Iranian Journal of Pharmaceutical Sciences
Spring 2012: 8(2): 121-128
ijps.sums.ac.ir

Original Article

Study of Aptamer-Attached Juglone in Different pH Ranges and Ionic Concentrations of Buffers

Mehdi Saberian[a,b], Davoud Asgari[a], Yadollah Omidi[a], Hossein Hamzeiy[b,*]

[a]*Research Center for Pharmaceutical Nanotechnology,*
[b]*Pharmacology and Toxicology Department, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran*

Abstract

Electrochemical aptamer-based sensors attract a lot of interest as useful methods because of their low cost, accuracy, sensitivity, and simplicity. An electro-active redox molecule comprises the main part of the electrochemical-based sensors. Ferrocene is one of the most popular redox molecule used in biosensor fabrication. But, instability of ferrocenium ion in strong nucleophilic reagents and chloride containing solutions is one of the main problems of this redox molecule. In this study, Juglone is used as an effective quinone redox molecule for aptasensor designing in different pH ranges and different concentrations of chloride ion. The voltammetric studies showed that the electrochemical response of sensor increased by raising the buffer ionic concentration and the sensor accuracy in 7.0 to 8.0 pH range as well. According to the findings, Juglone could be used as an effective redox molecule in high concentrations of chloride containing solutions in the 7.0 pH.

Keywords: Aptamer; Aptasensor; Juglone; pH; Redox.
Received: October 16, 2011; *Accepted:* February 23, 2012.

1. Introduction

The pH of biological samples may be considered as one of the main problems in the analytical experiments. The saliva has a pH in 6.0 to 7.0 ranges; the pH of the small intestine is acidic because of hydrochloric acid secretion in the stomach, and the large intestine has a pH of about 8.0. The pH of the body fluids, especially urine, can also be affected by different conditions and unwanted contaminations. For example, substances such as drugs, food elements and drinks influence the pH of urine and result in complications in the analytical determinations [1-6]. Therefore, in the sensor fabrication, the pH independency could be considered as a main point, and designing a pH independent biosensor would be a big progress in this field.

Novel aptamer-based sensors (aptasensors) have been introduced as a good candidate for this subject. Aptasensors are a kind of affinity based biosensors that use aptamers as recognition elements. Among optical, electrochemical, and other approaches, electrochemical based aptasensors attract a lot of interest as useful methods because of

*Corresponding author: Prof. Hossein Hamzeiy, Pharmacology and Toxicology Department, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz-Iran
Tel. (+98) 411 337 2251; Fax: (+98) 411 334 4798
Email: hamzeiy@tbzmed.ac.ir

their low cost, accuracy, sensitivity and simplicity [7-12]. In the electrochemical approaches, the electro-active redox molecules have been used as the transducer part in several different ways, i.e. label free, biomolecule-attached, or surface-attached [13]. Biomolecule-attached redox molecules are very common in biosensor designing and ferrocene is used as the most popular redox molecule in this field [14-18]. However, the instability of the ferrocenium ion in the strong nucleophilic reagents and chloride containing buffer solutions is one of the main problems related this molecule [14, 19, 20]. Therefore, the quinine derivatives were proposed as an alternative biomolecular-attached redox species [21]. Since, the quinine derivatives show electro-activity in a wide range of pH [22, 23], these redox molecules may be able to solve the mentioned problems simultaneously: (1) using a sensor for a specific target in different pH ranges; (2) and in the chloride containing buffers without any intervention with the contents.

Juglone, a 5-hydroxy derivative of 1,4-naphthoquinone, is a natural electro-active quinine that is used as a surface attached redox molecule in several studies by us and others [16, 24, 25]. In this study the potential usage of Juglone as an attached redox molecule in different concentration of chloride containing buffers has been investigated and the ability of the fabricated sensor in the different pH ranges was also tested.

2. Materials and methods

2.1. Materials

Codeine phosphate (99%) was provided from Temad Company, Tehran, Iran. 6-Mercaptohexanol (97%) was purchased from Sigma-Aldrich, Germany. N-hydroxysuccinimide (≥99%), 3-mercaptopropionic acid (≥98%) and 3-Mercaptopropionic acid (3-MPA) synthesis grade were purchased from Merck, Germany. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and 5-Hydroxy- 1,4-naphthoquinone (97%; Juglone) were supplied by Acros, Belgium, and were used without further purification. The specific RNA-aptamer sequence for codeine [26] (5'-SHC6-GGG ACA GGG CUA GCU UAG UGC UAU GUG AGA AAA GGG UGU GGG GG-C7NH2-3') was synthesized by Microsynth, Switzerland, with a C6 aliphatic thiol and a C7 primary aliphatic amin modifications in the 5' and 3' termini, respectively.

2.2. Buffer preparations

A range of buffers and aqueous solutions were prepared during the work. For preparing the phosphate buffer in different pH ranges, a mixture of monobasic dihydrogen phosphate (KH_2PO_4) and dibasic monohydrogen phosphate (K_2HPO_4) were used. A 1 M stock solutions of KH_2PO_4 and K_2HPO_4 were made separately by sterile deionized water (with 0.05 µs/cm electrical conductivity) at 25 ºC. Then, the appropriate volumes of these solutions were mixed and diluted to 1 liter by sterile deionized water to reach to a 0.1 M

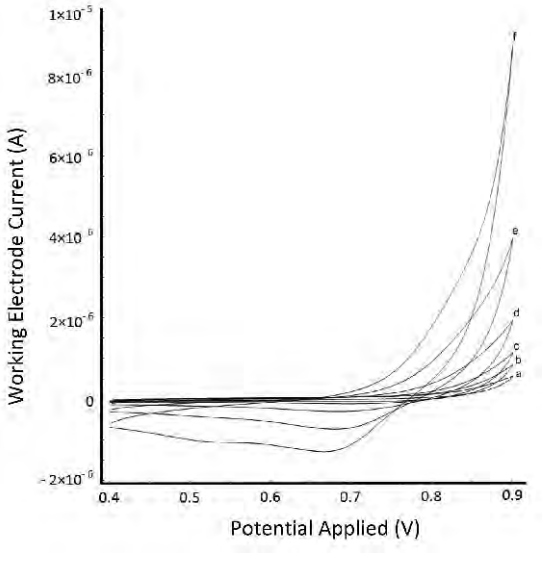

Figure 1. The aptasensor was tested against 10 µM codeine phosphate (Metrohm Autolab 302N potentiometer output) in the presence of different concentrations of NaCl. a: 0.0, b: 0.1 M, c: 0.2 M, d: 0.5 M, e: 1.0 M, and f: 2.0 M.

desire phosphate buffer. The ratio of the needed KH_2PO_4 and K_2HPO_4 solutions was calculated by the Henderson-Hasselbalch equation [27-29]. For accurate adjusting of the pH, 1 M HCl and 3 M NaOH solutions were used.

2.3. Instrumentation and procedures

Electrochemical measurements were performed by a Metrohm Autolab 302N Potentiostats-Galvanostats (The Netherlands). An Ag/AgCl/KCl 3 M reference electrode, a 2 mm diameter gold disk working electrode (purchased from Azar Electrode Co., Iran) and a platinum wire auxiliary electrode were used as customary electrodes in electrochemical experiments. The NOVA software (version 1.5, Eco Chemie BV, The Netherlands) was used for controlling the electrochemical procedures. All the electrochemical experiments were performed at room temperature and in the 50 ml of 0.1 M phosphate buffer solution (PBS).

The pH of all buffers and aqueous solutions were controlled by a Metrohm Autolab 827 pH lab pH meter (The Netherlands).

2.4. Preparing the electrode for electrochemical experiments

The impurities were removed by physical and electrochemical polishing of the working electrode for efficient immobilization of aptamer on the surface of the electrode [14, 16, 30, 31]. Then, A 50 µl volume of 5 µM modified RNA-aptamer was placed on the surface of polished electrode to form a monolayer of 5'-thiolated aptamer on the gold surface by self-assembling procedure for 18 h [32]. In the next step, the electrode was rinsed by the phosphate buffer (pH 7.0) several times to remove residues from the surface and make ready for the redox attachment. The preparation of the redox molecule, N-hydroxysuccinimide ester of β[(5-hydroxy–1,4-naphthoquinonyl) thio] propionic acid (Jug-PE), was performed by the previously reported procedure [33]. Then, the molecule was attached to the 3'-amin-modified terminus of the aptamer followed by treating with 2 mM 1-mercaptohexanol for two h [14, 15, 25]. In this stage, the electrode was ready and immediately used in experiments.

3. Results

3.1. Effect of ionic strength on the aptasensor's response

The effect of the ionic strength of the buffers on the cyclic voltammetry (CV) scan of Juglone was studied by taking and comparing the cyclic voltammetry scans in the potential range of +0.4 to +0.9 V and the scan rate of 0.15 V/s in the 0.1 M PBS containing 10 µM codeine phosphate in the presence of NaCl concentrations of 0.0, 0.1, 0.2, 0.5, 1, and 2 M (Figure 1). The maximum faradic currents of working electrode obtained were 5.75 µA, 8.56 µA, 11.5 µA, 19.5 µA, 39.7 µA, and 87.2 µA, respectively (Figure 2). The obtained data show a significant relation between the ionic strength of the buffer and the faradic current of working electrode.

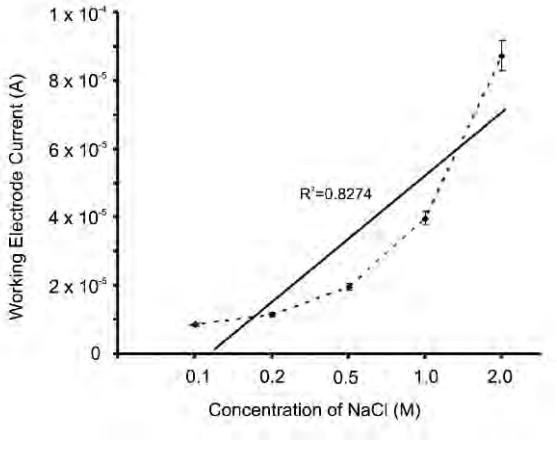

Figure 2. The aptasensor response to the different concentrations of NaCl. The maximum faradic currents of working electrode were obtained 5.75 µA, 8.56 µA, 11.5 µA, 19.5 µA, 39.7 µA, and 87.2 µA in the presence of 0.0, 0.1 M, 0.2 M, 0.5 M, 1.0 M, and 2.0 M NaCl respectively. The data show almost a linear relation between the NaCl concentration and faradic current of working electrode.

3.2. Effect of different concentrations of codeine phosphate on the response of aptasensor

The fabricated aptasensor was treated by the different concentrations of codeine phosphate (CP) to investigate the behavior of the sensor in the presence of its target. First, a background cyclic voltammetry scan was taken in 0.1 M PBS containing 2 M NaCl in the potential range of +0.4 to +0.9 V and the scan rate of 0.15 V/s. Then, the main scans were taken in the same condition in the presence of 10 nM, 50 nM, 100 nM, 500 nM, and 1000 nM of CP. The obtained data represent significant changes in the maximum faradic current of working electrode by increasing the CP concentration. In the presence of 10 nM of CP, an 11.4 µA faradic current was observed on the working electrode's voltammogram. Similarly, the 19.5 µA, 34.9 µA, 48.7 µA, and 75.5 µA faradic currents were observed, respectively, by adding 50 nM, 100 nM, 500 nM, and 1000 nM of CP to the medium (Figure 3). Based on the obtained data, it can be shown that the aptasensor shows a linear response to increasing concentrations of its specific target.

3.3. Effect of pH on the sensor's response

The ability of the aptasensor for CP detection in a wider range of pH was investigated by taking cyclic voltammetry scans in the presence of a high concentration of CP (10 µM). The background CV scan was taken in 0.1 M PBS containing 2 M NaCl and the main scan was performed in the same condition in the presence of CP. The electrochemical experiments were performed in the 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0 pH ranges. The CV of the working electrode did not show the expected reduction and oxidation peaks of aptamer-attached Juglone in the 5.5 and 6.0 pH ranges. The reduction and oxidation peaks of Juglone were appeared in pH 6.5, and a 35.42 µA faradic current of working electrode was observed. In pH 7.0, the sharpest oxidation and reduction peaks were observed and the maximum faradic current of working electrode reaches to 87.19 µA. By increasing the pH to 7.5, 8.0, 8.5, and 9.0, the maximum currents of working electrode produced were 77.61 µA, 73.79 µA, 35.98 µA, and 7.66 µA, respectively (Figure 4). The obtained data showed that the faradic current of the working electrode (i.e. sensitivity of the sensor) also started to reduce by rising pH. It means that, aptamer attached Juglone can only reach its maximum working sensitivity at pH 7.0.

4. Discussion

According to the obtained data, the maximum faradic current of working electrode has been increased by increasing the ionic strengths in the medium [14]. Although the adequate ionic strength of buffers is an essential parameter for electrochemical experiments [14], there are some limitations for the presence of large ionic concentrations in electrochemical based aptasensors. The high ionic environments could reduce the electrochemical responses of aptasensors via two main mechanisms. The aptamer-target complex forms by the electrostatic interactions, Van der Waals forces, hydrogen bonding, or a combination of these effects between aptamer and its own target [34-38].

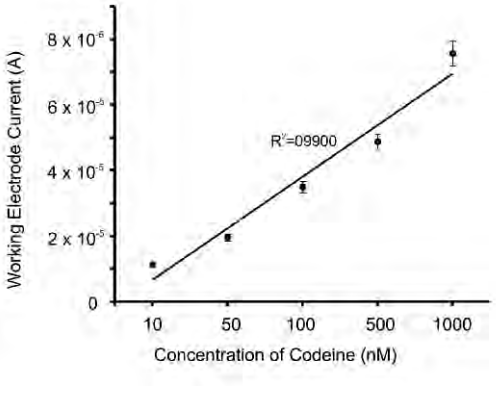

Figure 3. The fabricated aptasensor was used for detection of different concentrations of codeine. The obtained data demonstrated the linear response of sensor to the presence of its own target.

Also, the aptamer folding is being formed by pairing the organic bases with the hydrogen bonding [39]. Since, the concentrated ionic solution affects these forces [40, 41], the affinity of the aptamer to its target may be reduced because of two problems: (1) the organic bases could not pair completely and this phenomenon affects the complete folding of the aptamer. The unfolded aptamer could not have an effective affinity to its own target and this may lead to a reduction in the response of aptasensors [8, 14-16]. (2) The same effects may also reduce the affinity of the aptamer (if not affected) towards the target in high salt concentrations. By reducing the folding status of aptamer and aptamer-target complex formation, the faradic current of the working electrode decreases and the sensor would not work correctly. In brief, increasing the ionic concentration of buffers can have some limitations for electrochemical based aptasensors and these limitations are independent from the redox molecule.

The response of the fabricated sensor to different concentration of the codeine had been described by the previously published mechanism [33]. In the presence of higher concentrations of codeine, more complexes of aptamer-target are being formed and the faradic current of working electrode increases. The increase in response continues to reach a plateau which indicates the saturation of all immobilized aptamers by the target molecules. In another word, the fabricated aptasensor shows a linear response to the presence of different concentrations of its own target, i.e. codeine (Figure 3).

As mentioned before, pH is an important problem in the analytical experiments. The obtained data show that the sensor works accurately only at 7.0 to 8.0 pH ranges. Despite of Juglone ability to show electro-activity in a wide range of pH [22, 23], the aptasensor did not showed enough sensitivity. This phenomenon may occurred by the influence of pH not only on the aptamer-target complex formation but also on the aptamer folding. However, the quinone based redox molecules have potential usage in a wide pH ranges and more experiments with different molecules are needed to be done in future works [22, 23].

5. Conclusion

Despite of the sensitivity of the aptasensors to large concentration of ions in the environment, the Juglone was used successfully in a 2 M NaCl containing PBS buffer. Therefore, the ability of this redox molecule in a high chloride containing solution is a considerable result. As mentioned before, Ferrocene which is a chloride sensitive redox molecule, can be replaced by Juglone as an appropriate alternative attached redox molecule in the RNA or DNA-based biosensors for usage in the chloride containing buffers and solutions. Another main point is the pH dependency of the aptamer folding and aptamer-target complex formation, and should be considered more carefully in selecting redox molecules for aptasensor designing.

Acknowledgment

The authors would like to thank the Research Center for Pharmaceutical Nanotechnology and vice chancellor for research of Tabriz University of medical sciences, IRAN, for financially supporting

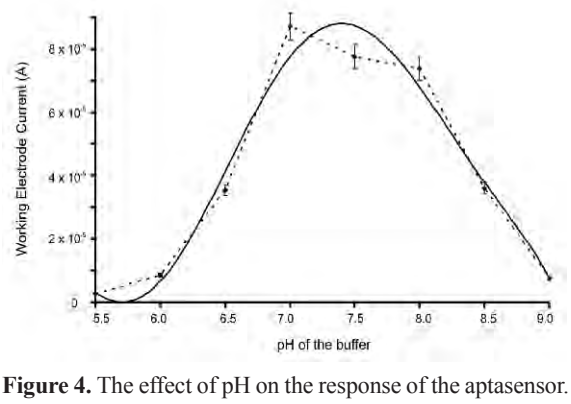

Figure 4. The effect of pH on the response of the aptasensor. The aptasensor was used to detect codeine in the different pH ranges, i.e. 5.5 to 9.0. The obtained data show an optimum response of the sensor only in pH range of 7- 8.

this project as a part of Ph.D. thesis (Mehdi Saberian). Also we would like to thank Exir Pharmaceutical Co., IRAN, for providing codeine phosphate.

References

[1] Tadasi PS. A pH curve of human resting saliva sampled with a small paper slip and its medical application. *Pathophysiol* 2002; 8: 283-90.

[2] Neyraud E, Bult JHF, Dransfield E. Continuous analysis of parotid saliva during resting and short-duration simulated chewing. *Arch Oral Biol* 2009; 54: 449-56.

[3] Koff SG, Paquette EL, Cullen J, Gancarczyk KK, Tucciarone PR, Schenkman NS. Comparison between lemonade and potassium citrate and impact on urine pH and 24-hour urine parameters in patients with kidney stone formation. *Urol* 2007; 69: 1013-6.

[4] Eisner BH, Porten SP, Bechis SK, Stoller ML. Diabetic kidney stone formers excrete more oxalate and have lower urine pH than nondiabetic stone formers. *J Urol* 2010; 183: 2244-8.

[5] Chang IH, Lee YT, Lee DM, Kim TH, Myung SC, Kim YS. Metabolic syndrome, urine pH, and timedependent risk of nephrolithiasis in Korean men without hypertension and diabetes. *Urol* 2011; 78: 753-8.

[6] Guyton AC, Hall JE. *Textbook of medical physiology. Philadelphia:* W.B. Saunders Company, 2000; pp. 738-53.

[7] Song S, Wang L, Li J, Fan C, Zhao J. Aptamer-based biosensors. *TrAC-Trend Anal Chem* 2008; 27: 108-17.

[8] Lee JO, So HM, Jeon EK, Chang H, Won K, Kim YH. Aptamers as molecular recognition elements for electrical nanobiosensors. *Analyt Bioanal Chem* 2008; 390: 1023-32.

[9] Tombelli S, Minunni M, Mascini M. Analytical applications of aptamers. *Biosens Bioelectro* 2005; 20: 2424-34.

[10] Velasco-Garcia MN. Optical biosensors for probing at the cellular level: a review of recent progress and future prospects. *Semin Cell Dev Biol* 2009; 20: 27-33.

[11] Electrochemical biosensors: recommended definitions and classification (Technical Report). *Pure Appl Chem* 1999; 71: 2332-48.

[12] Wang J. Electrochemical biosensors: towards point-of-care cancer diagnostics. *Biosens Bioelectron* 2006; 21: 1887-92.

[13] Odenthal KJ, Gooding JJ. An introduction to electrochemical DNA biosensors. *Analyst* 2007; 132: 603-10.

[14] Xiao Y, Lai RY, Plaxco KW. Preparation of electrode-immobilized, redox-modified oligonucleotides for electrochemical DNA and aptamer-based sensing. *Nat Protoc* 2007; 2: 2875-80.

[15] Ferapontova EE, Olsen EM, Gothelf KV. An RNA aptamer-based electrochemical biosensor for detection of theophylline in serum. *J Am Chem Soc* 2008; 130: 4256-8.

[16] Baker BR, Lai RY, Wood MS, Doctor EH, Heeger AJ, Plaxco KW. An electronic, aptamer-based small-molecule sensor for the rapid, label-free detection of cocaine in adulterated samples and biological fluids. *J Am Chem Soc* 2006; 128: 3138-9.

[17] Wang X, Dong P, Yun W, Xu Y, He P, Fang Y. A solid-state electrochemiluminescence biosensing switch for detection of thrombin based on ferrocene-labeled molecular beacon aptamer. *Biosens Bioelectron* 2009; 24: 3288-92.

[18] Li Y, Qi H, Peng Y, Gao Q, Zhang C. Electrogenerated chemiluminescence aptamer-based method for the determination of thrombin incorporating quenching of tris(2,2'-bipyridine)ruthenium by ferrocene. *Electrochem Commun* 2008; 10: 1322-5.

[19] Hurvois JP, Moinet C. Reactivity of ferrocenium cations with molecular oxygen in polar organic solvents: decomposition, redox reactions and stabilization. *J Organomet Chem* 2005; 690: 1829-39.

[20] Prins R, Korswagen AR, Kortbeek AGTG. Decomposition of the ferricenium cation by nucleophilic reagents. *J Organomet Chem* 1972; 39: 335-44.

[21] Chatterjee M, Rokita SE. The role of a quinone methide in the sequence specific alkylation of DNA. *J Am Chem Soc* 1994; 116: 1690-7.

[22] Ngameni E, Tonle IK, Nanseu CP, Wandji R. Voltammetry study of 2-Hydroxy-3-isopropenyl -1,4-naphthoquinone using a carbone paste electrode. *Electroanal* 2000; 12: 847-52.

[23] Jahan D, Raoof B, Golabi SM. Electrochemical properties of carbon-paste electrodes spiked with some 1,4-naphtoquinone derivatives. *Bull Chem Soc Jpn* 1995; 68: 2253-61.

[24] Paulsen MT, Ljungman M. The natural toxin juglone causes degradation of p53 and induces rapid H2AX phosphorylation and cell death in human fibroblasts. *Toxicol Appl Pharm* 2005; 209: 1-9.

[25] March G, Nool V, Piro B, Reisberg S, Pham MC. Nanometric layers for direct, signal-on, selective, and sensitive electrochemical detection of oligonucleotides hybridization. *J Am Chem Soc* 2008; 130: 15752-3.

[26] Win MN, Klein JS, Smolke CD. Codeine-binding RNA aptamers and rapid determination of their binding constants using a direct coupling surface plasmon resonance assay. *Nucleic Acids Res* 2006; 34: 5670-82.
[27] Online Source: http://psiweb.unl.edu/cahoon/files/phosphate%20buffer.pdf. Available at 1/28/2012.
[28] Online Source: http://en.wikipedia.org/wiki/Henderson%E2%80%93Hasselbalch_equation. Available at 1/28/2012.
[29] Po HN, Senozan NM. The Henderson-Hasselbalch equation: Its history and limitations. *J Chem Educ* 2001; 78: 1499-503.
[30] El-Deab MS, Ohsaka T. Molecular-level design of binary self-assembled monolayers on poly-crystalline gold electrodes. *Electrochim Acta* 2004; 49: 2189-94.
[31] Merrill DR, Stefan IC, Scherson DA, Mortimer JT. Electrochemistry of gold in aqueous sulfuric acid solutions under neural stimulation conditions. *J Electrochem Soc* 2005; 152: 212-21.
[32] Wink T, Van-Zuilen SJ, Bult A, Van-Bennekom WP. Self-assembled monolayers for biosensors. *Analyst* 1997; 122; 43-50.
[33] Saberian M, Hamzeiy H, Aghanejad A, Asgari D. Aptamer-based nanosensors: juglone as an attached-redox molecule for detection of small molecules. *Bioimpacts* 2011; 1: 31-6.
[34] Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. *Nat* 1990; 346: 818-22.
[35] Ellington AD, Szostak JW. Selection in vitro of single-stranded DNA molecules that fold into specific ligand-binding structures. *Nature* 1992; 355: 850-2.
[36] Hermann T, Patel DJ. Adaptive recognition by nucleic acid aptamers. *Sci* 2000; 287: 820-5.
[37] Tuerk C, Gold L. Systemic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 1990; 249:505-10.
[38] Toulme JJ, Daguer JP, Dausse E. Aptamers: ligands for all reasons. In: Mascini M, (editor). Aptamers in bioanalysis. New Jersey: *John Willy & Sons INC* 2009; pp. 3-30.
[39] Berg JM, Tymoczko JL, Stryer L. Biochemistry. New York: W.H. *Freeman and Company* 2002; 117-42.
[40] Strehlitz B, Stoltenburg G. SELEX and its recent optimisations. In: Mascini M, (editor). Aptamers in bioanalysis. New Jersey: John Wiley & Sons, *INC* 2009;31-61.
[41] Stoltenburg R, Reinemann C, Strehlitz B. SELEX-A (r)evolutionary method to generate high-affinity nucleic acid ligands. *Biomol Eng* 2007; 24: 381-403.