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Short Review

Recent Advancement in Various Electrochemical and Immunosensing Strategies for Detection of Chloramphenicol

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The application of Chloramphenicol (CAP), a broad spectrum antibiotic, has been restricted in poultry, aquatic and other food producing animals in EU, Canada and US completely due to its numerous toxic, adverse and fatal side effects in human and veterinary. Hence, there is a constant requirement for accurate, simpler, faster and improved analytical method for its detection. Several methods have been developed and appeared in literature for the detection of CAP. Among all the techniques, the immonosening and electrochemical methods have been considered the foremost methods because of their unique features. The immunosensor show great sensitivity and selectivity, on the other hand low cost, easy operation, fast response time and excellent potential for miniaturization and construction for portable equipment applications make electrochemical sensors as preferred choice. Thus, this mini review brings the various immunosensing and electrochemical strategies applied for the preparation of CAP sensor in last twelve years (from 2000-2013) on one platform.

Keywords: Chloramphenicol, electrochemical methods, immunosensor, antibody, nanostructures,

Abbreviations: CAP, Chloramphenicol; SWCNTs, Single-walled carbon nanotubes; MWCNTs, Multi-walled carbon nanotubes; AUNPs, Gold nanoparticles; MIP, Molecular imprinted polymer; OWLS, Optical waveguide light mode spectroscopy; QCM, Quartz crystal microbalance; HGNs, Hollow gold nanospheres, GCE, Glassy carbon electrode

1. INTRODUCTION

With growing concerns over food safety and the need to increase sample-throughput in analytical testing laboratories, there is a constant requirement for accurate, simpler, faster and improved analytical methods. The complexity of food matrices and the presence of much potential interference, require specific and selective methods of analysis. Chloramphenicol (CAP), a broad spectrum antibiotic, has been extensively used in human and veterinary. However, due to some

potential fatal side effects such as aplastic anemia (a rare but fatal blood disorder), agranulocytosis, and dosage independent suspected carcinogenity in humans, it is used in serious infections only (typhoid fever and meningitis and so on). Consequently, the use of CAP has been strictly restricted in poultry, aquatic and other food producing animals in EU, Canada and US completely. Since, its toxic effects are not dose-dependent but rather related to the hypersensitivity of certain individuals; any detectable amount of this drug is reportable. Recently, European Commission established maximum residue performance limit (MRPL) for CAP detection in food products at 0.3μg/kg [32].

Due to the significance of CAP, numerous techniques and methods have been extensively utilized to detect it including various chromatographic techniques [1], microbiological [2], chemiluminescence [3], chemiluminesence based immunoassays [4], and MIP based method [5]. These methods are routinely used and offer precise and accurate determination of CAP. Among all the techniques, the use of antibody-antigen complex based immunosensors and electrochemical sensors approaches are at the forefront owing to numerous striking and unique features. The immunosensor show great sensitivity and selectivity, on the other hand low cost, easy operation, fast response time and excellent potential for miniaturization and construction for portable equipment applications make electrochemical sensors as preferred choice.

In order to benefit from these techniques, several reports have been appeared in literature focusing on different fabrications and detection principle strategies including amperometric immunosensor [9], composite of single wall carbon nanotube-gold colloids-ionic liquid modified electrodes [21], electrochemical detector with flow injection analysis [25], electrochemically activated carbon fiber microelectrodes [32], application of bare gold electrode [35], have been proposed to determine CAP. Furthermore, due to some complexity, lack of high sensitivity and selectivity towards CAP, several MIP based reports were published using electrochemical sensor. For example, a MIP based nano-Composite Carbon Paste Potentiometric Sensor [24], MIP-Carbon Nanotubes-AuNPs modified electrode [29] were prepared.

The object of this mini review is to provide comprehend details of all the electrochemical and immunoassay methods utilized in last twelve years (from 2000-2013). This review will describe all the methods categorically and has been written in a summary style. The focus of this work is to collect and present all types of electrochemical as well as immunoassay methods which used antibody-antigen complex, Quartz crystal microbalance, piezoelectric and micro-cantilever techniques, utilization of nanostructures, and molecular imprinted polymer formats and applied them into immunosensensing, amperomteric and voltammetric approaches and so on. The whole review has been distributed on various detection principles and sensor fabrication methods as discussed in the following text.

2. DETERMINATION BY IMMUNOSENSOR METHODS

Among numerous electrochemical method used for determination of CAP, immunosensor methods have shown greater amount of interest among scientists because they offer high selectivity and sensitivity. For the preparation of immunosensor, the following types of strategies were reported in the literature.

2.2. Lable free aptasensor

Aptasensor can be defined as nucleic based receptors obtained through a combinatorial selection process known as Systemic Evolution of Ligands by Exponential Enrichment (SELEX).

Very recently, Pilehvar et al. reported a label-free and binding-induced conformational change aptamer sensor providing limit of detection (LOD) of 1.6nM in the presence of thiamphenicol (TAP) and florfenicol (FF). The authors have utilized cyclic voltammetry (CV) and square wave voltammetry (SWV) to observe and characterize the interfacial changes during aptamer immobilization and specific binding affinity between CAP and aptamer.

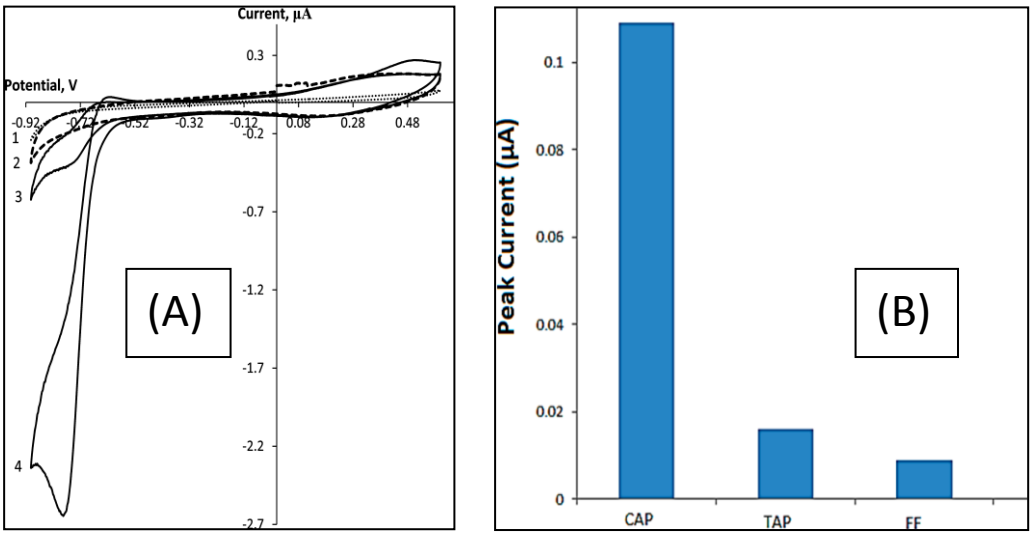

Figure 1. (A) Cyclic Voltammograms of an unmodified gold electrode in a blank solution (2), and in the presence of 1×10^{-6} mol L^{-1} CAP (3).The behavior of an aptamer-modified gold electrode in (1) a blank buffer solution and in the presence of 1×10^{-6} mol L^{-1} CAP is shown as curve 4 and (B) the selectivity of the DNA aptamer (1×10^{-6} mol $L^{-1}$of CAP, TAP, and FF) (reproduced with permission from reference [6]).

Figure 1 (A) depicts the CV response obtained with unmodified and aptamer modified gold electrode in the absence (blank) and presence of CAP solution. It is obvious from curve 4 that aptamer modified sensor offered highest response. This sensor selectivity has been shown in Figure 1 (B) and it is clear that system showed excellent selectivity for CAP compared to other members of phenicol class with similar structure. The applicability of proposed method was investigated by spiking the milk samples successfully [6].

In another report, a novel electrochemical aptesensor was developed by Yan et al. based on target-induced strand release (TISR) as shown in Figure 2. The authors used the selected and designed DNA aptamer sequence called as TISR (biotinylated detection probe) which could bind specifically with CAP compared to several other available aptamers.

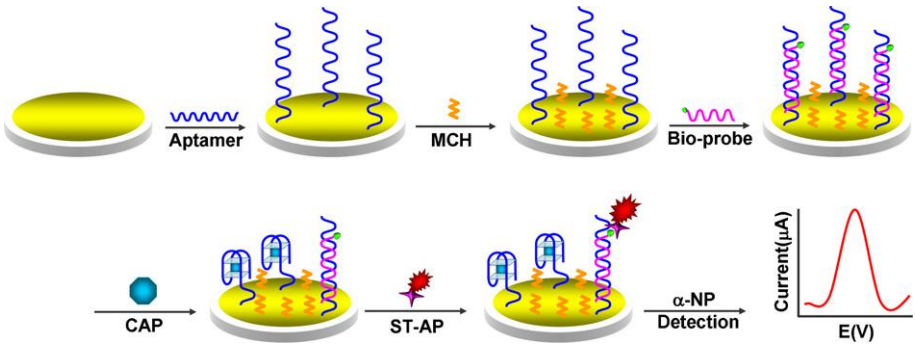

Figure 2. Schematic representation of the electrochemical aptasensor based on target-induced strand release for the detection of CAP (reproduced with permission from reference [7]).

The Gold electrode was modified with detection probe and utilized in CV technique. To confirm the successful stepwise modification, electrochemical impedance spectroscopy (EIS) was employed as shown in Figure 3 (A). Furthermore, the square wave voltammetry (SWV) analysis corresponding to various sensor modification steps results have also been assembled in Figure 3 (B). Under optimized experimental conditions, the TISR modified sensor showed excellent performance with wide linear range of CAP concentration with detection limit of 0.29nM. The real analytical evaluation was conducted by using six CAP-free honey samples with various CAP concentrations and their results complied with conventional LC-MS/MS technique [7].

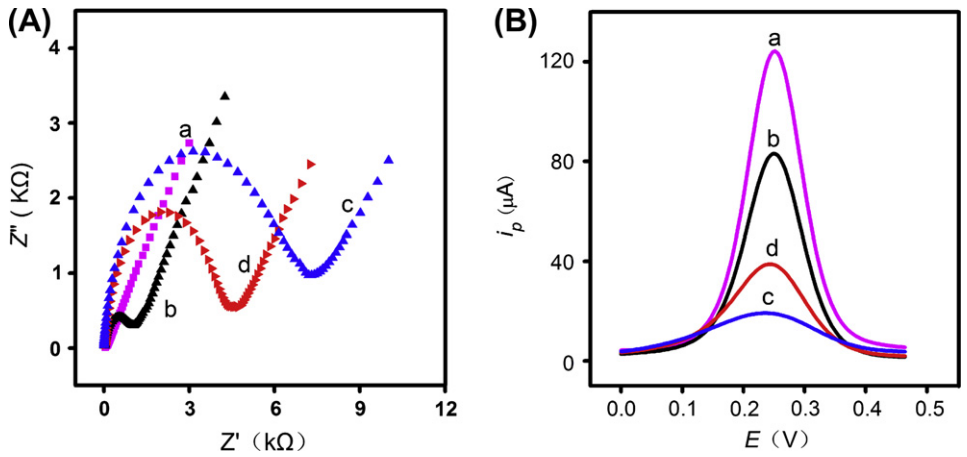

Figure 3. EIS (A) and SWVs (B) of bare gold electrode (a), aptamer modified electrode (b), dsDNA-modified electrode (c) and after reaction with CAP (d) in 0.5 mM $Fe(CN)_6^{3-/4-}$ containing 0.4 M KCl (2) (reproduced with permission from Reference [7]).

2.2. Antibody-nanostructure based immunosensor

In antibody-nanosystem approach, either antibody of CAP (anti-CAP) was entrapped or immobilized over nanostructure materials.

Yang et al. prepared a disposable reagentless immunosensor based on competitive immunoassay by modifying a screen printed carbon electrodes (SPCEs). Firstly, SPCEs were modified with a combined nanostructure of magnetic nanoparticle composite synthesized by entrapment of ferrocene (Fc) and carbon nanotubes (CNTs) into nafion (Nf) and an interface assembly of Fe_2O_3/Au composite nanoparticles coated with CAP-bovine serum albumin via an external magnetic field. The resulting amperometric immunosensor was characterized by using CV and DPV techniques and yielded excellent performance based competitive immunoreaction system and worked well in the range of 0.2~80.0 ng/mL and detection limit was about 0.11 ng/mL was obtained. This fabricated sensor was used to detect CAP in milk by spiking method with excellent precision and sensitivity [8].

A different and less complex approach for the determination of CAP was reported by Kim et al. They proposed an amperometric CAP immunosensor based highly sensitive hydrazine (Hyd) based-hydrogen peroxide sensor. The detection of CAP was based on competitive immuno-interactions between the free- and labeled-CAP for active sites of the anti-CAT using a hydrazine label, which catalyzed the electrochemical reduction of H_2O_2.

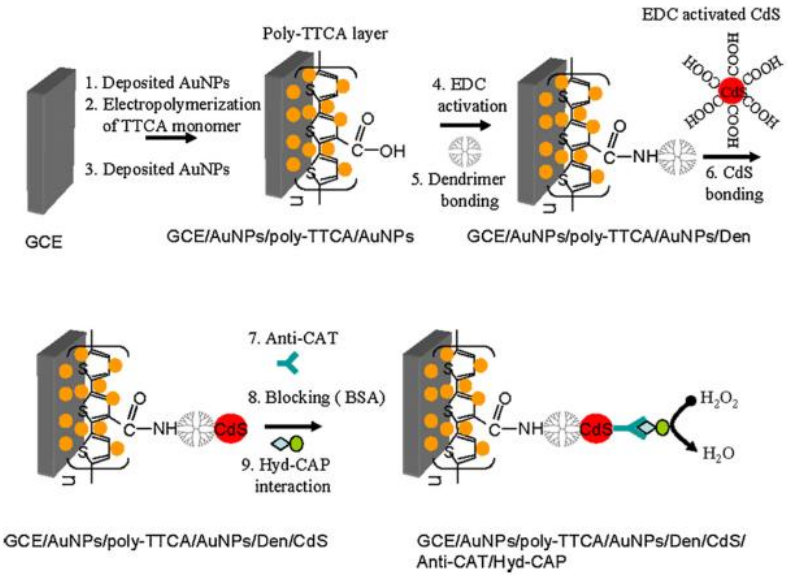

Figure 4. A Schematic illustration of the fabrication of CAP immunosensor based on AuNPs/Den/CdS modified conducting polymer (reproduced with permission from Reference [9].

In their fabrication approach shown in Figure 4, authors covalently immobilized anti-CAP acetyl tranferase (anti-CAT) antibody on cadmium sulfide nanoparticles (CdS)-modifed amine-terminated G4 poly(amidoamine) dendrimer (G = 4; PAMAM(NH_2) bonded to the poly 5,2':5', 2"-terthiophene-3'-carboxylic acid so that large number of available carboxylic acid functionalities could enhance the amount of hydrazine labeled-CAP interacted with the probe surface. This increased the immunosensor response due to the catalytic reduction of H_2O_2 in CV sweeping. Figure 5 shows the SEM, HR-TEM and EDS profiles of various modification steps. In addition to these techniques, a detailed characterization of modified sensor including XPS, cyclic voltammetry, chronoamperometry, and quartz crystal microbalance (QCM) techniques were carried out. Moreover various experimental

parameters such as the amount of anti-CAT, the pH, the temperature, the applied potential, etc. were optimized to determine the detection of limit. Authors claimed that the proposed immunosensor exhibited wide linear range of CAP concentration between 50 pg/mL and 950 pg/mL with excellent LOD of 45 pg/mL. Moreover, the immunosensor was also applied to determine the concentration of CAP in real meat samples [9].

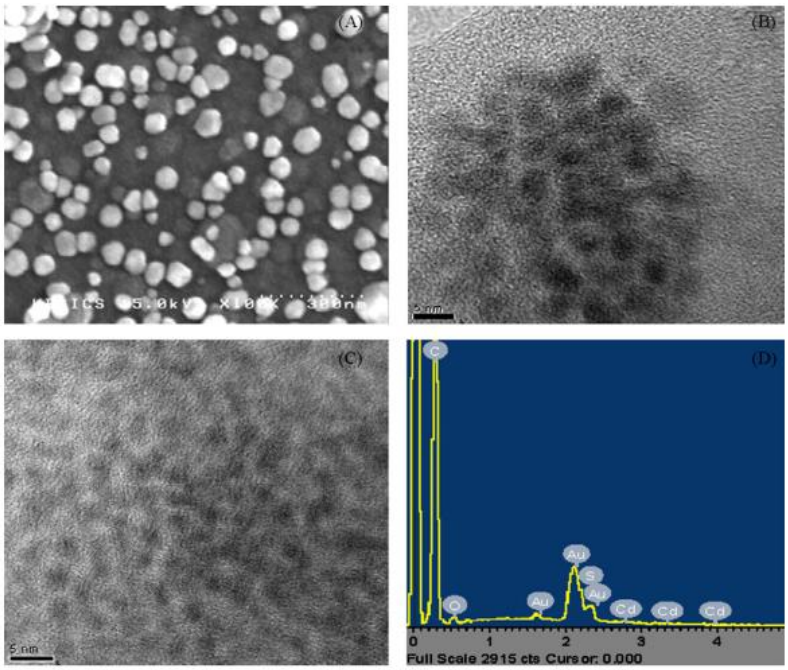

Figure 5. SEM image of electrodeposited AuNPs on the poly-TTCA film, HR-TEM images of (b) Den bonded on the AuNPs/poly-TTCA/AuNPs and (c) CdS nanoparticles bonded on the Den/AuNPs/poly-TTCA/AuNPs, and (d) EDS spectrum of the CdS immobilized on the den layer (reproduced with permission from Reference [9]).

Zhang et al. introduced a novel and quite interesting strategy for the preparation of label- free CAP immunosensor based on a composite composed of HGNs and chitosan. According to them, hollow interiors of HGNs depicted numerous advantages compared to their solid counterparts including their unique structural and optical properties, low density, high specific surface area and reduction of costs etc.

The GCE was modified by entrapping the monoclonal antibody to chloramphenicol (anti-CAP) in HGNs/chitosan composite. For the synthesis of HGNs, the authors utilized an already reported method where HGNs were simply synthesized using a procedure that involved the template-engaged replacement reaction between Co nanoparticles (sacrificial templates) and an aqueous $HAuCl_4$ solution. The stepwise modification of sensor was checked by EIS and UV-Vis spectroscopy. The structure and morphology of these HGNs were investigated by TEM. The determination of CAP was carried out by realizing the change in $K_3[Fe(CN)]_6$ as redox marker using DPV. The proposed label-free CAP immunosensor exhibited a highly sensitive response toward CAP in a linear range of 0.1–

1000 ng mL with the detection limit of 0.06 ng mL-1. The real applicability of immunosensor was realized by investigating the CAP in meat samples with précised results. Furthermore, the specificity of present immunosensor was ascertained among several antibiotic interferents such as streptomycin, ampicillin, tetracycline, neomycin with same concentration [10].

2.3. Antibody-immobilized piezoelectric principle based immunosensor

The QCM type immunosensors exhibit outstanding performance owing to the utilization of mass-sensitive detector based on an oscillating piezoelectric quartz crystal that resonates at a fundamental frequency. The QCM are extensively used as suitable transducers for affinity based biosensors exploiting an antibody as the biological component which could detect the micro mass changes and physical properties of micro mass layer deposition over quartz crystal surfaces in real time monitoring without labeling. Thus, few QCM based immunosensors have been developed for determination of CAP.

In 2004, Park et al. developed a batch type anti-CAP immobilized QCM sensor for CAP. For the realization of CAP sensor, firstly, self-assembled monolayers (SAMs) of various types of thiols and sulfides were chemisorbed over gold electrode surface of piezoelectric crystals followed by covalent attachment of anti-CAP. The responses of CAP sensors fabricated by use of various thiols and sulfides were compared. The detection limit of the proposed sensor was estimated to ~ 10^{-5} M. However, this detection limit was not enough for a direct measurement of CAP in real samples [11].

In a report presented by Adanyi et al. two different techniques including piezoelectric QCM and the optical waveguide light mode spectroscopy (OWLS) were utilized to fabricate the CAP immunosensor.

For the fabrication of QCM sensor, sulfide based SAM was formed by chemisorption followed by immobilization of anti-CAP antibody with the help of 3-mercaptopropionic acid (3-MPA) activated by dimethylaminopropyl-ethylcarbodiimide–hydroxysuccinimide ester (EDC-NHS). On the other hand, the OWLS immunosensor system was prepared by introducing the amine terminated c-aminopropyltriethoxysilane (APTS) over integrated optical waveguide chips. These chips were cross-linked by using glutaraldehyde (GA) followed by anti-CAP antibody immobilization. It was found that QCM based CAP immunosensor provided linear response for CAP between 5×10^{-5} M and 5×10^{-6} M while the OWLS immunosensor exhibited excellent and wide linear response for CAP between 10^{-7}-10^{-3} M. The authors claimed that both the techniques are stable, simple, rapid and economical as they do not require any labeling chemistry [12].

In another report based on QCM method, Sun et al. used an interesting fabrication procedure for CAP immunosensor. In order to enhance the sensitivity of QCM CAP biosensor, three-dimensional porous polystyrene (PS) fibrous membrane was deposited onto a gold QCM electrode via electrospinning technique. The immobilization of anti-CAP antibody was carried out by two strategies. In first strategy, SAM of 3-mercaptopropionic acid (3-MPA) was deposited on PS-modified QCM sensor by chemisorption followed by antibody immobilization. On the other hand, PS-modified QCM was activated by polyethyleneimine–glutaraldehyde (PEI-GA) and subsequent covalent

immobilization of antibody. However, the antibody functionalized MPA biosensor showed increased sensitivity compared to antibody functionalized PEI-GA biosensor toward CAP. The various properties of PS fibrous membrane including solvent ratio, pore volume and specific surface area (SSA) and effect of activation time were optimized to obtain best sensitivity. In order to observe the influence of various weight ratio of DMF/THF in solvent on electrospun PS membranes morphologies, FE-SEM images of were obtained and shown in Figure 6.

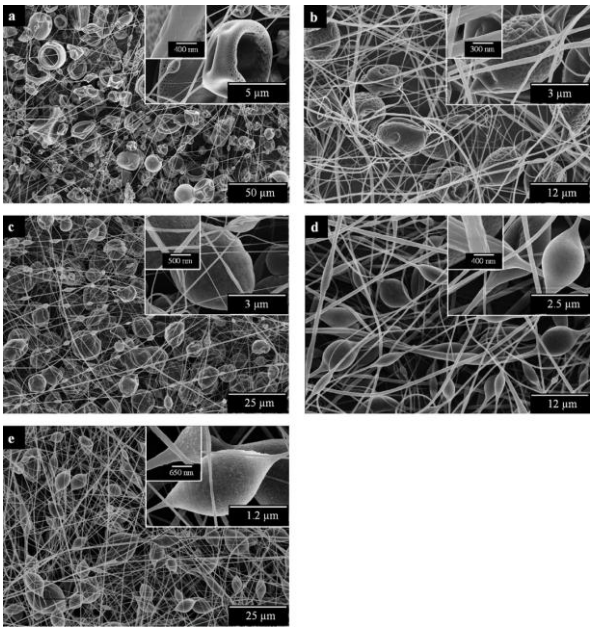

Figure 6. FE-SEM images of electrospun PS membranes formed from 10 wt% concentration with various weight ratios of DMF/THF in solvent: (a) 0/4, (b) 1/3, (c) 2/2, (d) 3/1, and (e) 4/0. (reproduced with permission from Reference [13]).

Figure 7 represents the real time response of QCM biosensors with and without PS membranes to increasing concentration CAP with application of different frequency range. The biosensor depicted nice linearity over the range concentration range of CAP between of 5–100 ppb. The as-prepared biosensor showed quick response (2–3 s) to CAP, with a detection limit of 5 ppb with optimal structure and activation time. In addition, the biosensor also exhibited good selectivity toward CAP when analyzed with other antibiotics at a concentration between 5 to 200 ppb [13].

Recently, a promising approach was developed by Karaseva et al. by using piezoelectric immunosensor for CAP. In this approach, firstly, the pre synthesized pyrrole monomer was electropolymerized over a gold surface of QCM electrode by cyclic voltammetry in order to obtain a stable and thin receptor layer for anti-CAP immobilization. This polypyrrole modified electrode was activated with GA followed by covalent immobilization of anti-CAP conjugate.

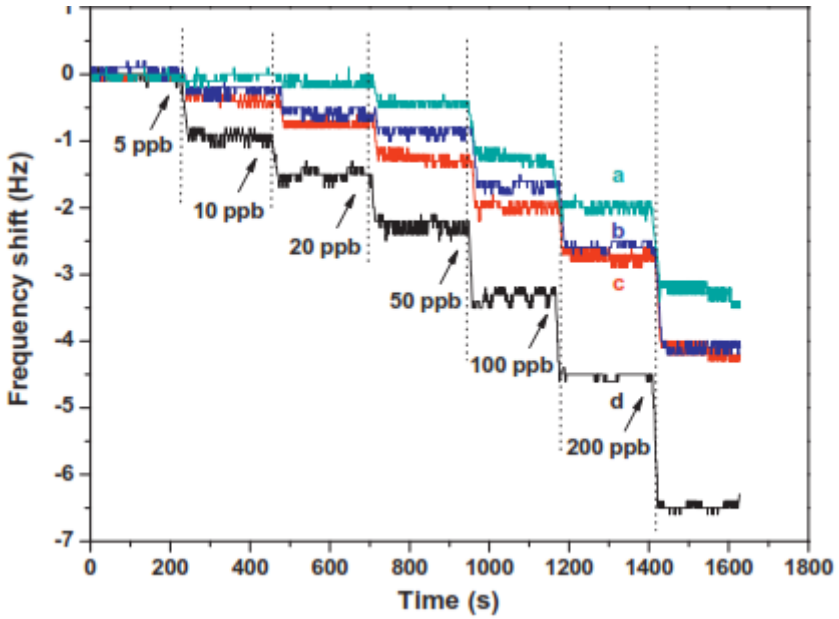

Figure 7. Response of QCM biosensors with various coatings exposed to CAP: (a) with-out PS membranes, (b) PS membranes with a loading of 1108 Hz and SSA of 16 m^2/g,(c) PS membranes with a loading of 595 Hz and SSA of 43 m^2/g, and (d) PS membranes with a loading of 1092 Hz and SSA of 43 m^2/g. The MPA immobilization method of anti-CAP is used; the activation time is 60 min (reproduced with permission from Reference [13]).

The variations in morphologies of piezoelectric sensor with different types of solvent systems were investigated by using atomic force microscopy (AFM). The CAP was determined in a competitive immunoassay format with a receptor layer of hapten–protein conjugate of CAP and soybean trypsin inhibitor. The QCM properties were optimized and a LOD of 0.2 ng/mL was obtained for CAP with linear calibration curve in the range of 0.5-100 ng/mL. The accurate determination of CAP was also performed in various real samples of meat, milk, egg and honey successfully under MRPL [14].

2.4. Surface Plasmon resonance (SPR) assay based CAP sensor

SPR- based biosensors have shown enormous potential and superior capability as an ultrasensitive assay format which can offer sensitivity up to femto (f) level for qualitative and quantitative monitoring of several biomolecules as well as small compounds without labeling. Few SPR-based biosensors for CAP have been appeared in literature as discussed in the following paragraphs.

A highly sensitive method for the detection of CAP was introduced by Yuan et al. in 2008 based on a SPR- principle which afforded a LOD approximately 17.5 fg/mL in honey spiked samples. For the fabrication of SPR-biosensor, firstly, chloramphenicol-oligoethylene-ovalalbumin (CAP-OEG-OVA) conjugate was synthesized separately in the laboratory. Then, mixed self -assembled monolayer (mSAM) of 11-mercaptoundecanol (11-MUOH), 16-mercaptohexadecanoic acid (16-MHA) was

formed followed by covalent immobilization of CAP-OEG-OVA conjugate through OEG linker on the mSAM. The detection of CAP was conducted by competitive assay format where mouse anti-chloramphenicol anybody (mAb, anti-CAP) and IgG/nanogold particles of two sizes i.e. 10 and 40 nm were used as sequential binding for rapid detection of CAP. The application of nanogold of 40 nm enhanced the signal response obtained from CAP SPR- biosensor. The proposed senor was highly stable (~ 400 binding regeneration cycles) and offered a LOD for CAP ~ 0.74 fg/mL with wide linear CAP concentration between 1-1000 fg/mL [15].

In 2009, Raz et al. proposed a microarray biosensor specific to several antibiotics simultaneously based on imaging surface plasmon resonance technique (iSPR). The multiplex immunodetection of various antibiotics including CAP was performed by creating seven microarrays on a single sensor chip. The immobilization of targeted compound was carried out using an amine-reactive hydrogel surface through conventional EDC-NHS chemistry. For the immunodetection of CAP, D-(-)- threo-2-amino-1-(*p*-nitrophenyl)-1,3-propanediol (CAP-base) was used as ligand. The immobilization condition of CAP compound was confirmed for compound solubility and spot formation on HCX hydrogel. At the time of monitoring of CAP, specific antibody (anti-CAP for CAP) was utilized for binding and spot (image) was immediately captured by using IBIS (IBIS technologies B.V. Hengelo, The Netherlands) iSPR system. The immobilization efficiency, spot-to-spot cross-contamination, and antibody's cross-reactivity were confirmed by serial injections of the corresponding antibodies. Finally, the iSPR method was applied for the quantitative determination of CAP and other antibiotics simultaneously in milk samples via multiplexed competitive immunoassay with single senor chip [16].

In a recent report of Fernandez et al. a portable multichannel SPR immunosenosr was develop which could be utilized on site analysis of CAP in milk. In the fabrication step of sensor chip, six portable channels were prepared on the plasmon of gold diffraction grating followed mixed self-assembled layer (m-SAM) of two different marcapto alkyl reagents containing PEG. Finally, haptenized proteins were covalently biofunctionalized. At the time of analysis, the sample mixed with specific antibody was injected into sensor system and a LOD of 0.26 μgL^{-1} was achieved. The advantages of this system lie in the fact that simultaneous detection of several antibiotics can be performed precisely and no clean up steps are required for real sample analyses except just dilution [17].

2.5 Microcantilever approach based Immonosensor

Recently, a microcantilever immunosensor was prepared for the detection of CAP based on direct competitive enzyme-linked immunosorbent assay (dc ELISA) technique. In this report, Au surface of microcantilever was modified with anti-CAP and protein A via sulfhydrylation reagent 2-iminothiolan hydrochloride. The use of sulfhydrylation reagent improves CAP sensitivity by 1.7 fold. Figure 8 (B) shows the as-synthesized microcantilever deflection vs. time at varying concentrations of CAP. It was found that the proposed immunosensor performed well and offered LOD of 0.2 ng/mL for

CAP. Thus, this research proved the suitability of microcantilever types of immunosensor for the detection of small biologically valuable molecules [18].

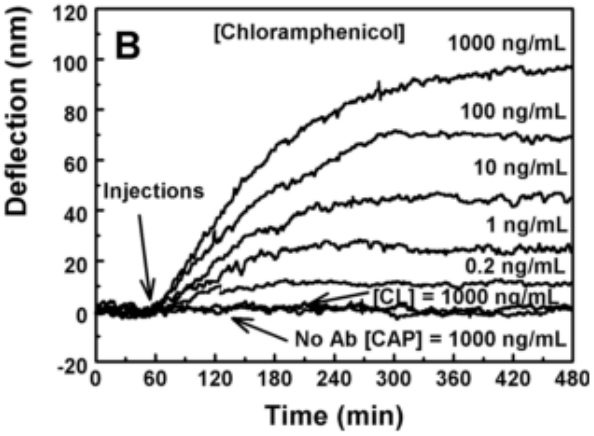

Figure 8. Microcantilever deflection vs. time at varying concentrations of CAP (B). The cantilever was functionalized with anti-CAP antibody via protein A (reproduced with permission from Reference [18]).

2.6 Impedance spectroscopy based immunosensor

Chullast et al. prepared a label-free immunosensor based on impedimetric system. The authors obtained outstanding LOD of 1 ×10-16 M for CAP. The authors followed a multilayer fabrication scheme. Firstly, a self-assembled thiourea monolayer (SATUM) was deposited over precleaned surface of gold electrode followed by adsorption of AuNPs and mercaptosuccinic acid (MSA).

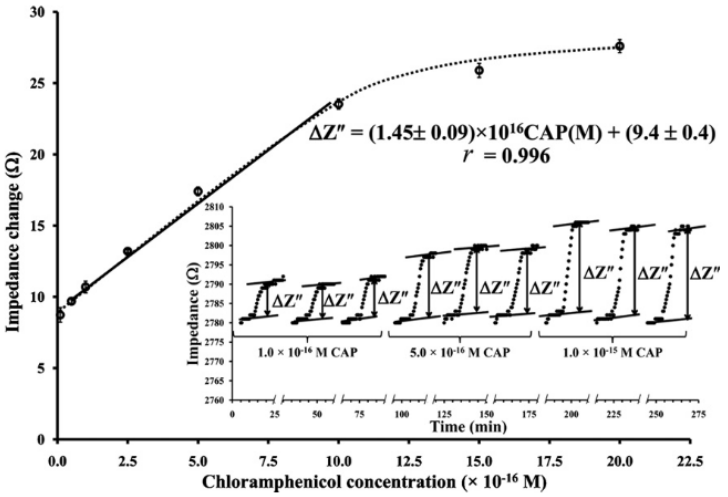

Figure 9. A calibration plot of SATUM/AuNPs/MSA modified electrode obtained from the impedimetric immunosensor under optimum conditions: 10 mM PBS pH 7.00 containing 2.7 mM KCl and 137 mM NaCl, flow rate 100 μL min^{-1}, sample volume 450μL and 50 mM NaOH as regeneration solution. Inset shows example of the impedance changes (ΔZ") caused by the binding of analyte (CAP)-immobilized anti-CAP interaction (reproduced with permission from Reference [19]).

Then, the resulting surface was activated through EDC-NHS chemistry and anti-CAP antibody was immobilized over it. At the time of impedimetric detection, the increase in the value of impedance signaled the formation of anti-CAP antibody- antigen at the electrode surface. A calibration plot of fabricated impedimetric sensor has been shown in Figure 9 under optimum experimental conditions. The inset of Figure 9 shows the impedance changes (ΔZ") caused by the binding of analyte (CAP)-immobilized anti-CAP interaction. Figure 10 clearly provides evidence of striking LOD of 1×10^{-16} M for CAP with excellent regression coefficient (r^2) value of 0.996. The proposed sensor was very selective and stable as it could be regenerated 45 times with a relative standard deviation (RSD) of less than 4%. The real usefulness of this method was assessed by spiking the shrimp samples with known concentrations of CAP. The obtained results were well coherent with those obtained by HPLC [19].

3. NANOSTRUCTURE BASED APPROACHES

In last two decades, nanostructured materials have received surge of interest owing to their remarkable properties and have been continuously utilized as efficient electron mediators for the fabrication of highly sensitive chemical and biosensors [20].

A composite film of SWCNTs-AuNPs and ionic liquid has also been applied for the determination of CAP by Xiao et al. Firstly, the SWCNTs-AuNPs hybrid was synthesized by adding suitable amount pretreated SWCNTs into synthesized AuNPs colloids. To this hybrid solution, commercially available ionic liquids 1-octyl-3-methylimidazolium hexafluorophosphate ($OMIMPF_6$) was dispersed by ultrasonication resulting into uniform suspension of SWCNTs-AuNPs- $OMIMPF_6$.

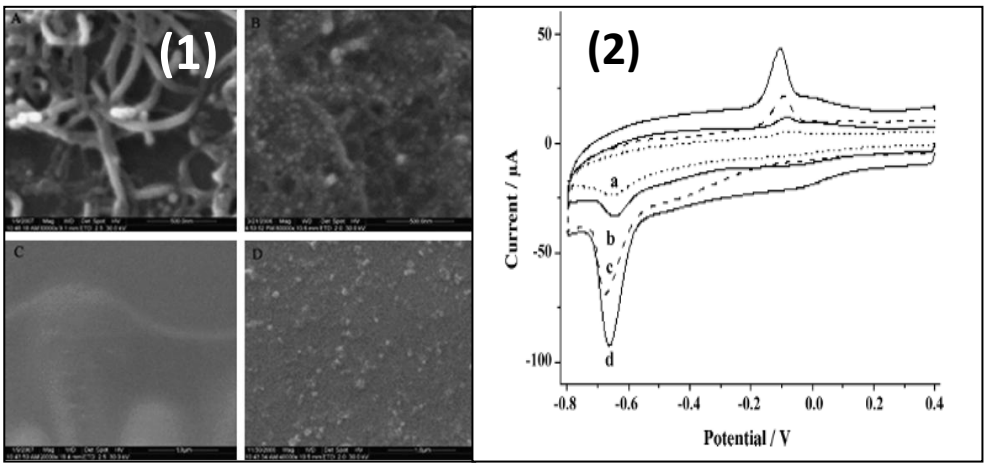

Figure 10. (1) SEM images of SWCNTs film (A), GNP- SWCNTs hybrid film (B), $OMIMPF_6$ film (C) and $OMIMPF_6$-GNP- SWCNTs hybrid film (D) and (2) Cyclic voltammograms of SWCNTs /GCE (a), GNP– SWCNTs /GCE (b), $OMIMPF_6$– SWCNTs /GCE (c), $OMIMPF_6$– GNP– SWCNTs /GCE (d) in 5.0×10^{-6} M CAP solution; Scan rate 0.1 V; supporting electrolyte 0.10 M phosphate buffer solution (pH 7.0); accumulation time: 150 s (reproduced with permission from Reference [21]).

Finally, the as-synthesized composite was used to modify the GCE for further study. The modified GCE electrode was characterized and its voltammetric behavior was studied in the presence of CAP. The SEM images of successive modification steps of GCE have been illustrated in Figure 10 (1). The performances of various modified electrodes such as bare GCE, bare Gold electrode, $OMIMPF_6$/GCE and SWCNTs/GCE were compared, however, no appreciable signals were recorded. Figure 10 (2) shows CV response of various stages of modified GCE including SWCNTs /GCE, GNP–SWCNTs /GCE, $OMIMPF_6$– SWCNTs /GCE and $OMIMPF_6$–GNP– SWCNTs /GCE in 5.0×10^{-6} M CAP solution.

It can be deduce easily that $OMIMPF_6$–GNP–SWNT/GCE provides highest detection of CAP. In order to achieve maximum and beneficial response of CAP, the various experimental parameters including Influence of ratio of $OMIMPF_6$ in the composite film, different types of ionic liquids, potential scan rate, pH of solution and accumulation time of CAP were studied. The proposed method showed linear current relationship to concentrations of CAP from 1.0×10^{-8} to 6.0×10^{-6} M and the LOD was estimated to be 5.0×10^{-9} M. The authors selected a wide range of interference species and showed that reported method exhibited good selectivity for 1.0×10^{-6} M CAP against 100 fold of various interferences such as uric acid, glucose, *V*c, V_{B1}, V_{B6}, V_{B12}, xanthine, hypoxanthine, cysteine, oxytetracycline, chlorotetracycline, clindamycin, streptomycin, puromycin, Ca^{2+}, Cd^{2+}, Co^{2+}, Fe^{3+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}; 10-fold of *p*-nitroaniline, p-nitrophenol do not interfere with the determination of CAP, however, *p*-nitrobenzoic acid, nitrobenzene and methylparathion showed severe interference. Furthermore, the real applicability of proposed method was investigated by spiking the milk samples with excellent recoveries [21].

An interesting sandwich nanohybrid approach of combination of single-walled carbon nanohorns (SWNHs), titania (TiO_2) and porphyrin was recently reported for the fabrication of highly sensitive amperometric biosensor for CAP. Firstly, the SWNHs were functionalized by using hydroxyferriprotoporphyrin followed by spontaneous adsorption of TiO2 nanoparticles over porphyrin bound carboxylate groups via dentate binding. The resulting sandwich nanohybrid showed superior electrocatalysis activity toward reduction of CAP. The as-prepared sensor offered a detection limit of 0.9 nM in just 5 s. The senor showed good reproducibility with RSD of only 4.6% and retained 91.7% of initial response after 25 days. Moreover, the proposed amperometric biosensor was very specific toward CAP when investigated in the presence of several interfering species such as ions (Na^+, K^+, Mg^{2+}, Cu^{2+},Ca^{2+}, Zn^{2+}, NH_4^+, SO_4^{2-}, PO_4^{3-}), saccharide (sucrose, glucose), and antibiotic (chlorotetracycline, streptomycin, penicillin) at 1000 times concentration of CAP [22].

In one of the simple approach, MWCNTs were used to modify the GCE for the detection of CAP by Lu et al. It was shown that use of MWCNTs decreased the CAP reduction overpotential significantly. Some of the experimental parameters such as value of buffer pH, scan rate, and amount of modifier, on the determination of CAP were optimized to achieve best response for CAP. The CAP reduction peak current showed good linear concentration range of CAP over 3×10^{-7} to 1.2×10^{-5} M with a LOD of 4.5×10^{-8} M when the signal to noise ratio was 3. Due to small RSD of 5.3%, it could be deduced that the proposed research exhibited good reproducibility. Furthermore, this method showed excellent selectivity and very high recoveries of CAP (~100%) was achieved when few eye drops solutions were evaluated for spiked concentrations of CAP [23].

Another approach for the determination of CAP based on nano-composite carbon paste potentiometric sensor was introduced in literature by Ganjali et al. Firstly, artificial host for CAP based MIP was prepared with the utilization of CAP as template, MAA as functional monomer, EDMA as cross linker, AIBN as polymerization initiator and chloroform as solvent. The as-prepared monomer solution was homogenized and polymerization was carried out for 24 h at 60°C. Finally, obtained polymer was washed and templates were extracted thoroughly. Then, nano-composite was prepared by mixing appropriate amounts of CAP-MIP along with graphite powder, paraffin oil or ionic liquids (IL), nano-silica and MWCNTs. After homogenization of the mixture, the resulting paste was carefully packed into the plastic tube tip to and copper wire was inserted into opposite end of the carbon paste electrode (CPE) to establish electrical contact. Finally, the external surface of MIP-CPE was polished and smoothed followed by conditioning for about 40 h by soaking it in a 1.0×10^{-3} M of CAP solution. The determination of CAP was investigated with potentiometer and best results were optimized based on various electrode compositions, response time and pH of CAP solution. The proposed CAP sensor was utilized with CAP concentration in the range of 1.0×10^{-6} to 1.0×10^{-2} mol/L and offered excellent nernstian response of 59.1±0.4 mV/ decade.

The sensor exhibited less than 10^{-4} potentiometric selectivity coefficients, evaluated by matched potential method (MPM) with Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Cl^{-}, CO^{3-}, Co^{2+} and glucose. The sensor showed average lifetime from 4-10 weeks. Finally, the analytical effectiveness of potentiometric CAP sensor was tested in determination of CAP concentrations in various pharmaceutical drugs satisfactorily [24].

4. FLOW INJECTION ANALYSIS (FIA) DETECTION OF CAP BASED ON ELECTROCHEMICALLY MODIFIED TECHNIQUE

In these approaches, the electrochemical properties were studied using cyclic voltammetry modified electrodes followed by determination of CAP using FIA system.

A preanodized wall-jet screen-printed ring disk carbon electrode (SPRDE) was utilized for the detection of CAP by FIA in aerobic conditions. The preanodised carbon electrode was utilized in order to reduce the CAP overpotential and to detect CAP in aqueous medium selectively. Furthermore, it is well known from the literature that according to its typical electrochemical behavior, CAP is irreversibly reduced from nitro group ($-NO_2$) to a hydroxylamine group (−NHOH) followed by reversible oxidation of –NHOH group to nitroso (–NO) group referred as two well defined redox couple. However, it is found that the irreversible reduction peak of CAP is susceptible to the O_2 oxidation which occurs close to the reduction potential of CAP in most of the electrochemical studies.

Hence, this study showed an interesting approach where the irreversible reduction of nitro group ($-NO_2$) to a hydroxylamine group (−NHOH) of CAP was carried out at disk electrode and the following reversible oxidation of –NHOH group to nitroso (–NO) group was monitored at the ring electrode, thus the interference of dissolved oxygen was completely avoided. This strategy could enable the accurate determination of CAP by FIA under aerobic conditions. The proposed method furnished linear calibration range of 0.1-20 μM with LOD of 0.074μM. The practical applicability of proposed method was investigated in several veterinary pharmaceuticals successfully [25].

In a similar approach, Chuanuwatanakaul et al. proposed a boron-doped diamond thin-film (BDD) electrode and its utilization for the detection of CAP equipped with FIA system as an amperometric detector. Firstly, authors studied the electrochemical behavior of CAP using cyclic voltammetry and hydrodynamic voltammetry and determination of CAP was performed using FIA system with BDD electrode as an amperometric detector. The CV offered a linear current response between 0.1-10 mM with excellent regression coefficient value of 0.9990. The experimental parameters including buffer concentration, variation in ethanol (organic modifier) amount and pH values were optimized to acquire maximum CAP response. Secondly, the BDD electrode was also applied in FIA system in order to determine the CAP amount in standard samples of sterile eye drops and milk samples. The FIA system showed linear response over the concentration range of 0.1-50 μM with r^2 value of 0.9948 and the LOD of 0.03 μM was obtained [26].

5. CAP DETECTION BASED ON MOLECULAR IMPRINTED POLYMER (MIP) MODIFIED ELECTRODE

Molecular imprinting technique is considered an effective approach where any template molecule is introduced in a mixture of monomer and cross-linker dissolved in a solvent resulting into three-dimensional polymer matrix. After removal of the template from as–prepared polymer, the permanent cavities of the original template is formed which are capable to rebind selectively to the template molecules. The obtained polymer, referred as MIP, demonstrates high stability and robustness in harsh synthesis and analysis environment [27]. Although several immunosensors and biosensors based on the utilization of anit- CAP antibodies have been appeared with excellent sensitivity and selectivity but those methods are very susceptible to harsh experimental and surrounding environment thus reducing sensors life time and stability. Hence, MIPs based electrochemical sensors are considered suitable for the detection of several biologically valuable analytes. Hence, due to their high selectivity and stability, MIPs have also been applied for determination of CAP.

Mena et al. prepared CAP imprinted polymers and utilized them as a selective solid phase extraction (SPE) media for the on-line clean up and as a preconcentration of CAP before voltammetric detection of CAP. Firstly, the polymerization was performed to synthesize CAP imprinted polymer with suitable monomer, cross-linker and polymerization initiator at 60°C for 48 h. The non-imprinted polymer (without CAP inclusion) was also prepared for comparison study. Then, the as-synthesized MIPs were packed in microcolumns for solid extraction procedure of CAP and retained CAP was collected with methanol followed by square wave voltammetric detection at preactivated cylindrical carbon fibers microelectrodes (CFMEs). The influence of sample pH values, amount of methanol and binding and extraction parameters were tuned to obtained maximum benefit from the method. Taking consideration of CAP elution time and different sample volumes in SPE, ~ 96 % recoveries of CAP was acquired from 250 mL sample volume of 3.0×10^{-8} M/L (9.7 μg/L) with enrichment factor of 500. The proposed method showed good selectivity for CAP in the presence of few structurally similar analytes including chloramphenicol diacetate, chloramphenicol base and thiamphenicol. Moreover,

this method was very useful for clean-up as well as preconcentration of CAP in ophthalmic and spiked milk samples [28].

Zhao et al. developed CAP electrochemical sensor based on the integration of CAP imprinted polymer with MWCNTs (*c*-MWCNTs) doped with AuNPs on a GCE. A Schematic diagram for the fabrication of MIP/*c*-MWCNTs-AuNPs/GCE electrode has been shown in Figure 11. Prior to AuNPs-doped c-MWCNTs, the c-MWCNTs were modified to expose carboxylic functionalities for improved AuNPs doping. Then, as-prepared AuNPs-doped c-MWCNTs suspension was casted 5 times on GCE followed by dipping of this modified electrode in a separately prepared monomer mixture for 1 min. Finally, the electrode was taken out and polymerization reaction was carried out at 60°C for 24 h under N_2 atmosphere. After 24 h, the templates and residual reactant were washed by ethanol under magnetic stirring for 12 h. The non-imprinted sensor was also prepared for the comparison purpose. The analytical performance of as-fabricated sensor was conducted based on the differential pulse voltammetry method (DPV) using a traditional three electrode system. The proposed CAP electrochemical sensor showed good performance and exhibited linear current response over the concentration range of 0.1 to 100 mg/L and a detection limit of 0.024 mg/L with r^2 value of 0.9962. Furthermore, good selectivity and interference immunity against few structurally similar compounds and antibiotics were achieved. The applicability of sensor was investigated using some natural seawater spiked with known concentrations of CAP and results were found to be in agreement with HPLC method and could be utilized for onsite detection of CAP of a real aqueous solution without the need for other complicated and expensive equipment [29].

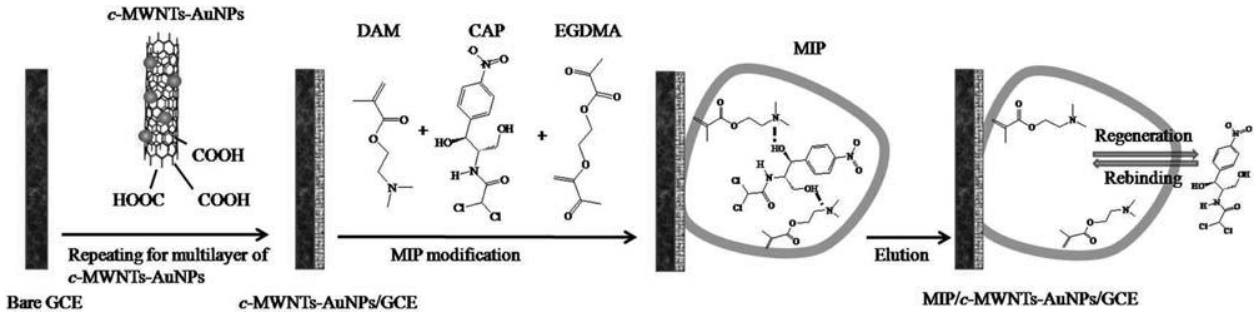

Figure 11. Schematic diagram for the fabrication of MIP/*c*-MWCNTs-AuNPs/GCE electrodes (reproduced with permission from Reference [29]).

For the determination of CAP, an interesting method was explored by Alizadeh et al. The authors prepared the CAP based molecular imprinted polymer separately and then added the as-synthesized CAP MIP to the CPE to acquire a stable MIP-CP sensor for CAP. For comparison study, various types of sensor were prepared including non-imprinted (without CAP molecules, NIP-CP) and only carbon paste (CP) electrodes. To achieve the best results, various constituents of MIP-CP composition such as amount of MIP, graphite and n-eicosane and experimental conditions including CAP incubation time, CAP solution pH and stirring rate and finally analyte extraction conditions were tuned and results were compared. Under optimal experimental parameters, the proposed CAP sensor

exhibited a linear response over CAP concentration in the range of 8×10^{-9} to 1.0×10^{-6} M with a detection limit of 2×10^{-9} M (S/N = 3) by DPV. In addition, the CAP sensor was very immune against some nitroaromatic compounds like metronidazole, para-nitrophenol and nitrobenzene and the current responses were negligible. The analytical applicability was performed with some spiked milk samples and excellent recoveries of CAP were obtained with less than 5% RSD. This method also prevents the problem of sensor leaching as usually observed with simple coated or casted GCE [30].

6. MISCELLANEOUS METHODS

This section of the review will focus on some miscellaneous electrochemical procedures appeared in literature for the determination of CAP.

In 2000, Jin et al. determined CAP by separation using capillary zone electrophoresis followed by end-column amperometric detection at a carbon fiber micro-disk electrode at a constant potential of -1.00 V with deoxygenation of CAP solution. The carbon fiber micro-disk electrodes were prepared with the help of 6 μm carbon fiber inserted into a fused silica capillary. This fused silica capillary was again inserted into glass capillary of large diameter and electrical contact was made by using copper wire. Finally, the protruding carbon fiber was trimmed and polished to get carbon fiber micro-disk electrodes. The separation of CAP was carried out using fused silica capillary of 25 μm I.D. The voltammetric behavior of CAP was investigated by linear sweep voltammetry. It is worth mentioning that when the area of prepared electrode was less than 1.1 mm^2, the interference of oxygen was negligible. The separation and detection condition were optimized and calibration curve was plotted. The CAP concentration range was linear from 5×10^{-6} to 1×10^{-3} M/L with LOD of 9.1×10^{-7}M/L. The usefulness of proposed method was tested in human serum by standard addition method and 99% recovery of CAP was achieved [31].

Another work of carbon fiber micro electrode was reported by Agui et al. in 2002. In their work, they electrochemically activated the cylindrical carbon fiber microelectrodes of 8 μm I.D by immersing them into a 0.05 mM/L buffer solution of $H_2PO_4^-/HPO_4^{2-}$ of pH 7.8, and by five successive square-wave (SW) voltammetric scans. The Scanning electron micrograph for an activated carbon fiber has been depicted in Figure 12 showing numerous fractures and fissures that enhanced the fibers surface area which in turn provided much increased cathodic current response of CAP. Hence, the activation method was optimized in order to acquire the best results and electrochemical behavior of CAP studied on as-prepared activated carbon fiber microelectrodes. The authors were able to get linear concentration response of CAP over the range of 1.0×10^{-7} to1.0×10^{-5} M/L. The LOD was calculated to be 4.7×10^{-8} M/L. The Proposed method was applied to test the validity of method on two spiked samples of milk successfully with recoveries of CAP over 97% [32].

In a report published by Chai et al. the effect of a cationic surfactant was explored on the voltammetric determination of CAP at GCE. In this report, authors developed a method to improve the sensitivity of CAP detection in biological samples by applying a common cationic surfactant, cetyltrimethyl ammonium bromide (CTAB). It was shown that only high concentration of CAP can be

detected easily using bare GCE, however, addition of CTAB in sample solutions could improve the sensitivity of CAP significantly.

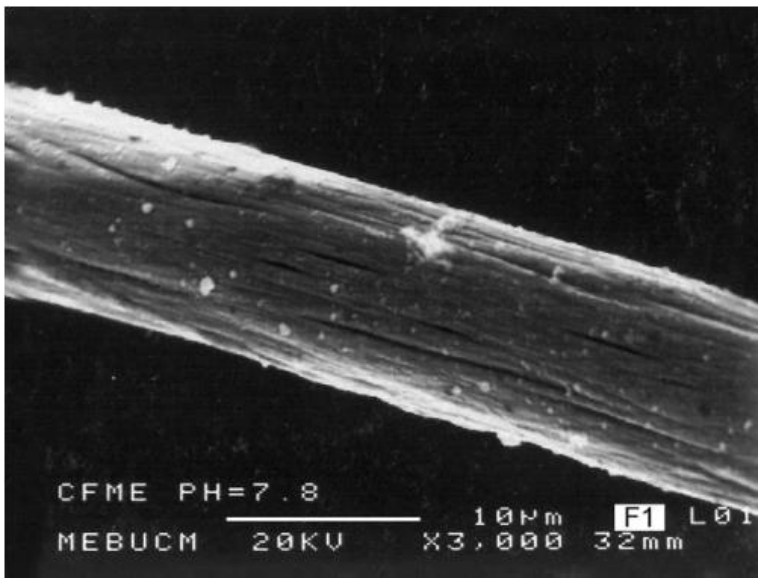

Figure 12. SEM for an activated carbon fiber (reproduced with permission from Reference [32]).

Besides CTAB, some other long chain cationic surfactant including dodecyl trimethyl ammonium bromide, n-Octyltrimethylammonium bromide, octadecyl trimethyl ammonium bromide, cetylpyridinium bromide and anionic surfactants such as nafion were investigated in the voltammetric determination of CAP at GCE. It was found that nafion-modified GCE was not able to detect CAP. However, among all the cationic surfactant, only CTAB exhibited best response which could be attributed to suitable length of alkyl chain and structure of hydrophilic groups of CTAB which may provide variation in inductive effect and steric hindrance. The effect of pH of solution and supporting electrolytes were also optimized. The calibration plot was linear from 0.0026 mg/L to 8 mg/L and the LOD was found to be 0.83 μg/L. The method validation was carried out by spiking the milk samples with various concentrations of CAP and average recovery ratio of 96.8 % was obtained [33].

Another simple approach for the detection of CAP was proposed by Codognoto et al. by applying the self-assembled monolayers (SAMs) of 2-mercapto-5-methylbenzimidazole (MMB). Before modification, the bare gold electrode was pretreated in piranha solution under mild heating to remove contaminants followed by its activation by repetitive CV scanning in 0.5 M/L sulfuric acid. The self-assembled monolayers of MMB was physically adsorbed over activated AuE by immersing it in freshly prepared ethanolic solution of MMB solution for 24 h and finally modified electrode was rinsed thoroughly before use.

The SAMs were characterized by CV and EIS and electrochemical behavior of CAP was studied by CV. Finally, MMB modified electrode was coupled with FIA system and its performance was investigated by using two CAP containing ophthalmic solution through amperometric detection method successfully and the obtained results were according to the results of HPLC method. The

method exhibited linear response from 0.050 to 1.000 μM/L and LOD of 44μM/L. This method was rapid and economical and SAMs were able to prevent the electrode from fouling [34].

In a recent report by pilehvar et al. a bare gold electrode was used to detect several phenicol family drugs including CAP by CV and SWV. It was shown that CAP shows well defined redox reaction in the presence of its derivatives in TRIS buffer solution. The authors obtained linear calibration graphs over the concentration range of 2.5 to 7.4 μM/L and a LOD of 1 μM/L was achieved [35].

7. CONCLUSION

This review provides the comprehensive details of detection of CAP from 2000 to 2013. The detection of CAP was performed by sensors by applying various fabrication procedures. The immunosensor based research was on forefront. Although, these approaches afford high selectivity and selectivity, but suffer from stability against harsh environment and have lower shelf life. To enhance the stability of system, several nanostructures based sensing methods were introduced, however, these systems sometimes lack selectivity. To improve sensitivity, selectivity and stability of CAP sensors, MIP in conjugation with different nanostructures were reported in literature. It was claimed that these types of sensor perform extremely well against harsh conditions of sensing units including highly acidic and basic buffer and wide range of temperature. Moreover, the imprinted cavities complementary to CAP offer excellent selectivity with good sensitivity.

It could be realized from the literature study that detection of CAP requires not only the sensitivity but also the selectivity of the system in real sample analysis, hence, MIP based sensor could be in demand keeping the stability issue in mind.

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