

1-1-2013

Establishment of an electrochemical RNA aptamer-based biosensor to trace nanomolar concentrations of codeine

MEHDI SABERIAN

DAVOUD ASGARI

YADOLLAH OMIDI

JALEH BARAR

HOSSEIN HAMZEIY

Follow this and additional works at: <https://journals.tubitak.gov.tr/chem>

 Part of the [Chemistry Commons](#)

Recommended Citation

SABERIAN, MEHDI; ASGARI, DAVOUD; OMIDI, YADOLLAH; BARAR, JALEH; and HAMZEIY, HOSSEIN (2013) "Establishment of an electrochemical RNA aptamer-based biosensor to trace nanomolar concentrations of codeine," *Turkish Journal of Chemistry*. Vol. 37: No. 3, Article 5. <https://doi.org/10.3906/kim-1209-45>
Available at: <https://journals.tubitak.gov.tr/chem/vol37/iss3/5>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Chemistry by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Establishment of an electrochemical RNA aptamer-based biosensor to trace nanomolar concentrations of codeine

Mehdi SABERIAN^{1,2}, Davoud ASGARI¹, Yadollah OMIDI¹, Jaleh BARAR¹,
Hossein HAMZEY^{2,*}

¹Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences,
Tabriz, Iran

²Pharmacology and Toxicology Department, Faculty of Pharmacy, Tabriz University of Medical Sciences,
Tabriz, Iran

Received: 23.09.2012 • Accepted: 08.03.2013 • Published Online: 10.06.2013 • Printed: 08.07.2013

Abstract: Codeine is an opium alkaloid with a great potential of abuse among opioid consumers. Detection of codeine is a routine procedure in the military and government of some countries for personnel recruitment. Therefore, a specific, selective, and easy to use method would be an important improvement in such detection procedures. According to previous reports, short single-stranded DNA or RNA sequences with high affinity and specificity to their targets, aptamers, could be used for designing an accurate and specific biosensor for codeine. This study introduces an aptamer-based biosensor for codeine detection in nanomolar concentrations by an electrochemical method, and ferrocene carboxylic acid was used as the redox molecule. The data show an improvement in codeine detection in comparison with the previous reports by other methods. The fabricated aptasensor offers a simpler and faster method with a lower limit of detection for codeine. The results demonstrate the reliability and robustness of the constructed aptasensor in codeine detection and measurement.

Key words: Aptasensor, aptamer, SELEX, codeine

1. Introduction

Aptamers are single-stranded DNA or RNA nucleic acid sequences with specific binding capacity and high affinity to special targets; these characteristics make them analogous to antibodies. Generation of aptamers starts with a random pool of oligonucleotides with about 10^{14} to 10^{16} different sequences. The generation is an in vitro process of selection called Systematic Evolution of Ligands by Exponential Enrichment (SELEX).^{1–3} The specific binding property provides several advantages for these sequences in comparison with antibodies. For example, they have smaller sizes than antibodies and can be immobilized more densely on the surface of the electrode in biosensors.^{4,5} Because aptamers have specific affinity to their targets even at very low concentrations, they attract further interest in the biosensor designing field.^{6–8} Nowadays, increasing numbers of aptamer-based sensors have been introduced^{9,10} and some of them could detect the presence of their own targets in picomolar¹¹ or even subpicomolar¹² concentrations.

Codeine phosphate is an alkaloid obtained from the opium poppy of *Papaver somniferum*. Since it has analgesic, sedative, and antitussive properties, it is used as a component of several pharmaceutical preparations.^{13–15} About 10% of oral doses of codeine is metabolized into morphine,¹⁴ and so this compound

*Correspondence: hamzeiy@tbzmed.ac.ir

has a great potential for abuse by morphine, heroin, and opium consumers. Therefore, it can be an important subject in forensic and toxicology fields. The misuse of codeine as an illegal relief for withdrawal symptoms of opioid dependency is a known problem. It is also mandatory to test for in the drug testing programs of military and workplace recruitments in 40 $\mu\text{g/L}$ ranges (100 nM) in some countries.¹⁶ Although the detection methods for codeine have progressed over time,¹⁷ further development of more specific, rapid, and simpler methods would be useful.

This study describes the construction of a new electrochemical aptamer-based sensor (aptasensor) for codeine detection by an improvement in the limit of detection. Ferrocene carboxylic acid is used as the redox molecule in the electrochemical experiments.

2. Experimental

2.1. Materials

Codeine phosphate (99% from Temad Co., Iran), ferrocene carboxylic acid (98% from Alfa Aesar, USA), N-hydroxysuccinimide ($\geq 99\%$ from Merck, USA), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (synthesis grade from Merck), and 6-mercaptohexanol (97% from Sigma-Aldrich, USA) were purchased and used without further purification. A previously reported RNA aptamer sequence¹⁸ was used by adding a C₆ aliphatic thiol modifier and a C₇ primary aliphatic amine modifier at its 5'-terminus and 3'-terminus, respectively (5'-SHC₆-GGG ACA GGG CUA GCU UAG UGC UAU GUG AGA AAA GGG UGU GGG GG-C₇NH₂-3', synthesized by Microsynth AG, Switzerland).

2.2. Instrumentation and procedures

Electrochemical measurements were performed using a Metrohm Autolab 302N potentiostat-galvanostat (Eco Chemie BV, the Netherlands) with 3 customary electrodes: an Ag/AgCl/KCl 3 M reference electrode, a platinum wire auxiliary electrode, and a 2 mm gold disk electrode as the working electrode (purchased from Azar Electrode Co., Iran). Total control of the electrochemical procedures was carried out by NOVA software (version 1.5, Eco Chemie BV). Cyclic voltammetry (CV) procedures were performed in the -0.1 to 0.6 V potential range using a 50 mV/s scan rate. All experiments were performed at room temperature on 50 mL of different concentrations of codeine phosphate in 0.1 M phosphate buffer solution containing 1 M sodium chloride, pH 7.0 .

2.3. Preparation of buffers and solutions

Main aptamer stock solution (100 μM) was prepared by adding nuclease-free deionized water (purchased from CinnaGen, Iran) to lyophilized aptamer and stored at -80 °C for future use. The 0.1 M phosphate buffer (pH 7.0) was used for subsequent dilution to the required concentrations. Aqueous buffers and solutions were prepared by sterile deionized water (with 0.05 $\mu\text{s/cm}$ electrical conductivity) and all equipment was washed by diethylpyrocarbonate-treated water ($\geq 97\%$ from Sigma-Aldrich) to deactivate any RNase before use.

2.4. Polishing the surface of the electrode

The efficient immobilization of 5'-thiol modified aptamer on the gold electrode depends on the smoothness and cleanness of its surface. For removing impurities and reaching a clean and mirror-like surface, the electrode was polished by physical and electrochemical procedures, respectively.¹⁹ At the first step, the electrode was polished sequentially with physical rubbing by a 2500 emery paper and 0.05 μm alumina, respectively. The electrode

was then put in a 70% ethanolic ultrasonic bath (ethanol and deionized water, 70:30 v/v) for 10 min to remove residues from the surface.²⁰ The chemical polishing was fulfilled by subsequent cyclic oxidation and reduction of the electrode: 100 scans in 0.5 M NaOH, 100 scans in 0.5 M H₂SO₄, 100 scans in 0.2 M H₂SO₄, and 500 to 1000 scans in 0.05 M H₂SO₄ to reach to a stable gold reduction peak at 0.85 V (potential range of -0.3 to 1.5 V and scan rate of 150 mV/s).^{21,22}

2.5. Immobilization of aptamer on the surface of the electrode

Immobilization of the modified RNA aptamer was carried out by self-assembling of its 5'-thiolated terminus on the surface of the gold electrode.²³ For self-assembled monolayer formation, a 50 μ L volume of 5 μ M modified RNA aptamer was placed on the mirror luster surface of the electrode overnight. The surface was then rinsed with phosphate buffer (pH 7.0) several times to wash the unbound aptamers. Ferrocene carboxylic acid N-hydroxysuccinimide ester (FcNHS: the redox molecule) was synthesized according to the previously reported procedure¹⁹ and made ready for the 3'-terminus attachment. The modified electrode was dipped into a 1 mM FcNHS solution (dissolved in a mixture of 0.1 M phosphate buffer and dimethylformamide, 5:1 v/v) for 12 h to complete the 3'-terminus reaction. The electrode was then rinsed with phosphate buffer (pH 7.0) gently and was treated with 2 mM 1-mercaptohexanol in phosphate buffer (pH 7.0) for 2 h.^{19,24,25} By performing these procedures, the electrode was made ready and was immediately used in experiments.

2.6. Detection of codeine

The responses of the aptasensor were investigated by comparing a background CV scan with a main CV scan that was taken in the absence and in the presence of codeine, respectively. First, a background scan was taken in 0.1 M phosphate buffer solution containing 1 M NaCl (pH 7.0). A proper concentration of codeine phosphate was then added to the solution and the second CV scan was taken after a 3 min incubation time for codeine-aptamer complex formation.¹⁹ Percentage of the faradic current change in the presence versus the absence of codeine phosphate was considered as an index for the aptasensor response.

2.7. Reproducibility studies and regenerating method

Electrochemical experiments were performed 3 times separately for each concentration to validate the reproducibility of the responses and the average of the collected data was considered as the result.

To ensure that any change in the faradic current of the working electrode is not related to interventional factors such as degradation of the sensor, regeneration studies should be a part of any biosensor designing.¹¹ First, a main CV scan was taken in the presence of a high concentration of codeine phosphate (2 μ M). The codeine phosphate treated sensor was then stirringly regenerated by immersing in the 80 °C heated 0.1 M phosphate buffer for 5 min followed by washing with deionized water 3 times.^{19,26} The regeneration capability of the aptasensor reached a minimum after 5 stages (see Section 3).

3. Results

3.1. Sensitivity of the sensor in the presence of codeine

For investigating the sensitivity of the aptasensor for codeine, CV measurements were performed in 0.1 M phosphate buffer solution containing 1 M NaCl (pH 7.0) in the absence and in the presence of codeine phosphate, respectively. The CV scans were taken in a potential range of -0.1 to 0.6 V and at a scan rate of 50 mV/s. The

results showed a significant increase in the faradic current of the working electrode in the presence of codeine phosphate (from 600 nA of background to 1.2 μ A). This increase indicated the sensitivity of the fabricated aptasensor to its target (Figure 1).

3.2. The linearity of the aptasensor's responses to the presence of its target

Main CV scans were performed in 0, 100 pM, 500 pM, 1 nM, 2 nM, 10 nM, 100 nM, 500 nM, 1 μ M, 2 μ M, 10 μ M, and 100 μ M concentrations of codeine to find out the lower and upper limits of detection of the fabricated aptasensor.

The obtained data showed that there were no significant changes in the faradic current of the working electrode in the presence of 0 to 1 nM of codeine phosphate. The first significant change was observed in the presence of the 2 nM concentration of the target and the increase in the responses continued up to 2 μ M. Lower and upper limits of detection were determined between 2 nM and 2 μ M of codeine for the fabricated aptasensor (Figure 2).

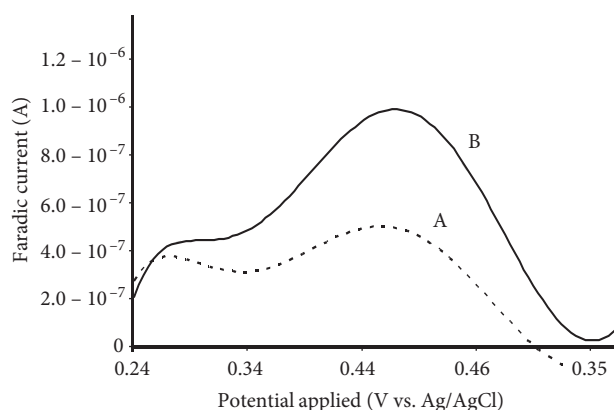


Figure 1. The sensitivity of the sensor to codeine. The applied potential versus current diagram represents the faradic current of the working electrode in the absence (A) and in the presence (B) of 10 μ M of codeine. The obtained data show a significant increase in the faradic current of the working electrode after adding codeine to the experimental environment.

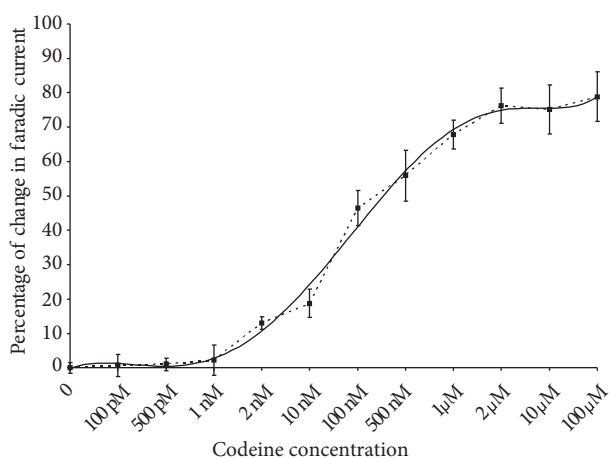


Figure 2. The linearity of the aptasensor's responses to the different concentrations of codeine. The slope of the graph starts to increase in the presence of 2×10^{-9} M of codeine phosphate. This increase continues up to 2×10^{-6} M. In concentrations of more than 2 μ M of codeine phosphate, all the immobilized aptamers are involved completely and the sensor becomes saturated (potential range: -0.1 to 0.6 V, scan rate: 50 mV/s).

3.3. Selectivity of the sensor to similar molecules

To investigate the sensitivity of the fabricated aptasensor to similar molecules, electrochemical experiments were separately performed by using 50 nM of codeine, morphine, tramadol, and naloxone. As expected, the aptasensor showed a significant sensitivity to the presence of codeine. The sensitivity of the aptasensor was partial to the presence of morphine, but naloxone and tramadol could not trigger any effect. Percentages of obtained faradic current changes were 39%, 12%, 2%, and 1% for codeine, morphine, tramadol, and naloxone, respectively (Figure 3).

3.4. Regeneration of the aptasensor

Regeneration capability of the aptasensor was examined by comparing the CV scan of a $2 \mu\text{M}$ codeine phosphate sample with a background CV scan. The data represent a 73% change in the faradic current of the working electrode in the presence of codeine phosphate at the first CV scan. The 1st, 2nd, 3rd, and 4th stages of regeneration examinations were then performed and the percentage of changes in faradic current of the working electrode were obtained as 50%, 28%, 11%, and 3% for them, respectively. The data showed a continuous decrease in the response of the regenerated sensor to a constant concentration of codeine phosphate (Figure 4).

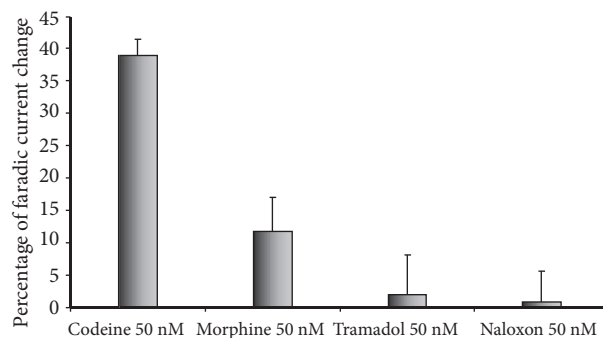


Figure 3. The selectivity studies of the sensor. A comparison study was done with codeine, morphine, tramadol, and naloxone. In the presence of 50 nM codeine, a considerable increase was observed on the main scan compared to the background scan (Figure 1). While the response of the sensor to 50 nM morphine was somewhat lower, the responses to the same amounts of tramadol and naloxone were negligible.

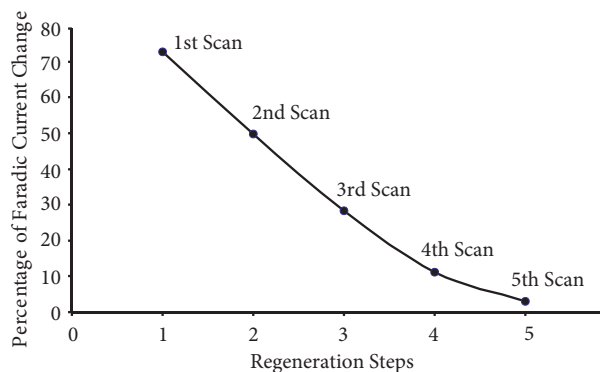


Figure 4. The regeneration of the sensor. The fabricated aptasensor was treated by $2 \mu\text{M}$ of codeine for 5 stages. The percentage of faradic current change in every stage resulted in a steady decrease (potential range: -0.1 to 0.6 V, scan rate: 50 mV/s).

4. Discussion

As mentioned above, the fabricated aptasensor showed a significant sensitivity to the presence of its own target, codeine. The background scan represents a faradic current equal to 592 nA for the working electrode (the dotted line in Figure 1) related to the distance between the redox molecule and the surface of the electrode. After codeine–aptamer complex formation, the spatial structure of the immobilized aptamer changes and it sets the redox molecule to the surface of the electrode, similar to the previously described mechanisms (Figure 5).^{21,25} This kind of change causes a significant electron transfer from the redox molecule to the surface of the electrode. As a result, the faradic current of the working electrode rises to $1.14 \mu\text{A}$.

Since the increase in the faradic current of the working electrode is proportional to the numbers of aptamer–codeine complexes,²⁷ the aptasensor's responses should be linear. The obtained data confirmed the linearity and showed that the responses of the aptasensor increase by the target addition. Significant change in the faradic current starts in the presence of 2 nM codeine phosphate (i.e. the lower limit of this sensor). By increasing the concentration of the target, the faradic current of the working electrode was increased up to a plateau related to $2 \mu\text{M}$ codeine phosphate and higher concentrations (Figure 2). Because all the immobilized aptamers became saturated in the presence of $2 \mu\text{M}$ of the target, the higher concentrations of codeine could not have any effect on the faradic current of working electrode. Therefore, the lower and higher limits of

the aptasensor were determined as 2×10^{-9} M and 2×10^{-6} M, respectively. This lower limit shows an improvement in the codeine detection in comparison with the previous reports for high-performance liquid chromatography (HPLC), capillary electrophoresis, flow injection analysis, miniaturized devices, and sequential injection analysis (SIA) (Table). Although the limit of detection in 2 cases of HPLC and SIA is less than 2×10^{-9} M, the rapidness and simplicity of the fabricated aptasensor are important advantages.

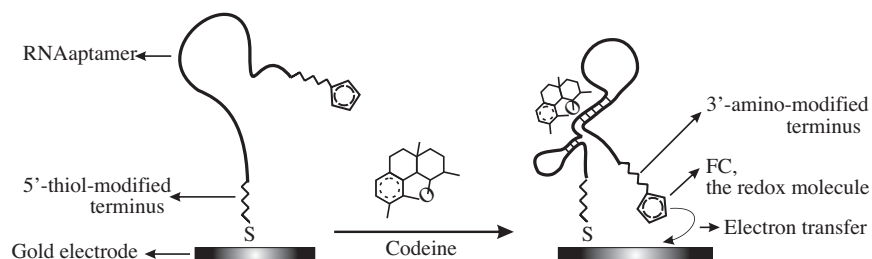


Figure 5. The probable mechanism for the fabricated aptasensor. In the presence of codeine, the spatial structure of the aptamer changes and it sets the redox molecule (FC) to the surface of the electrode. This results in an increase in the faradic current of the working electrode.

Table. Detection of codeine by the previous methods.

Method	Limit of detection	Reference
Capillary electrophoresis	5×10^{-8} M	[33]
Flow injection analysis	2×10^{-6} M	[17]
	1×10^{-7} M	[17]
	3×10^{-7} M	[17,34]
	1×10^{-8} M	[35]
	2×10^{-8} M	[17]
	5×10^{-8} M	[36]
HPLC	5×10^{-9} M	[37,38]
	2×10^{-6} M	[17]
	5×10^{-7} M	[17]
	5×10^{-9} M	[39]
Miniaturized devices	5×10^{-10} M	[40]
	1×10^{-4} M	[41]
	2×10^{-5} M	[17]
SIA	8×10^{-7} M	[42]
	1×10^{-8} M	[34]
	5×10^{-10} M	[43]

The specificity studies showed that the agonist of opioid receptors, tramadol, did not intervene in the process of codeine detection by the fabricated aptasensor. This was predictable because the chemical structure of tramadol is completely different from that of codeine.²⁸ In the case of naloxone, an antagonist for the opioid receptors, although its chemical structure is very similar to codeine,^{15,28} this molecule did not interact with the aptasensor, either. However, in the case of morphine, an opioid receptor agonist with a very similar structure to codeine, the sensor was affected partially.

In brief, the fabricated sensor was partially influenced by morphine and could not distinguish between morphine and codeine as we expected. Although morphine and codeine are only different in a methyl group,

a very specific aptamer should be able to distinguish between them in theory.^{7,29} A specific aptamer for theophylline is the best example for this case. Theophylline and caffeine are only different in a methyl group, but the aptamer was claimed to have about 10,000-fold more affinity to theophylline.³⁰ Since codeine is metabolized to morphine in the body,²⁸ the response of the sensor in the body fluids and urine sample would be relatively unspecific. However, it is valuable in the abuse detection point of view. Thus, the fabricated aptasensor is not 100% specific for codeine measurements in the presence of morphine. This problem probably originates from the counter selection stage against morphine in the SELEX process, which may have been done relatively poorly.¹⁸ To solve this problem, the SELEX process should be done using another pool of RNA library. The data in the related paper¹⁸ show that the aptamer has 16% affinity to morphine.

As reported before, the response of a regenerated sensor should be at least 95% of the first detection for introducing it as a multiple use sensor.^{19,21,26} However, the regeneration studies showed that the response of the fabricated aptasensor to its target reduced after each regeneration step. The obtained data represented only 69% recovery for the first stage of regeneration and the proportion of recovery for the 2nd to 4th stages of regeneration were 57%, 39%, and 26 %, respectively. Despite working under RNase free conditions, this problem may have been raised because of the susceptibility of the sensor to degradation by remaining trace nucleases. Therefore, the fabricated sensor could not be reused and is only appropriate for single-use applications.

Since response of the aptamer to the target is very quick, the fabricated aptasensor is comparable with the antibody-based rapid strips. Although the instrumentation for the electrochemical aptasensors is more complicated, progress in the aptamer-based strip construction may solve this problem^{31,32} and there will certainly be a possibility to introduce the new generation of aptamer-based rapid strips, i.e. aptastrips. The aptastrips would be more accurate than the antibody-based strips for of several reasons. The aptamers are much smaller than antibodies and can be immobilized more densely than antibodies on the surface. Moreover, lack of batch-to-batch variation, longer shelf lives, and possibility of aptamer selection for a wide range of targets such as ions, toxic compounds, and even a whole cell are some of the advantages of aptastrips.^{1,2,6}

5. Conclusion

The fabricated biosensor represents an improvement in the limit of detection of codeine regarding most of the previously reported methods. Rapidness and simplicity of the method can also be mentioned as an important advantage for this sensor. Although the sensor could not be regenerated, it may be useful in single-use strip-based aptasensor design (aptastrips). Aptastrips could be mentioned as good candidates for rapid and accurate detection of codeine in military, workplace, and medical services recruitments.

Acknowledgment

The authors would like to thank the Research Center for Pharmaceutical Nanotechnology and the vice chancellor of research of Tabriz University of Medical Sciences, Iran, for financially supporting this project as a part of a PhD thesis (Mehdi Saberian). The authors also thank Exir Pharmaceutical Co., Iran, for providing codeine phosphate and Ayoub Aghanejad for assisting in chemical synthesis procedures.

References

1. Nguyen, T. H.; Hilton, J. P.; Lin, Q. *Microfluid. Nanofluid.* **2009**, *6*, 347–362.
2. Tombelli, S.; Minunni, M.; Mascini, M. *Biosens. Bioelectron.* **2005**, *20*, 2424–2434.

3. Stoltenburg, R.; Reinemann, C.; Strehlitz, B. *Biomol. Eng.* **2007**, *24*, 381–403.
4. Strehlitz, B.; Nikolaus, N.; Stoltenburg, R. *Sensors* **2008**, *8*, 4296–4307.
5. Rowe, W.; Platt, M.; Day, P. J. R. *Integr. Biol.* **2009**, *1*, 53–58.
6. Lee, J. O.; So, H. M.; Jeon, E. K.; Chang, H.; Won, K.; Kim, Y. H. *Anal. Bioanal. Chem.* **2008**, *390*, 1023–1032.
7. Cho, E. J.; Lee, J. W.; Ellington, A. D. *Annu. Rev. Anal. Chem.* **2009**, *2*, 241–264.
8. Sassolas, A.; Blum, L. J.; Leca-Bouvier, B. D. *Electroanal.* **2009**, *21*, 1237–1250.
9. Hianik, T.; Wang, J. *Electroanal.* **2009**, *21*, 1223–1235.
10. Wei, F.; Ho, C. M. *Anal. Bioanal. Chem.* **2009**, *393*, 1943–1948.
11. Lai, R. Y.; Plaxco, K. W.; Heeger, A. J. *Anal. Chem.* **2007**, *79*, 229–233.
12. Hansen, J. A.; Wang, J.; Kawde, A. N.; Xiang, Y.; Gothelf, K. V.; Collins, G. *J. Am. Chem. Soc.* **2006**, *128*, 2228–229.
13. Hsu, A. F.; Liu, R. H.; Piotrowski, E. G. *Phytochemistry* **1985**, *24*, 473–476.
14. American Society of Health-System Pharmacists, *AHFS Drug Information*, Bethesda, MD, USA, 2010.
15. Sweetman, S. C. (ed). *Martindale: The Complete Drug Reference*, Pharmaceutical Press, London, 2011.
16. Kim, I.; Barnes, A. J.; Oyler, J. M.; Schepers, R.; Joseph, R. E. J.; Cone, E. J.; Lafko, D.; Moolchan, E. T.; Huestis, M. A. *Clin. Chem.* **2002**, *48*, 1486–1496.
17. Francis, P. S.; Adcock, J. L.; Costin, J. W.; Purcell, S. D.; Pfeffer, F. M.; Barnett, N. W. *J. Pharmaceut. Biomed.* **2008**, *48*, 508–518.
18. Win, M. N.; Klein, J. S.; Smolke, C. D. *Nucleic Acids Res.* **2006**, *34*, 5670–5682.
19. Xiao, Y.; Lai, R. Y.; Plaxco, K. W. *Nat. Protoc.* **2007**, *2*, 2875–2880.
20. El-Deab, M. S.; Ohsaka, T. *Electrochim. Acta* **2004**, *49*, 2189–2194.
21. Baker, B. R.; Lai, R. Y.; Wood, M. S.; Doctor, E. H.; Heeger, A. J.; Plaxco, K. W. *J. Am. Chem. Soc.* **2006**, *128*, 3138–3139.
22. Merrill, D. R.; Stefan, I. C.; Scherson, D. A.; Mortimer, J. T. *J. Electrochem. Soc.* **2005**, *152*, E212–E221.
23. Wink, T.; Van Zuilen, S. J.; Bult, A.; Van Bennekom, W. P. *Analyst* **1997**, *122*, 43R–50R.
24. March, G.; Noel, V.; Piro, B.; Reisberg, S.; Pham, M. C. *J. Am. Chem. Soc.* **2008**, *130*, 15752–15753.
25. Ferapontova, E. E.; Olsen, E. M.; Gothelf, K. V. *J. Am. Chem. Soc.* **2008**, *130*, 4256–4258.
26. Zhao, G. C.; Yang, X. *Electrochem. Commun.* **2010**, *12*, 300–302.
27. Thevenot, D. R.; Toth, K.; Durst, R. A.; Wilson, G. S. *Pure Appl. Chem.* **1999**, *71*, 2332–2348.
28. Fries, D. S. *In Foye's Principles of Medicinal Chemistry*; Lemarke, T. L.; Williams, D. A., Eds.; Wolters Kluwer Health, Philadelphia, 2008.
29. Proske, D.; Blank, M.; Buhmann, R.; Resch, A. *Appl. Microbiol. Biot.* **2005**, *69*, 367–374.
30. Jenison, R. D.; Gill, S. C.; Pardi, A.; Polisky, B. *Science* **1994**, *263*, 1425–1429.
31. Liu, J.; Mazumdar, D.; Lu, Y. *Angew. Chem. Int. Ed.* **2006**, *45*, 7955–7959.
32. Mazumdar, D.; Liu, J.; Lu, G.; Zhou, J.; Lu, Y. *Chem. Commun.* **2010**, *46*, 1416–1418.
33. Barnett, N. W.; Hindson, B. J.; Lewis, S. W.; Purcell, S. D. *Anal. Commun.* **1998**, *35*, 321–324.
34. Barnett, N. W.; Bos, R.; Brand, H.; Jones, P.; Lim, K. F.; Purcell, S. D.; Russell, R. *Analyst* **2002**, *127*, 455–458.
35. Brown, A. J.; Lenehan, C. E.; Francis, P. S.; Dunstan, D. E.; Barnett, N. W. *Talanta* **2007**, *71*, 1951–1957.
36. Barnett, N. W.; Hindson, B. J.; Lewis, S. W.; Jones, P.; Worsfold, P. J. *Analyst* **2001**, *126*, 1636–1639.
37. Barnett, N. W.; Hindson, B. J.; Lewis, S. W.; Purcell, S. D.; Jones, P. *Anal. Chim. Acta* **2000**, *421*, 1–6.
38. Qiu, B.; Chen, X.; Chen, H. L.; Chen, G. N. *Luminescence* **2007**, *22*, 189–194.
39. Adcock, J. L.; Francis, P. S.; Agg, K. M.; Marshall, G. D.; Barnett, N. W. *Anal. Chim. Acta* **2007**, *600*, 136–141.
40. Costin, J. W.; Lewis, S. W.; Purcell, S. D.; Waddell, L. R.; Francis, P. S.; Barnett, N. W. *Anal. Chim. Acta* **2007**, *597*, 19–23.
41. L'Hostis, E.; Michel, P. E.; Fiaccabrino, G. C.; Strike, D. J.; Rooij, N. F.; Koudelka-Hep, M. *Sensor. Acuat. B* **2000**, *64*, 156–162.
42. Greenway, G. M.; Nelstrop, L. J.; Port, S. N. *Anal. Chim. Acta* **2000**, *405*, 43–50.
43. Gorman, B. A.; Barnett, N. W.; Bos, R. *Anal. Chim. Acta* **2005**, *541*, 119–124.