performed in Milli-Q water having a resistivity of 18.2 MΩ cm. The glassware was rinsed with aqua regia prior to use.

2.2. Instrumentation

The UV-Visible measurements were recorded on JASCO V-530 spectrophotometer. TEM analyses were carried out using Hitachi H-7500 microscope. HPLC chromatograms were obtained from Waters HPLC using Waters 2996 Photodiode Array Detector.

2.3. Gold Nanoparticles Synthesis

AuNPs were synthesized by citrate reduction of chloroauric acid (Bala et al., 2015). Briefly, 100 mL of aqueous 0.01% $HAuCl_4$ solution was heated to boiling followed by the rapid addition of 2 mL of 1% trisodium citrate solution. The color change from pale yellow to red indicated the formation of AuNPs. The solution was further boiled for an additional 10 min and allowed to cool at room temperature under stirring. The colloids were stored in dark bottles at 4 °C.

2.4. Analysis of malathion

Stock solution of malathion was prepared in acetone and stored at 6° C. Varied dilutions of malathion were then prepared in phosphate buffer (pH 7.32). For malathion analysis, 10 µL of 50 nM aptamer was mixed with 50 µL of different malathion concentrations, diluted with 100 µL phosphate buffer and incubated for 1 h at room temperature. Afterwards, 200 µL of 15 nM PDDA was added into the solution and the solution was again incubated for 30 min. Finally, 600 µL of 5 nM AuNPs were added followed by the UV–vis measurements. Various pesticides such as atrazine, chlorosulfuron, 2,4-D, diuron and phorate were used to test the selectivity of the biosensor.

2.5. Determination of malathion in spiked samples

The practicability of the aptasensor was evaluated by performing the analysis in real samples i.e. food and water. The lake water was obtained from Sukhna Lake, Chandigarh, India and filtered using 0.4 µm filter in order to remove any suspended impurities. Subsequently, the lake water was spiked with different malathion concentrations followed by the analyses as described above. Apple was chosen as the matrix to test the feasibility of the biosensor in food. Firstly, the apple was cut and crushed into a homogenate followed by extraction with methanol. The sample was then filtered to remove the solid part and mixed with active charcoal to eliminate colored impurities. Finally, the solvent was evaporated and the residue was diluted with water. Malathion was then spiked in the samples and detected using the proposed assay.

2.6. Validation of the proposed assay with conventional technique

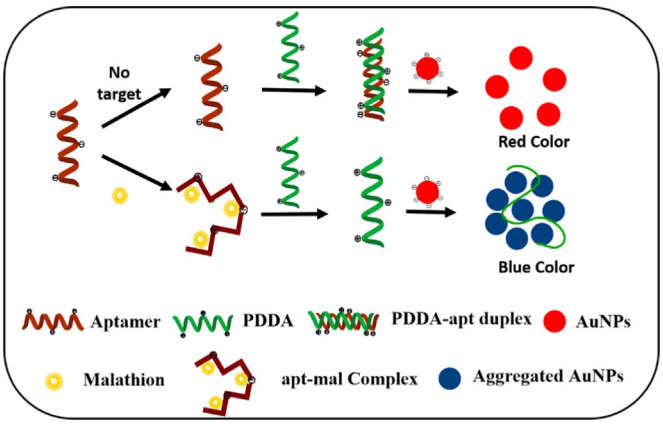
The performance of the aptasensor was validated with the conventional HPLC. For that, prior to use, all the malathion samples were filtered using 0.4 µm filter. Water-acetonitrile system was used as the mobile phase to carry out the analysis.

3. Results and discussion

The strategy for the colorimetric detection of malathion is illustrated in Scheme 1. In the absence of malathion, the aptamer is free and hybridize to form a duplex with the cationic PDDA owing to the interaction of negatively charged phosphate backbone of aptamer with PDDA. Thus, the aggregation of AuNPs is prevented due to the lack of sufficient PDDA. However, upon the addition of malathion, the aptamer forms a complex with malathion which, in turn, makes the PDDA free and results in the aggregation of AuNPs. Consequently, the remarkable change in the color of the AuNPs from red to blue is evident from naked eyes. The color of the solution is dependent on the concentration of PDDA which is directly linked to the concentration of malathion. Hence, the present methodology can be employed for detecting the presence of malathion colorimetrically.

3.1. Optimization of the reaction conditions

In order to develop a highly sensitive aptasensor for malathion,



Scheme 1. Schematic illustration of a colorimetric aptasensor based on gold nanoparticles for the detection of malathion. In the absence of malathion, the aptamer interacts only with the polymer and hence, the gold nanoparticles are well dispersed due to lack of sufficient amount of PDDA. However, in the presence of malathion, the aptamer interacts with the malathion and free PDDA aggregate the AuNPs, thereby leading to the color change of the solution from red to blue.

all the reaction conditions were carefully optimized. To optimize polymer concentration, various PDDA concentrations i.e. 2, 5, 10, 15, 20, 25 and 30 nM were incubated with 5 nM AuNPs for 15 min. It was observed that 15 nM PDDA was sufficient enough to aggregate the AuNPs and change the red color of the AuNPs to blue (See Fig. S1 in Supplementary data). Hence, 15 nM PDDA was used for all the experiments. After careful examination of polymer concentration, aptamer concentration was optimized that can bind to PDDA and keep the AuNPs red in color. For this, different aptamer concentrations were incubated with 15 nM PDDA for 30 min followed by the addition of AuNPs. As clear from the Figure (See Fig. S2 in Supplementary data), 50 nM aptamer was successfully able to hybridize with PDDA and prevented the aggregation of AuNPs thereby maintaining the red color of the AuNPs.

3.2. Colorimetric detection of malathion

After the careful optimization of all the reaction conditions, the aptasensor was then tested for its sensitivity employing UV–vis spectroscopy, TEM and visual detection. The biosensor was treated with a series of varied malathion concentrations ranging from 0.5 pM to 1000 pM and subsequently the UV–vis measurements were recorded. As clear from the Fig. 1(A) upon increase in malathion concentration, the characteristic peak of the AuNPs at 518 nm decreased while a new band emerged around 630 nm representing the aggregation of AuNPs. Accordingly, the color of the nanoparticle dispersions went an obvious change from red to purple to blue. In order to quantify malathion, ratio of the absorbance of aggregated peak to the characteristic AuNPs peak i.e. A_{630}/A_{518} was plotted and a calibration curve was obtained that fitted best to the logarithmic concentration of malathion. Fig. 1 (B) shows that A_{630}/A_{518} is proportional to the log concentration of malathion with a regression coefficient $R=0.9965$.

Moreover, the respective values of A_{630} and A_{518} were also plotted versus the logarithmic concentration of malathion and the results obtained were in accordance with the principle explained above (See Fig. S3 in Supplementary data). The detection limit of the aptasensor came out to be 0.06 pM which was calculated using the formula 3α/slope (Wu et al., 2012; Bala et al., 2016) where α represents the standard deviation of the instrument and slope is obtained from the linear calibration plot. The results obtained clearly indicate that this biosensor can be potentially used to detect malathion with high sensitivity.

The aggregation phenomenon was further verified by analyzing the samples through TEM. The well dispersed nature of the AuNPs is evident from Fig. 2(a) that clearly indicate that the particles are uniformly distributed and spherical in nature.

Upon the addition of PDDA, the electrostatic interactions between PDDA and AuNPs resulted in the aggregation of the particles thereby rendering the solution blue color [Fig. 2(b)]. However, in the presence of aptamer, the PDDA formed a duplex structure with aptamer and therefore, the dispersity of the particles was maintained due to lack of sufficient PDDA and the solution remained red in color [Fig. 2(c)]. On the contrary, upon malathion addition, the formation of malathion-apt complex rendered the PDDA free leading to the aggregation of particles and change in the color of the solution to blue [Fig. 2(d)].

3.3. Specificity of the assay

Further, the selectivity of the proposed assay was also evaluated by subjecting the biosensor to malathion and several other non-target pesticides. Fig. 3 clearly shows that the biosensor responded only to malathion whereas response of the biosensor for other pesticides was almost negligible.

Moreover, the color change from red to blue was observable only for malathion while the remaining solutions remained red. The results further demonstrate that the proposed method is specific only for malathion and the interference arising from non-target pesticides is insignificant. The selectivity of the proposed aptasensor was further tested by increasing the concentration of the non-target pesticides to 100 folds. The results clearly showed that even at higher concentrations i.e. 1 mM of the interfering pesticides, the aptasensor had negligible interference (See Fig. S4 in Supplementary data).

3.4. Malathion detection in water and food samples

The applicability of the aptasensor was then assessed by detecting malathion in water and food samples. For this, standard malathion concentrations were spiked in lake water and apple. The above real samples were then detected visually by observing change in the color of the solution whereas quantification was carried out using the calibration plot obtained (See Figs. S5 and S6 in supplementary data). The results clearly demonstrate that the proposed method can be easily applied for the rapid and efficient determination of malathion residues in practical applications though the sample pre-treatment for food sample may lead to some loss of malathion. In order to confirm the applicability of the proposed colorimetric aptasensor in real samples, the present methodology was validated using conventional technique HPLC. It was observed that the present biosensor offered high sensitivity as compared to HPLC since lower malathion concentrations that were non-detectable in HPLC were easily detectable with the present aptasensor.

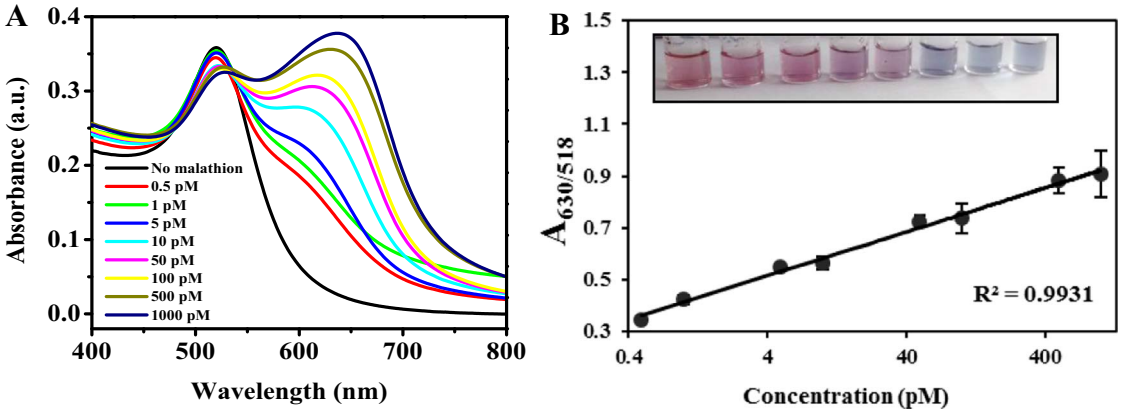


Fig. 1. Sensitivity of the proposed aptasensor for malathion detection. (A) Absorbance spectra of AuNPs in the presence of increasing malathion concentrations. (B) Calibration plot of the aptasensor. The values of A_{630}/A_{518} was fitted to a Logarithmic plot with a correlation coefficient of 0.9965. Inset shows the corresponding color changes.(For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

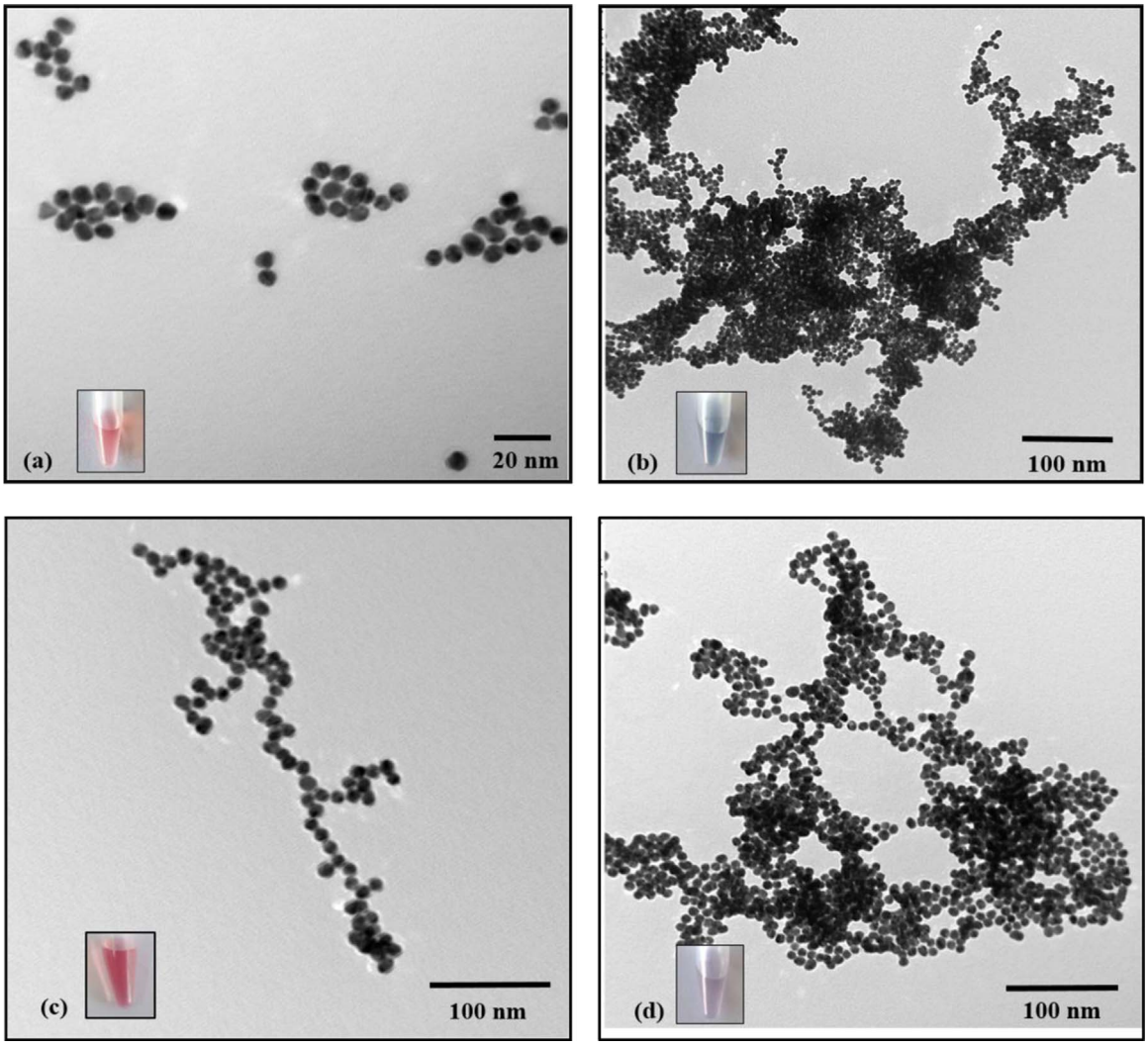


Fig. 2. The transmission electron microscope (TEM) images of gold nanoparticles treated with various substances. (a) AuNPs, (b) AuNPs on the addition of 15 nM PDDA, (c) AuNPs after treatment with 15 nM PDDA and 50 nM aptamer and (d) AuNPs after treatment with 50 nM aptamer, 10 nM malathion and 15 nM PDDA. Inset shows the respective images.

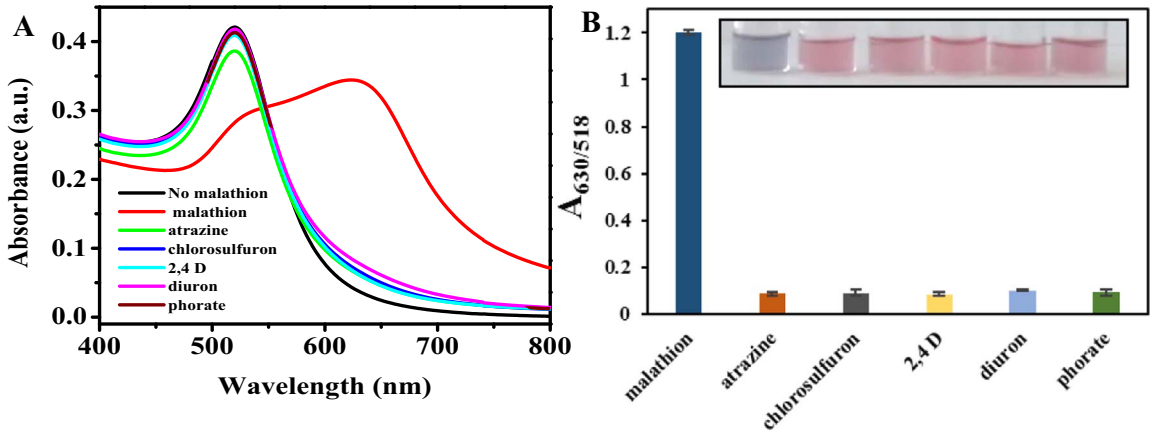


Fig. 3. Selectivity of the assay for the detection of malathion. (A) The absorbance spectra of AuNPs with various pesticides. The concentration of all the pesticides, PDDA and aptamer was 2 μM, 15 nM and 50 nM respectively. (B) Relative response of the aptasensor on treatment with different pesticides. Inset shows the corresponding images.

Table 1 shows the comparison of the present assay with HPLC. As evident from the Table, the observed values came closer to the added values further suggesting that the aptasensor can be easily employed for the ultrasensitive detection of malathion in real samples.

4. Conclusion

In summary, we have demonstrated a novel and highly sensitive colorimetric aptasensor for the detection of malathion, a toxic

Table 1.
Determination of malathion residue in spiked samples.

No.	Malathion added	Malathion found		Recovery (%)		RSD (%)
		Present method	HPLC	Present method	HPLC	
1	5 pM	5.2 pM	Non-detectable	104	–	3.91
2	50 pM	44.5 pM	55.6 pM	88	110	3.08
3	100 pM	102.6 pM	110 pM	102	110	7.91

pesticide using gold nanoparticles and positively charged polymer. The polymer when associated with the aptamer prevents the aggregation of the gold-nanoparticles while no such inhibition is observed when the aptamer specific pesticide is added to the solution, thereby changing the color of the solution from red to blue. Our results proved that the proposed sensor is very specific and has high sensitivity with the detection limit as low as 0.06 pM. Moreover, the method was also applicable to real sample signifying the reliability of the assay. The experimental results reported here will offer the possibility of a highly ultrasensitive, selective, rapid and reliable monitoring method of malathion detection in environmental samples.

Acknowledgments

This study was supported by the Department of Science & Technology (DST) INSPIRE of India, (Grant No. IFA12-CH-52) and partly by Science Education & Research Board (SERB) of India (Grant F. No.SB/SO/BB/0040/2013). RB thanks University Grants Commission (UGC), India, for research fellowship.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.05.042.

References

Bala, R., Sharma, R.K., Wangoo, N., 2015. Sens. Actuators B 210, 425–430.
Bala, R., Sharma, R.K., Wangoo, N., 2016. Anal. Bioanal. Chem. 408, 333–338.
Barahona, F., Bardliving, C.L., Phifer, A., Bruno, J.G., Batt, C.A., 2013. Ind. Biotechnol. 9, 42–50.
Berijani, S., Assadi, Y., Anbia, M., Milani Hosseini, M.R., Aghaee, E., 2006. J. Chromatogr. A 1123, 1–9.
Bouchard, M.F., Bellinger, D.C., Wright, R.O., Weisskopf, M.G., 2010. Pediatrics 125, 1216–1226.
Boyd-Boland, A.A., Magdic, S., Pawliszyn, J.B., 1996. Analyst 121, 929–937.
Brito, N.M., Navickiene, S., Polese, L., Jardim, E.F.G., Abakerli, R.B., Ribeiro, M.L., 2002. J. Chromatogr. A 957, 201–209.
Chen, Z., Huang, Y., Li, X., Zhou, T., Ma, H., Qiang, H., Liu, Y., 2013. Anal. Chim. Acta 787, 189–192.
Chen, Z., Tan, Y., Zhang, C., Yin, L., Ma, H., Ye, N., Qiang, H., Lin, Y., 2014. Biosens. Bioelectron. 56, 46–50.
Freeman, R., Liu, X., Willner, I., 2011. J. Am. Chem. Soc. 133, 11597–11604.
Gabaldon, J.A., Maquieira, A., Puchades, R., 2007. Talanta 71, 1001–1010.
Ho, M.Y., D'Souza, N., Migliorato, P., 2012. Anal. Chem. 84, 4245–4247.
Jiang, X.X., Shi, H.Y., Wu, N., Wang, M.H., 2011. Food Chem. 125, 1385–1389.
Kuang, H., Wang, L.B., Chen, W., Ma, W.W., Liu, L.Q., Ma, W., Zhao, Y.A., Zhu, Y.Y., Lau, P.S., Li, Y.F., 2011. Curr. Org. Chem. 15, 557–575.
Lau, P.S., Li, Y.F., 2011. Curr. Org. Chem. 15, 557–575.
Lin, T.J., Huang, K.T., Liu, C.Y., 2006. Biosens. Bioelectron. 22, 513–518.
Liu, C., Jia, Q., Yang, C., Qiao, R., Jing, L., Wang, L., Xu, C., Gao, M., 2011. Anal. Chem. 83, 6778–6784.
Liu, H., Xiang, Y., Lu, Y., Crooks, R.M., 2012. Angew. Chem. Int. Ed. 51, 6925–6928.
Mionettol, N., Marty, J.L., Karube, I., 1994. Biosens. Bioelectron. 9, 463–470.
Qian, G., Wang, L., Wu, Y., Zhang, Q., Sun, Q., Liu, Y., Liu, F., 2009. Food Chem. 117, 364–370.
Shi, H., Zhao, G., Liu, M., Fan, L., Cao, T., 2013. J. Hazard. Mater. 260, 754–761.
Suri, C.R., Boro, R., Nangia, Y., Gandhi, S., Sharma, P., Wangoo, N., Rajesh, K., Shekhawat, G.S., 2009. Trends Anal. Chem. 28, 29–39.
Weerathunge, P., Ramanathan, R., Shukla, R., Sharma, T.K., Bansal, V., 2014. Anal. Chem. 86, 11937–11941.
Wei, H., Li, B.L., Li, J., Wang, E.K., Dong, S.J., 2007. Chem. Commun. 36, 3735–3737.
Wu, Y., Zhan, S., Wang, F., He, L., Zhia, W., Zhou, P., 2012. Chem. Commun. 48, 4459–4461.
Zhang, C., Wang, L., Tu, Z., Sun, X., He, Q., Lei, Z., Xu, C., Liu, Y., Zhang, X., Yang, J., Liu, X., Xu, Y., 2014. Biosens. Bioelectron. 55, 216–219.