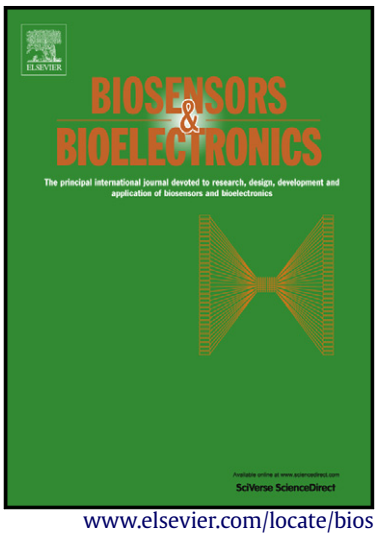

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PII: S0956-5663(13)00902-0
DOI: http://dx.doi.org/10.1016/j.bios.2013.12.031
Reference: BIOS6448

To appear in: *Biosensors and Bioelectronics*

Received date: 3 October 2013
Revised date: 22 November 2013
Accepted date: 11 December 2013

Cite this article as: Saurabh K. Yadav, Bharati Agrawal, Pranjal Chandra, Rajendra N. Goyal, *In vitro* chloramphenicol detection in a *Haemophilus influenza* model using an aptamer-polymer based electrochemical biosensor, *Biosensors and Bioelectronics,* http://dx.doi.org/10.1016/j.bios.2013.12.031

In vitro chloramphenicol detection in a *Haemophilus influenza* model using an aptamer-polymer based electrochemical biosensor

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Abstract

A sensitive and selective electrochemical biosensor is developed for the determination of chloramphenicol (CAP) exploring its direct electron transfer processes in *in-vitro* model and pharmaceutical samples. This biosensor exploits a selective binding of CAP with aptamer, immobilized onto the poly-(4-amino-3-hydroxynapthalene sulphonic acid) (p-AHNSA) modified edge plane pyrolytic graphite. The electrochemical reduction of CAP was observed in a well-defined peak. A quartz crystal microbalance (QCM) study is performed to confirm the interaction between the polymer film and the aptamer. Cyclic voltammetry (CV) and square wave voltammetry (SWV) were used to detect CAP. The *in-vitro* CAP detection is performed using the bacterial strain of *Haemophilus influenza*. A significant accumulation of CAP by the drug sensitive *H. influenza* strain is observed for the first time in this study using a biosensor. Various parameters affecting the CAP detection in standard solution and in *in vitro* detection are optimized. The detection of CAP is linear in the range of 0.1 to 2500 nM with the detection limit and sensitivity of 0.02 nM and 0.102 μA/nM, respectively. CAP is also detected in the presence of other common antibiotics and proteins present in the real sample matrix, and negligible interference is observed.

Keywords: *In vitro* detection; Aptamer; *Haemophilus influenza*; Chloramphenicol, Voltammetry

1. Introduction

Chloramphenicol (CAP) is a broad spectrum antibiotic, which inhibits the activity of both Gram-positive and Gram-negative bacteria (Wang et al., 1999). It is an antimicrobial drug which was first obtained from the culture of a soil bacterium *Streptomyces venezuelae* and was chemically synthesized in 1948 (Alemu and Hlalele, 2007). It is the drug of first choice for the treatment of vancomycin-resistant *Enterococcus*, tetracycline-resistant *Vibrio cholerae* and the patients having severe penicillin or cephalosporin allergy. Although CAP is clinically important, it has some serious harmful effects which form the basis of CAP detection in various clinical, environmental, and pharmaceutical samples. Many countries such as USA, Canada, and China have banned the use of CAP in the food producing animals. The United States Food and Drug administration has decided the "minimum required performance limit" (MRPL) of CAP as 0.3 μg kg^{-1} for the detection of its residues in food products (Pilehvar et al., 2012a; Karaseva et al., 2012). Thus, it is interesting and extremely important to develop a simple, sensitive, and selective method for CAP detection in the *in vitro* and real sample.

CAP has been detected using various techniques such as; gas chromatography-mass spectrometry (Li et al., 2006), liquid chromatography-tandem mass spectrometry (Forti et al., 2005), capillary electrophoresis with amperometric detection (Jin et al., 2000), enzyme immunoassay (Xu et al., 2012) and electrochemical sensors using variety of unmodified and modified electrodes (Pilehvar at al., 2012b; Wei et al., 2011; Borowiec et al., 2013). An aptasensor has also been reported in literature for the determination of chloramphenicol (Yan et al., 2012). A short review dealing with electrochemical and immunosensing strategies for the detection of CAP has also appeared in the literature (Zaidi, 2013). Recently a single wall carbon nanohorns –TiO_2 – porphyrin modified electrode has also been used for the determination of CAP and sandwitch nano structure of the electrode has been found to exhibit

good analytical performance (Tu et al. 2012). Although these methods can be used to detect CAP but they require multiple steps fabrication and long analysis time, thus limited in their real applications. Also, the reported immunosensors for the detection of CAP require animal models for the antibody production, hence are expensive. The antibodies used in the immunosensors fabrication can be easily denatured and hence affect its long term storage. Furthermore, the reported methods are not been extended for the *in vitro* CAP detection using bacterial cultures. Thus, it is desirable to develop a single step, sensitive, selective, quick, less expensive, and easily fabricable biosensor for CAP detection. To achieve a robust and simple detection method for CAP, an aptamer is one of the candidate probe materials because it has been widely used to detect important clinical molecules with different fabrication approaches (Chandra et al., 2013).

A polymer modified sensor is the class of chemically modified electrodes which comprise a novel approach to electrode system and are very useful in basic and applied electrochemical investigations (Rahman et al., 2008; Amare and Admassie, 2012a; Geto et al., 2012; Amare and Admassie, 2012b; Amare et al., 2011). Compared to the other electrodes, edge plane surface of pyrolytic graphite (EPPG) has been widely used due to its wide potential window and minimum possibility to show deteriorated response as a result of surface fouling. Hence, we used a poly-(4-amino-3-hydroxynapthalene sulphonic acid) (p-AHNSA) coated EPPG for the quantitative detection of CAP.

The resistance of CAP in different bacterial species has a significant impact on the public health and clinical implications, particularly in the areas of the world where these bacterial strains are endemic. The availability of rapid and sensitive screening methods for the detection of CAP resistance is extremely important. In the present study, we fabricated a simple and efficient aptamer based biosensor for the direct *in vitro* determination of CAP. Electro-polymerization of AHNSA onto the EPPG was carried out to increase the surface

area for the aptamer interaction due to the electrostatic interaction between sulphonic acid group and amino group, providing significant signal amplification, for the CAP detection. *H. influenza* was used to investigate the *in vitro* CAP detection ability of the biosensor. The experimental parameters affecting the CAP detection in standard solution and in *in* vitro detection were optimized and the detection limit of CAP was determined. The drug resistant and sensitive *H. influenza* strain was identified using the fabricated biosensor based on the CAP accumulation.

2. Experimental

2.1. Instrumentation

The electrochemical measurements were carried out using a BAS (West Lafayette, USA) CV-50W voltammetric analyzer. Voltammetric cell used was a single compartment glass cell containing aptamer immobilized polymer modified edge plane pyrolytic graphite sensor, Ag/AgCl (3 M NaCl) as reference electrode (model BAS MF-2050 RB-5B) and a platinum wire as counter electrode. The pH of buffer solutions was measured using pH meter (model Eutech pH 700). The pyrolytic graphite piece was obtained from Pfizer Inc., USA as a gift. The micro structure of working electrode surface was characterized using field emission scanning electron microscopy (Quanta 200 FE-SEM).

2.2. Chemicals and reagents

The aptamer sequence (Mehta et al., 2011) used in the present study was procured from Genxbio Health Sciences, India, and had the sequence:

(5′-NH_2-ACTTCAGTGAGTTGTCCCACGGTCGGCGAGTCGGTGGTAG-3′).

CAP and 4-amino-3-hydroxynaphthalene sulfonic acid were obtained from Sigma–Aldrich Inc., USA and used as received without further purification. Phosphate buffers of different pH and ionic strength (μ = 1.0 M) were prepared by the reported method (Christian and

Purdy, 1959) using analytical grade chemicals. The double distilled water was used to prepare the solutions.

2.3. Fabrication of aptamer/p-AHNSA/EPPG biosensor

Prior to modification, the edge surface of pyrolytic graphite was cleaned by rubbing it on an emery paper (P-400), washed with double distilled water and then dried. The electrochemical polymerization of AHNSA on the EPPG was carried out using CV in a solution containing 2 mM of AHNSA in 0.1 M nitric acid solution. The cyclic voltammograms were recorded by scanning the potentials between - 0.8 V and + 2.0 V at a scan rate of 0.1 Vs^{-1} for 15 scans. The film obtained was rinsed with double distilled water. In the next step electrode was cycled between - 1.0 and + 1.0 V at a scan rate of 0.1 Vs^{-1} in 0.5 M H_2SO_4 until a stable voltammogram is obtained. The polymer modified sensor was then rinsed with water, and dried under nitrogen. Next, the immobilization of aptamer on to the polymer modified EPPG was carried out by the spin coating technique. The appropriate aptamer concentration prepared in Tris EDTA buffer (pH 8.0) was coated on the polymer film and left over night. Thereafter, the modified electrode was washed with the same buffer followed by 0.1 M mercaptoethanol for 2 min to remove any unbound aptamer. The final fabricated electrode is termed as aptamer/p-AHNSA/EPPG biosensor.

2.4. Voltammetric procedure

The stock solution of 50 μM of CAP was prepared by dissolving the required amount in double distilled water. For electrochemical experiments, a required amount of the stock solution was further diluted by phosphate buffer solution to keep the ionic strength of buffer as 0.5 M. For the detection of CAP, the aptamer/p-AHNSA/EPPG was dipped in CAP solutions of various concentrations for appropriate time at room temperature and the electrochemical response of the biosensor was checked in a deoxygenated phosphate buffer to avoid the oxygen interference. The surface of aptamer/p-AHNSA/EPPG was cleaned after

each run by applying a potential of -100 mV for 120 s to remove the adsorbed CAP from the surface.

2.5. Preparation and processing of H. influenza bacterial cultures

Various strains of *H. influenzae* were cultured in the laboratory on a chocolate agar medium with added X(Hemin) & V(NAD) factors at 37°C in an enriched CO_2 incubator. The broth cultures were prepared in supplemented brain-heart infusion (sBHI) broth. The bacterial cultures were stored frozen in skim milk at - 80°C when not in use and inoculated onto solid media prior to each experiment. The bacterial cultures were grown to mid-log phase in sBHI and after centrifugation cells were treated with 10 μM CAP for different time intervals. After this step cells were centrifuged and CAP was assayed in the supernatant using the developed aptamer sensor. Before each experiment bacterial cells were counted using a hemocytometer under optical microscope.

3. Results and discussion

3.1. Characterization of the preparation process of the aptasensor

The developed biosensor was characterized using electrochemical techniques and quartz crystal microbalance studies. Figure 1 shows the repetitive cyclic voltammograms of 2 mM AHNSA in a 0.1 M nitric acid solution at bare EPPG. In the first scan AHNSA monomer shows two anodic peaks (E_p ~ +146 and ~ +415 mV) and three cathodic peaks (E_p ~ +44.5, ~ +331.9 and ~ +1384 mV). In the consecutive scans an additional anodic peak began to appear at 1706 mV. Upon continuous scanning, the peak current of these peaks increases indicating the formation of the conducting electrode surface. The peaks having peak potential 1384 and 1706 mV did not increase much as compared to other peaks as they were not related to the oxidation of 4-amino-3-hydroxynapthalene sulphonic acid (Amare et al., 2011).

After the polymerization step, the electrode was cycled in 0.5 M H_2SO_4 until a stable voltammogram was obtained. A typical cyclic voltammogram of p-AHNSA/EPPG observed in 0.5 M H_2SO_4 is presented in inset of Fig. 1. and shows three redox couples a-a', b-b' and c-c', which confirms the successful formation of the polymer film at the electrode surface. The polymer modified surface was then characterized by FE-SEM to confirm the formation of polymer film. A comparison of FE-SEM images of bare and polymer modified EPPG is presented in the Supplementary Information (SI 1) section. The morphology of the polymer modified EPPG surface shows layer of polymer and was completely different as compared to the bare EPPG surface. This was due to the coverage of the electrode surface by polymer film. These results clearly indicate that p-AHNSA has been successfully coated on the electrode surface.

Figure 1 here

Further studies were performed through QCM experiments to confirm the interaction between aptamer and p-AHNSA/EPPG surface based on the frequency change. The plot observed for change in Δf with time is presented in Supplementary Information (SI 2). In this case, the decrease in frequency reaches a complete steady state after about 45 min, with an overall frequency change (Δf) of 0.76 Hz, corresponding to a mass change (Δm) of 8.34 ± 0.31 10^{-10} g based on the following equation $\Delta m = \Delta f \times 5.608$ (ng/cm^2) / Hz x 0.196 cm^2. In this equation, Δf is the change in frequency, 5.608 (ng/cm^2)/ Hz is the sensitivity factor calculated from the physical constant for quartz, and 0.196 cm^2 is the electrode area (Lee, et al. 2010). The number of molecules per area for the aptamer on the surface was determined to be 1.8×10^{-10} mol/cm^2. These results confirm that the aptamer has successfully interacted with the p-AHNSA/EPPG surface.

3.2. Electrochemical behaviour of CAP at aptamer/p-AHNSA/EPPG

The cyclic voltammetric response of the aptamer/p-AHNSA/EPPG biosensor was studied in phosphate buffer of pH 7.2. No oxidation or reduction peak was observed in this case and hence, it was concluded that the polymeric layer was not electroactive in the neutral medium. Similar observation is also reported in the literature (Amare et al., 2011). The voltammetric studies of CAP were carried out by dipping the aptamer/p-AHNSA/EPPG electrode in CAP solutions. The fabrication of sensor, chemical structure of AHNSA and CAP and mechanism of biosensing of CAP by the sensor is shown in Fig 2. Initially the polymeric layer is formed at the surface of EPPG as shown in Fig 2 (A). The aptamer is then attached to the polymer modified surface of EPPG, which then captures CAP. The reduction of CAP then occurs to give voltammetric signal. The chemical structures of CAP and the monomer 4-amino-5-hydroxynaphthalene sulphonic acid are presented in Fig 2 (B) and (C) respectively. The *In vitro* sensing mechanism of CAP resistant and CAP sensitive *H. influenza* is shown in (D). In the case of CAP resistant *H. Influenza*, the concentration of CAP in the supernatant is much higher than CAP sensitive *H. Influenza*. Cyclic voltammetry (CV) is the most effective and versatile electro-analytical technique available for the mechanistic study of redox systems, hence, initially, cyclic voltammograms were recorded for CAP using aptamer/p-AHNSA/EPPG at pH 7.2. Fig. 3 shows the CV of 2 μM CAP solution at aptamer/p-AHNSA/EPPG. In the first negative scan only one reduction peak I_c is appeared at - 660 mV, which corresponds to the electrochemical reduction of NO_2 group of CAP to hydroxyl amine (Alemu and Hlalele, 2007; Zhang et al., 2000). In the reverse scan an oxidation peak (II_a) is observed at - 62 mV which formed a quasi-reversible couple with peak II_b (- 112 mV) observed in the cathodic scan. To confirm the quasi-reversible nature of the redox couple (II_a/II_b), effect of sweep rate was studied. It was found that in the low sweep rate range (10-150 mVs^{-1}), the peak potentials were practically constant with ratio of peak currents as 1.0. However, at higher sweep rates, the ΔEp value increased indicating quasi-

reversible nature of the redox couple. To confirm that peak II_a is not due to the oxidation of CAP, voltammogram was also recorded by initiating the sweep in the positive direction. No oxidation peak was noticed in this case, it was necessary to record peak I_c to see other peaks (II_a, II_b). When number of cycles increased, peak current of peaks II_a and II_b increased, whereas peak current of irreversible peak (I_c) decreased indicating that product of irreversible reduction is responsible for the formation of redox couple (II_a, II_b). The 4e, $4H^+$ reduction of the nitro group of CAP to hydroxylamine has been well documented at the mercury electrode in literature (Fossdal and Jacobson, 1977). Further oxidation of hydroxyl amine derivative in 2e, $2H^+$ reaction has been found to give nitroso derivative. The detailed mechanism for the reduction of CAP has already been reported in literature (Alemu and Hlalele, 2007).

To determine the nature of the electrode reaction of peak Ic, sweep rate studies were performed in the range 25 - 250 mVs^{-1}. The peak potential of peak Ic was found to shift to more negative potentials with increase in sweep rate. The linear dependence of Ep on log v can be represented by the relation:

E_p (mV) = 56.25 log v + 535.58 (R^2 = 0.973)

and confirmed the irreversible nature of peak Ic. On the other hand, the peak current was found to increase linearly with increasing sweep rate as shown in inset of Fig. 3. The dependence of scan rate on peak current of CAP is expressed by the equation:

i_p /μA = 1.766 [v] - 16.63

with the correlation coefficient of 0.979, where i_p is the peak current and v is scan rate in mVs^{-1}. To further confirm the mechanism of electrode reaction, a graph was plotted between log i_p and log v. The slope of this graph was found to be ~ 1.07 confirming that the electrode process is adsorption controlled (Gilbert et al., 2009; Wopshall and Shain, 1967). Control experiments were performed at bare EPPG and p-AHNSA/EPPG surfaces for 2.0 μM CAP to confirm the importance of aptamer modified electrode in the CAP detection. At the bare

EPPG and p-AHNSA/EPPG, the current signal observed was substantially lower as compared to the aptamer/p-AHNSA/EPPG modified surface (11.8, 24 and 120 μA respectively). A comparison of cyclic voltammogram of CAP at the background and p-AHNSA/EPPG is shown in SI-3. The E_p of main reduction peak (I_c) of CAP at these surfaces was -585, -580 and -660 mV respectively. This was possible due to the successful capturing of CAP due to the aptamer present on the sensing surface. Hence, a stable and higher signal was observed at aptamer/p-AHNSA/EPPG modified surface. The low current response at bare and polymer modified sensors is also likely due to non-specific adsorption response of CAP.

The optimized square wave voltammetric conditions to record square wave voltammograms were as follows: initial (*E)*: - 400 mV, final (*E*): - 1000 mV, square wave amplitude (E_{sw}): 25 mV, potential step (*E*): 4 mV, square wave frequency (*f*): 15 Hz. The experimental parameters for the analysis of CAP with the aptamer/p-AHNSA/EPPG were optimized in terms of the concentration of immobilized aptamer, temperature, and reaction times, where the CAP concentration was kept constant and were used throughout the studies (Supplementary information; SI-4).

Figure 2, 3 here

3.3. Analytical performance of aptamer/p-AHNSA/EPPG

To quantitatively assess the detection limit and dynamic range of CAP aptasensor, square wave voltammetry (SWV) was employed to determine the dependence of the peak current on the concentration of CAP. The sensor probe was dipped into the buffer (blank/no CAP) and square wave voltammogram was recorded. No reduction peak was observed because no CAP was captured by the aptamer/p-AHNSA/EPPG. Thereafter, the aptamer/p-AHNSA/EPPG was reacted with different concentrations of CAP and then square wave voltammograms were recorded, where a well defined reduction peak at peak potential ~ -590 mV was observed. Fig. 4 shows the square wave voltammograms, recorded using aptamer/p-

AHNSA/EPPG sensor after capturing various concentrations of CAP. A calibration plot (Fig. 4 inset) was obtained with the dynamic range between 0.1 – 2500 nM. The linear regression equation is expressed as follows:

i_p /μA = 0.102 [CAP nM] + 19.15

with a correlation coefficient of 0.995. The detection limit for CAP was determined to be 0.02 nM. (R.S.D. < 5%) based on the measurements performed five times for the standard deviation of the blank solution (95% confidence level, *k = 3, n = 5*). This detection limit is ~ 80 times lower as compared to the method recently reported for CAP detection using apta sensing (Pilehvar et al., 2012b). The detection limits of recently reported methods for CAP determination at other electrodes were, 1 μM at gold electrode (Pilehvar et al., 2012a), 0.59 μM at nitrogen doped graphene nanosheets (Borowiec at al., 2013), 0.34 nM at screen printed electrode (Yang et al., 2009), 0.29 nM at aptasensor based electrode (Yan et al., 2012) and 2 nM at molecularly imprinted polymer modified carbon paste electrode (Alizadeh et al., 2012). These detection values clearly indicate that the present sensor is more sensitive in comparison to recently reported sensors. The impact of other parameters such as pH of the supporting electrolyte and square wave frequency on the electrochemical response of aptamer/p-AHNSA/EPPG was also studied for the detection of CAP. These parameters significantly affected the sensor response and the results have been shown in Supplementary Information (SI-5).

Figure 4 here

3.4. Real sample analysis

In order to evaluate the practical applicability of the proposed method, three commercial medicinal samples containing CAP were examined. The CAP containing commercial medicinal tablets, viz. paraxin-250 (Piramal Healthcare Ltd. Baddi, Himanchal Pradesh), chloromycetin 250 mg and 500 mg capsules (Pfizer Ltd., Navi, Mumbai), were

purchased from the local market of Roorkee. Table 1 shows that the aptamer/p-AHNSA/EPPG sensor can detect CAP in various pharmaceutical samples irrespective of the type and formulation. We also analysed the CAP detection in the urine samples after spike and recovery method (figure not shown). For this purpose, the biosensor was used to detect the CAP concentration in 10-fold diluted urine sample. Under the optimized conditions, CAP was detected in the similar dynamic range as in the blank buffer. The linear regression equation for CAP detection in urine is expressed as follows: i_p /μA = 0.097 +18.01 [CAP nM] with a correlation coefficient of 0.987. The recovery in all the cases was more than 96.4 % and the relative standard deviations were less than 5 %. The results based on the recovery obtained clearly indicate that the developed sensor can detect CAP from the complex urine matrix. Interestingly, in medicinal as well as in the urine samples no foreign peak was observed which clearly indicated that other chemical and biomolecules do not interfere in CAP detection. One of the reasons for such non-interference can be explained on the basis of the fact that most of the compounds present in urine matrix are non-reducible in nature. A 3.9 % decrease in the CAP signal, however, was observed which was possibly due to the negligible electrode fouling by protein contents present in the urine sample.

Table 1 here

3.5. In vitro CAP detection H. influenza model

The *in vitro* detection of CAP was performed in the CAP treated *H. influenza* cells supernatant (CAP-s). In a control experiment CAP untreated supernatant was tested, where no CAP reduction peak was observed. This was due to the absence of CAP in the sample solution. In another experiment 30 min incubated CAP-s was analysed using the biosensor, where a clear CAP reduction peak was observed. Interestingly, the peak current was lower as compared to the only 5 min incubated CAP-s. The lower current signal in 30 min can be assigned to the higher CAP uptake with increase in the incubation time. Further, to confirm

the CAP uptake, *H. influenza* cells were treated with CAP with different incubation time from 5 min to 90 min and subsequently the supernatant was analyzed as mentioned above. During this experiment, the number of *H. influenza* cells, the CAP concentration, and the reaction time (between sensor probe and supernatant) were kept constant. The CAP reduction peak current gradually decreased with increase in the incubation time indicating thereby that the *H. influenza* strain under investigation was CAP sensitive (Fig. 5a). When another strain was examined under the similar experimental conditions the CAP concentration did not decrease with time (Fig. 5b). This was because the *H. influenza* was resistant to CAP and hence no CAP uptake was observed in this case. These results are extremely important in diagnosis and therapeutics due to their uniqueness in terms of simultaneous *in vitro* CAP detection and bacterial susceptibility / resistant diagnosis. To the best of our knowledge this is the first report, where CAP detection has been done in an *in vitro* model using an electrochemical biosensor.

Figure 5 here

3.6. Specificity, reproducibility, and stability of aptamer/p-AHNSA/EPPG

To assess the possibility of interference, the SWV response of the aptamer/p-AHNSA/EPPG was measured for tetracycline, kanamycin, chloramphenicol, neomycin, anthraquinone at 100.0 nM. These drugs did not interfere because the CAP selective aptamer could not interact with these drugs and aptamer–drug complex was not observed for these drugs. Also, no foreign peaks appeared in the real sample experiment indicating that other components present in the real sample matrix do not interfere with the sensor probe. These results indicate that proposed aptasensor has an excellent specificity for CAP detection.

The reproducibility of the method was determined by observing the current response at four prepared sensors for the replicate measurements. The good reproducible results were observed with relative standard deviation of only ~ 3.5 % which revealed acceptable

reproducibility of the method. Stability of aptamer/p-AHNSA/EPPG is a major issue due to its application in several pharmacological operations. In order to check the stability of aptamer/p-AHNSA/EPPG, the sensor was stored in pH 7.2 solution. The sensor is found to retain 97 % of its initial current upto 30 days indicating thereby, that the aptamer/p-AHNSA/EPPG sensor has very good stability and life length.

4. Conclusions

A facile, highly sensitive, selective, and stable biosensor for CAP detection using aptamer has been developed. The biosensor surface was successfully characterized using SEM, QCM, and electrochemical techniques. The detection limit of the proposed biosensor was 0.02 nM, which is lower than the previously reported results. A comparison of detection limit of the present sensor with recently reported methods for CAP determination at other electrodes clearly indicate that the present sensor is much more sensitive in comparison to other recently reported sensors. Also, the present biosensor exhibited long term stability up to 30 days and was successfully used to detect CAP in pharmaceutical and urine samples. The CAP biosensor was also successfully applied to detect CAP concentrations in *in vitro Haemophilus influenza* models to monitor the CAP uptake by the various strains of *Haemophilus influenza*. The CAP sensitive *H. influenza* strains were identified by this method and differentiated from the resistant *H. influenza* strains very effectively using an electrochemical biosensor. The strategy described here has many attractive features: simplicity, rapidity, low cost, and no requirement for a specific label (i.e., a fluorescent or reactive moiety), all of which indicate that this could be a useful method for diagnosis of CAP resistant *H. influenza* and other important CAP resistant bacterial isolates.

Acknowledgement

Authors (SKY and BA) are thankful to the CSIR, New Delhi for awarding Senior Research Fellowship. Financial assistance for this work was provided by the CSIR vide grant no. 01/2419/10-EMR-II.

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Table 1. Determination of CAP using aptamer/p-AHNSA/EPPG in different pharmaceutical tablets.

Sample	Stated content (mg)	Detected content* (mg)	Error (%)
Paraxin-250	250	248.7	-0.52
Chloromycetin-250	250	248.3	-0.68
Chloromycetin-500	500	502.8	0.56

* RSD for the determination was < ± 3.1% for n=5.

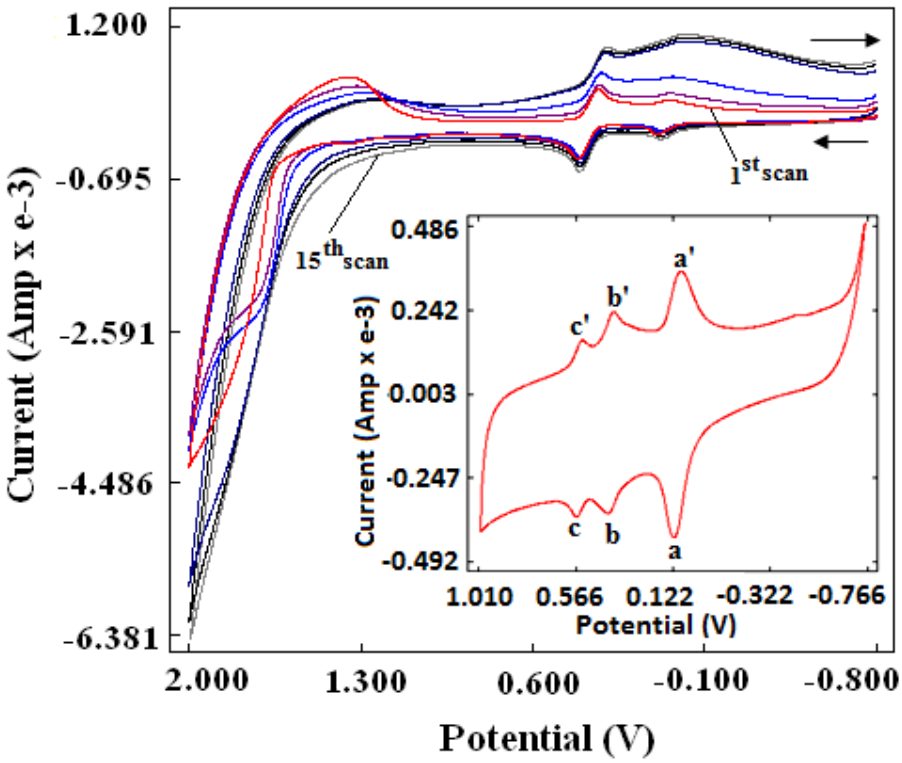

Fig.1: Cyclic voltammograms observed during electro polymerization of AHNSA in 0.1 M HNO_3 at scan rate of 0.1 Vs^{-1}; inset showing the stable cyclic voltammogram of polymer film in 0.5 M H_2SO_4.

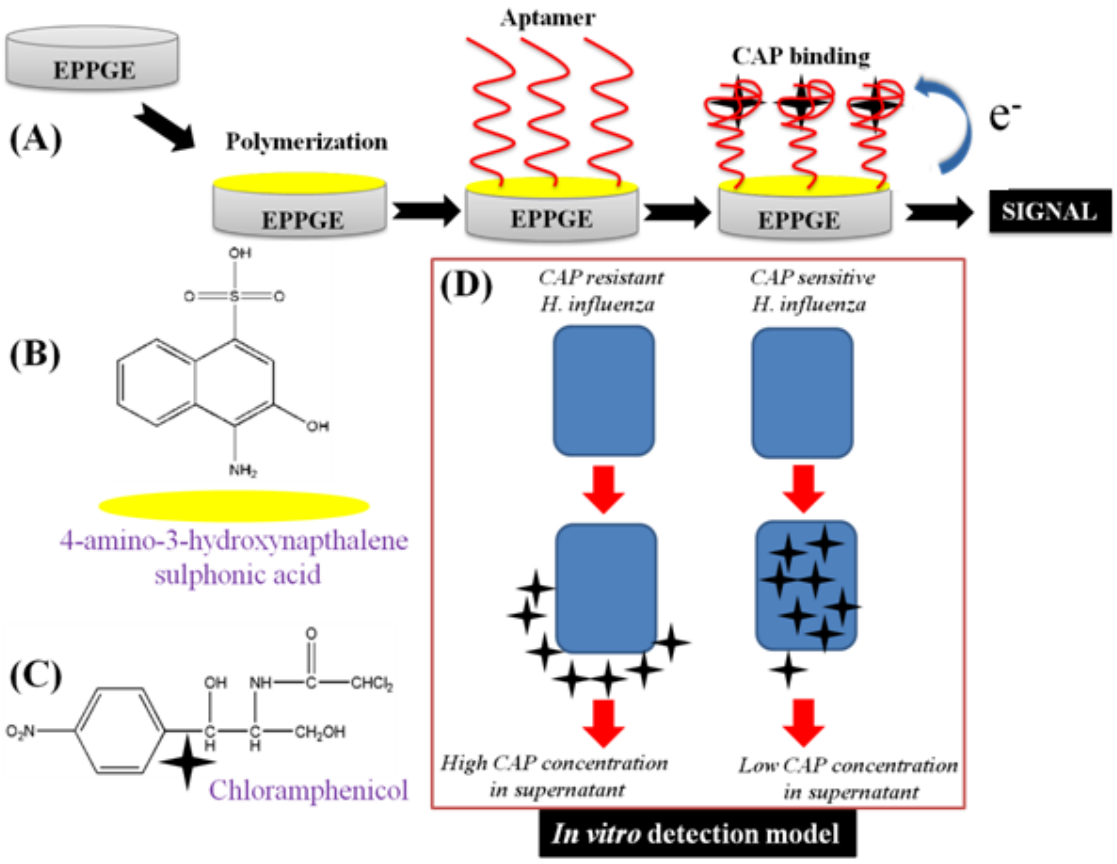

Fig. 2: (A) Schematic representation of the biosensor fabrication, (B) chemical structure of 4-amino-3-hydroxynapthalene sulphonic acid monomer, (C), chemical structure of chloramphenicol, (D) In vitro detection model for CAP detection using *H. influenza*

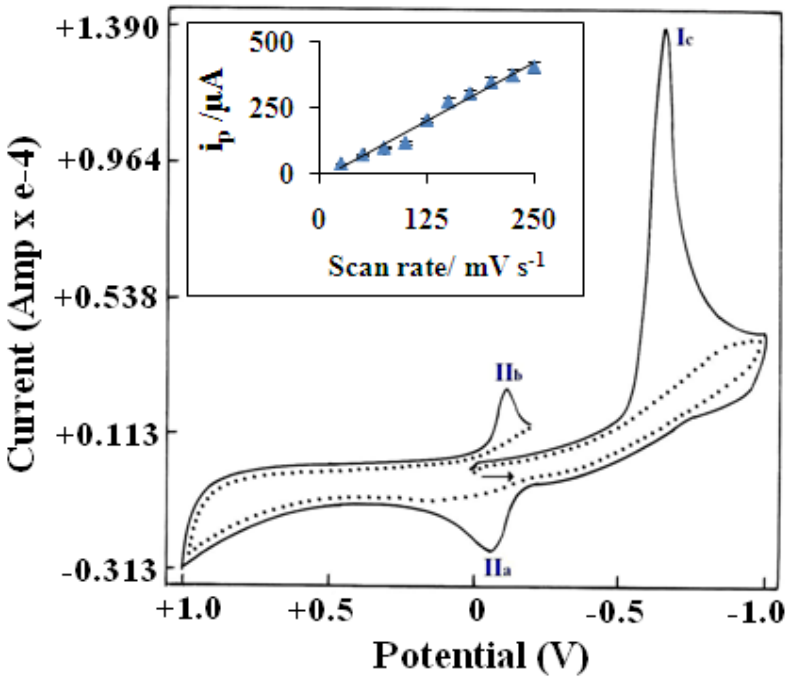

Fig.3: A typical cyclic voltammogram of 2 µM CAP at aptamer/p-AHNSA/EPPG in phosphate buffer of pH 7.20 at a scan rate of 0.1 Vs^{-1}. The background buffer is shown by dotted line. Inset is the graph between peak current and scan rate for peak I_c.

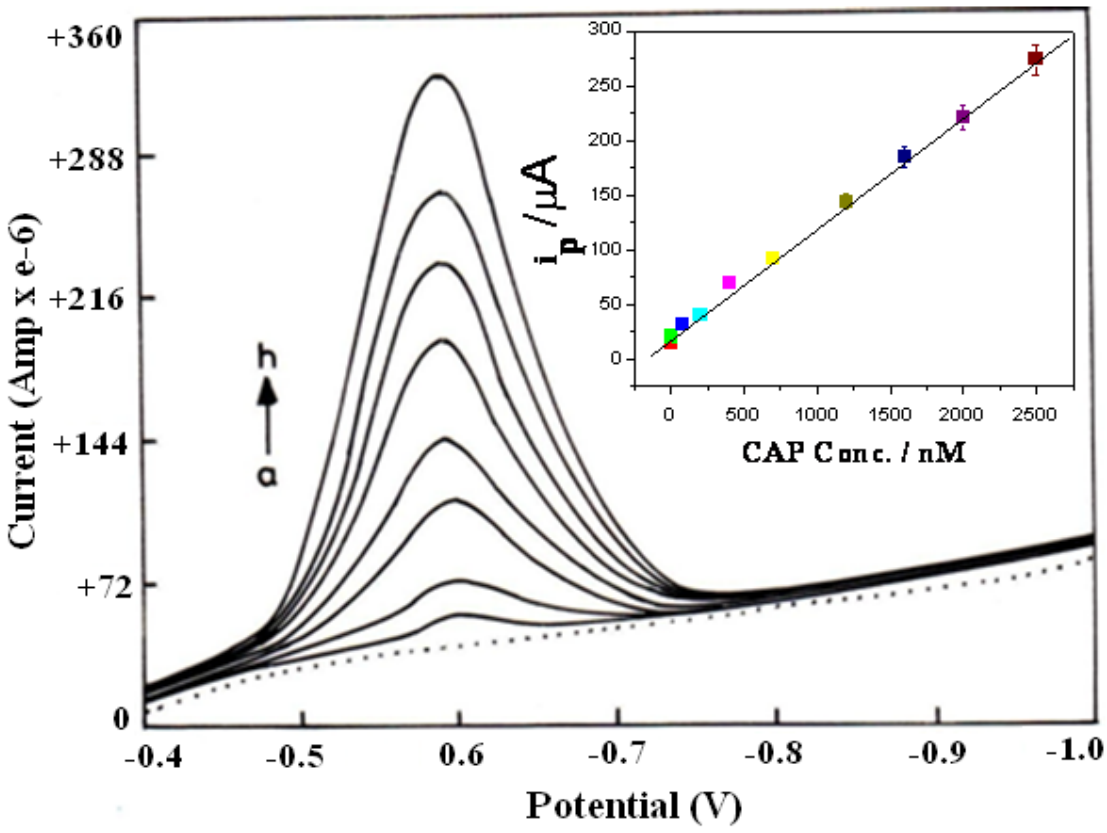

Fig. 4: Square wave voltammograms observed for background phosphate buffer (…) and on increasing the concentration of CAP. Curves were recorded at (a) 0.1, (b) 5, (c) 80, (d) 400, (e) 700, (f) 1600 (g) 2000 and (h) 2500 nM concentrations using aptamer/p-AHNSA/EPPG in phosphate buffer of pH 7.20; inset is the calibration plot.

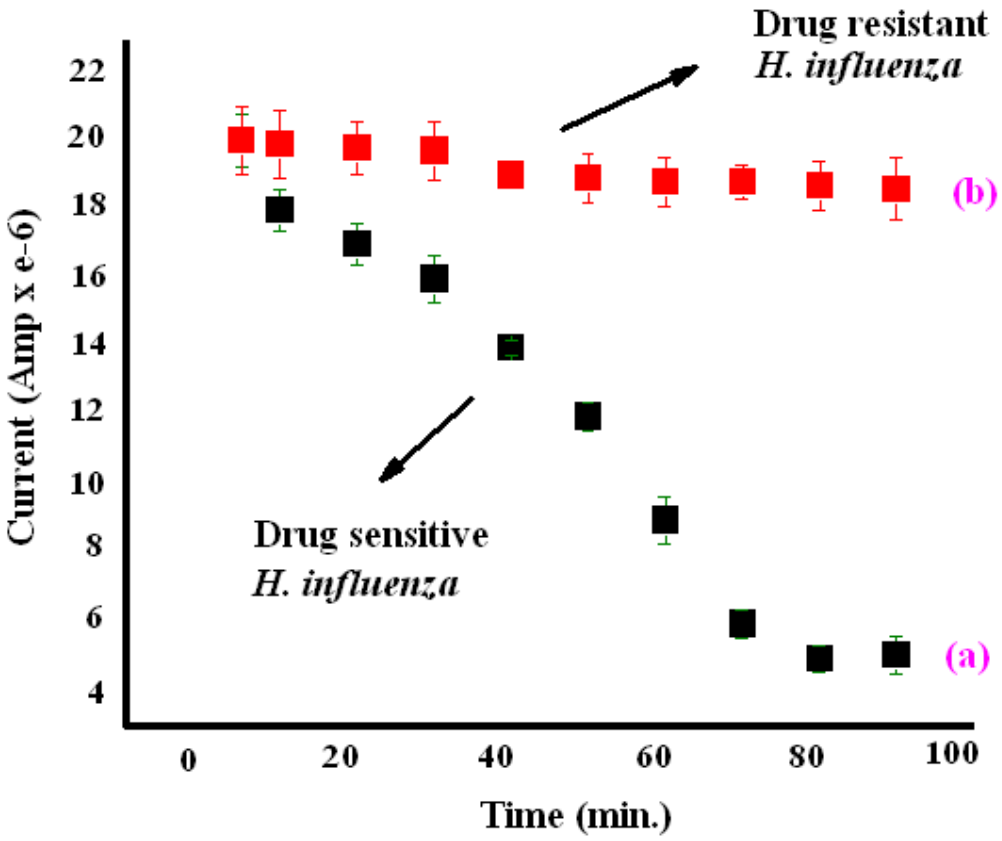

Fig. 5: *In vitro* analysis of CAP in drug sensitive (a) and drug resistant (b) *H. Influenza* isolates. CAP concentration was 5 nM. SWV signals were obtained at different incubation times using the biosensor.

Highlights

An aptamer based biosensor is developed for the determination of chloramphenicol > The sensor is characterized using FE SEM and quartz crystal microbalance > In vitro determination of chloramphenicol is reported in *Haemophilus influenza* model > Significant accumulation of chloramphenicol by the drug sensitive *H. influenza* strain is observed.