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(Electro)Sensing of phenicol antibiotics - a review

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## **Abstract**

The presence of residues from frequent antibiotic use in animal feed can cause serious health risks by contaminating products for human consumption such as meat and milk. The present article gives an overview of the electrochemical methods developed for the detection of phenicol antibiotic residues (chloramphenicol, thiamphenicol, and florfenicol) in different kinds of foodstuffs. Electrochemical sensors based on different biomolecules and nanomaterials are described. The detection limit of various developed methods with their advantages and disadvantage will be highlighted.

**Key-words:** chloramphenicol, thiamphenicol, florfenicol, antibiotics, electrochemical detection

## ***1- Introduction***

Antibiotics are one of the most frequently used pharmaceuticals in health care and veterinary practice. Particularly, food producing animals are treated with a wide range of antibacterial drugs in order to prevent infection diseases. However, overuse of antimicrobials leads to an increase of antimicrobial-resistant bacteria, increasing the chance for the individual to become infected (Wang et al., 2012). The main problems related to the illegal use of antibiotics include antibiotic residues and antibiotic resistance. Concerns associated with residues are about adverse human reactions. Additionally, antibiotic-resistant pathogens can be transferred through the food chain resulting in the risk of transfer of antibiotic-resistant genes from animal to human bacterial (gut) fauna. Consequently, efficiency of antibiotic therapy can be reduced in animals infected with resistant bacteria. Therefore, there is an urgent need to develop analytical devices for environmental monitoring of antibiotic residues with the possibility of on-site monitoring. In this respect, electrochemical sensors hold a great potential for effective monitoring of antimicrobials/antibiotics in the environment (Huet et al., 2010).

Phenicol is one of a broad and effective class of antibiotics, important in clinical veterinary practice because of their broad antibacterial spectrum, rapid absorption and wide distribution. They can easily transport into bacterial cells by passive or facilitated diffusion and interfere with the incorporation of amino acids which leads to growth inhibition of bacteria. However,

wide usage of these compounds leads to resistance phenomena and serious health problems in humans related to bone marrow functions and blood disorders. Consequently, misuse raised public concern (Lai et al., 2009).

This review focuses on the electrochemical sensing of phenicol antibiotics by summarizing the different electrochemical methods available to sense this group of antibiotics. The use of bio-recognition in the analysis of these antibiotics will be highlighted.

### ***1-1- History of phenicol antibiotics***

In the early 1980s different antibacterial classes were identified. Phenicols are one of the broad spectrum and effective category of antibiotics and chloramphenicol (CAP), thiamphenicol (TAP) and florfenicol (FF) (Table 1) belong to this family. All members are small lipid-soluble organic molecules containing neither acidic nor basic groups (Lai et al., 2009).

One of the oldest members of the phenicol family is CAP. It was first isolated from *Streptomyces venezuelae* in 1947 and named chloromycetin. CAP is active against to many strains of *Salmonella* species, while most strains of *Pseudomonas aeruginosa* are resistant. The drug is also very effective against all obligate anaerobes and decreases the growth of *Rickettsia* and *Chlamydia* species. It is a relatively cheap to produce and highly effective broad-spectrum antibiotic with excellent antibacterial and pharmacokinetic properties. These properties highlight the reasons why CAP was widely used for many years in veterinary both therapeutically and prophylactically. For these reasons, nowadays, this drug is produced widely for commercial use (Ehrlich et al., 1948; Smadel and Jackson, 1947).

FF is a broad-spectrum antibiotic with a range of activity against gram-negative and gram-positive organisms including some CAP resistant strains of bacteria, FF is not approved for use in humans but in 2010 the Bureau of Veterinary Drugs, Health Canada, has approved it for use in aquaculture. Once present in the body, FF is rapidly metabolized to a dominant metabolite, florfenicol amine which, like CAP ( $\text{Log } K_{o/w} = -1.14$ ), is lipid-soluble ( $\text{Log } K_{o/w} = -0.04$ ) and accumulates in fatty tissues (mostly in liver and kidney) of animals and humans (Lai et al., 2009; Smadel and Jackson, 1947).

TAP is a semi-synthetic derivative of CAP and has a similar antibacterial spectrum as CAP but with lesser activity. The first synthesis of TAP was reported in 1950s. It is an antimicrobial agent used for the treatment of respiratory and alimentary infectious diseases in cattle, pigs and poultry. TAP has a bacteriostatic effect against a broad range of micro-organisms. TAP is less lipid-soluble ( $\text{Log } K_{o/w} = -0.04$ ) compared to CAP (Sutter and Finegold, 1976; Van Beers et al., 1975).

### ***1-2- Legislation and regulations of phenicol antibiotics***

The occurrence of antimicrobial drug residues causes public health concerns. Therefore, many food safety programs have introduced values for maximum tolerable concentrations of antibacterial residues in food procuring animal, *i.e.* maximum residue levels (MRLs).

Since 1996 the European Union (EU) has introduced a program for monitoring veterinary drug residues, including antibiotics, which has been governed through Council Directive 2377/90/EC. This Directive defines the required frequency and level of

sampling, the necessary documentation, the investigation methodology, and the appropriate actions which have to be taken (EC reg. 2377/90, 1990; Annexure IV and 1430/94, 1994). Its Annexes present the following information:

- Annex I includes substances for which final MRLs have been established.
- Annex II includes substances which are allowed to be used in veterinary medicinal products for food-producing animals
- Annex III has been assigned provisional MRLs subject to a time limit, because they do not fully meet the criteria of Annexes I and II
- Annex IV lists substances for which no MRL could be introduced because residues of these substances at any detectable level lead to serious health problems

Council Directive 96/23/EC regulates the residue control of pharmacologically active compounds in products of animal origin. This Directive divides all residues into Group A compounds, which comprises prohibited substances (Annex IV) and Group B compounds, which includes all registered veterinary drugs (Annexes I and III) and other residues (2002/657/EC).

Commission Decision 2002/657/EC establishes criteria and procedures for the validation of analytical methods. In addition, Decision 2002/657/EC demonstrates criteria for the minimum required performance limits (MRPLs) for analytical methods applied to detect substances residues for which no MRLs have been fixed. This value is particularly important for substances

whose use is prohibited or not authorized in the EU legislations. According to this decision, analytical methods should be able to detect 95 % of non-compliant samples at the level of interest (*i.e.* MRL) (2002/657/EC).

Phenicol is listed in Annex I and IV since they can cause serious health problems for consumers. They can diffuse easily into the cells and bind to the 50S subunit of the 70S bacterial ribosome and prevent peptidyl-transferase activity, thus interfering with protein synthesis.. In this way they can cause severe side effects on human health (2003/181/EC) (Table 1).

CAP is hematotoxic for humans and can cause blood diseases in some patients treated with it. Residues of CAP can appear in animal derived products which is not due to (illegal) use, but rather due to the occurrence of CAP in the natural environment (Gowik, 2003). Once in the body, the p-nitro group changes into a toxic nitroso group. Consequently, it prevents mitochondrial protein synthesis in bone marrow cells that may result in bone marrow aplasia (loss of ability to produce blood cells) which leads to aplastic anemia. The dose of CAP responsible for these pathologies is not predictable, so that the use of this antibiotic for the treatment of food-producing animals in many countries such as EU, Canada and U.S. has been prohibited. The US national Toxicology Program classified CAP as a human carcinogen. The EU has recently introduced minimum required performance limits (MPRLs) for analytical methods used for the determination of CAP. It is included in Annex IV and a MRPL of  $0.3 \text{ g kg}^{-1}$  was established by the European Commission according to Regulation 675/92 and 1430/94, while China has an MRL level of  $0.5 \text{ g kg}^{-1}$  (Gowik, 2003).

Currently TAP and FF are used as alternatives to CAP for animal treatment in many countries. FF is a bacteriostatic antibiotic that prevents protein synthesis by binding to ribosomal subunits of bacteria, resulting in the inhibition of peptidyl-transferase and thereby preventing the transfer of amino acids to growing peptide chains and subsequent protein formation. The bacterial receptor that is the site of action for FF is the same as that for CAP. However, FF does not carry the risk of human aplastic anemia since it lacks the p-nitro group of CAP, which seems to be a key molecular feature for causing aplastic anemia. FF has a fluorine atom instead of the hydroxyl group located at C-3 in the structure of CAP. This may allow FF to be less susceptible to deactivation by bacteria with plasmid-transmissible resistance that involves acetylation of the C-3 hydroxyl group in CAP, and prevents their interaction with bacterial ribosomes. Therefore, the EU has established MRLs for FF. It is included in Annex of Council Regulation 2377/90 for bovine, porcine and chickens and in Annex for fish. The MRL currently established for FF is 200 g kg<sup>-1</sup> in the edible tissue of agriculture products (Samsonova et al., 2012).

TAP can cause reversible bone marrow depression but aplastic anemia has not been reported (Yunis et al., 1973; Samsonova et al., 2012). However, due to its efficiency in the treatment of diseases in a number of aquaculture species, there is the potential for illegal use of it in both the domestic and international markets. TAP, like FF, lacks the nitro group located on the CAP aromatic ring that has been associated with CAP-induced, non-dose-related, irreversible aplastic anemia. However, CAP and TAP also cause dose-dependent, reversible bone marrow suppression in some animals and people due to mitochondrial injury. The MRLs have been set for TAP at 50 g kg<sup>-1</sup> in food to date (Samsonova et al., 2012).



## ***2- Analysis of CAP residues***

To achieve the required level of consumer protection, sensitive and robust screening methods are needed for trace detection of CAP. Therefore, the analytical detection of phenicol residues in food needs innovative procedures which combine accuracy, precision, selectivity, and sensitivity with simplicity, rapidity and low cost. Ongoing approaches to detect these compounds are usually based on separation techniques (*i.e.* high performance liquid chromatography (HPLC), gas chromatography (GC), capillary zone electrophoresis, etc.) with different detectors (*i.e.* UV-vis, diode array, fluorescence, MS, etc.) that are expensive, time consuming and not useful for on-site analyses. An antibody-immobilized quartz crystal microbalance (QCM) system was developed for detecting CAP (Park et al., 2004). Compared to the bare QCM chip and the antibody-immobilized QCM chip, the latter showed greatly enhanced frequency shifts upon CAP injection. The detection limit reported by this method is  $3.2 \text{ mg kg}^{-1}$ . A simple, rapid capillary electrophoretic (CE) method for simultaneous analysis of CAP, TAP and FF in poultry tissues has been developed (Kowalski et al., 2008). The method is based on deproteinization by acetonitrile and a solid phase extraction (SPE) procedure. Calculated LOD with this method is  $1.5 \text{ ng g}^{-1}$  for CAP,  $3.2 \text{ ng g}^{-1}$  for TAP and  $7.4 \text{ ng g}^{-1}$  for FF. Nagata et al., 1996, presented the detection of CAP, TAP and FF residue in yellowtail fish muscles by capillary GC-MS. In this case, the detection limit was  $5 \text{ g kg}^{-1}$  and recoveries were more than 65%. Pfenning et al., 2000 reported a GC method determining residues of CAP, FF and TAP in shrimps, with meta-nitrochloramphenicol (mCAP) as the internal standard. The method detection limits (LOD) were calculated as 0.7, 1.4, 2.4, and  $1.3 \text{ ng g}^{-1}$  for CAP, FF, FFA, and TAP, respectively. Several studies were performed based on GC in combination with chemical ionisation (CI)-MS providing

excellent analyte detection up to  $0.1 \text{ g kg}^{-1}$ . Shen et al., 2009 developed a sensitive method using GC-negative chemical ionization mass spectrometry (GC-NCI/MS) for the simultaneous determination of CAP, TAP, FF, and florfenicol amine (FFA) at trace levels in muscle and liver. LODs of  $0.1 \text{ g kg}^{-1}$  for CAP and  $0.5 \text{ g kg}^{-1}$  for TAP, FF, and FFA were obtained. Limian Zhao, 2009 introduced a method for the simultaneous determination of phenicol residues in honey. The analyte samples are purified by liquid/liquid extraction and SPE and are quantified by liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in negative ion multiple reaction monitoring (MRM) mode. Gantverg et al., 2003 suggested that LC-APCI(+)QqQ-MS offered sensitivity and selectivity better than that of GC-MS. The LOD was  $0.02 \text{ g kg}^{-1}$ . Mottier et al., 2003 also reported an LC-tandem MS method for CAP in meat and seafood. The use of ESI(+)QqQ-MS allows quantification of CAP at the level of  $0.05 \text{ g kg}^{-1}$  in fish and shrimps. The recovery of CAP spiked into chicken meat was  $60 \pm 5\%$ . Ramos et al., 2003 also used LC-ESI(-)-MS for the determination of CAP in shrimps. In this case the LOQ was  $0.2 \text{ g kg}^{-1}$ . Van De Riet et al., 2003 used a LC-ESI(-)-MS to determine phenicols in farmed aquatic samples. Dumont et al., 2006 reported a rapid and sensitive screening qualitative method using a surface plasmon resonance (SPR) biosensor for determination of all phenicol antibiotic residues in shrimps from a single sample extract. However, most of these methods are expensive and time consuming or they need complicated sample extraction steps.

In contrast, electrochemical sensors have received great interest over recent decades, since such devices meet many of these qualities, including simplicity and rapidity. Indeed, due to these superior characteristics, electrochemical sensors have been applied to many fields of analysis including biomedical, pharmaceutical, food and environmental applications (Barceló, 2007).

Therefore, CAP, TAP and FF molecules can be detected through their electrochemical reduction. Fossdal and Jacobsen, 1971 and later Duda and Kucharska, 1999 detected CAP in food samples by means of differential pulse polarography with mercury drop electrodes. The measurements of CAP detection were done in basic food products such as boiled and smoked ham, breasts of turkey and chicken, beef sirloin and rump, pork shoulder, liver and kidneys, milk, eggs and mayonnaise. In the tested food products CAP is present in quantities from a few to several hundred  $\text{ng g}^{-1}$ . Agüi et al., 2002 and Mena et al., 2003 reported on the determination of CAP by square-wave voltammetry at electrochemically activated cylindrical carbon fiber microelectrodes in milk samples. In both cases, the LOD was  $0.015 \text{ ng L}^{-1}$  ( $4.7 \times 10^{-8} \text{ mol L}^{-1}$ ). Differential pulse voltammetry at a glassy carbon electrode was described by Chai et al., 2006. The detecting limit of this method is  $0.83 \text{ g L}^{-1}$ . Xiao et al., 2007 introduced the application of voltammetry at a single-wall carbon nanotube-gold nanoparticle-ionic liquid composite film modified glassy carbon electrode (limit of detection was  $0.83 \text{ g L}^{-1}$ ). The use of a cationic surfactant, cetyltrimethyl ammonium bromide, improved the signal related to the reductive peak and therefore significantly improved the sensitivity of the voltammetric determination of CAP. Chuanuwatanakul et al., 2008 proposed cyclic voltammetry at a boron-doped diamond thin-film electrode in flow-injection mode for the detection of CAP the limit of detection of  $10.59 \text{ g L}^{-1}$  ( $0.03 \times 10^{-6} \text{ mol L}^{-1}$ ) ( $S/N = 3$ ). This method has been applied to the determination of CAP in sterile eye drops and milk samples. A disposable electrochemical sensor was developed for the sensitive determination of nitro aromatic compounds including CAP (Chen et al., 2006). Sensor strips incorporated an electrochemically pre-anodized screen-printed carbon three-electrode configuration for direct detection of nitro aromatic compounds by square-wave voltammetry. The analysis was done in a single

run simply by measuring the ratio of peak currents between analytes of interest and internal standard. The LOD for CAP was  $136 \text{ g L}^{-1}$  ( $0.42 \times 10^{-1} \text{ mol L}^{-1}$ ).

The combination of biomaterial such as antibodies and aptamers with electrochemical sensors (electrochemical biosensors) has been reported for the sensitive and selective determination of phenicols. A sensor modified with antibody was introduced for the detection of ultra trace amount of CAP in an impedimetric system (Chullasat et al., 2011). The LOD was found to be of  $1.0 \times 10^{-16} \text{ mol L}^{-1}$ . Recently, Pilehvar et al., 2012 reported an electrochemical aptasensor for the sensitive detection of CAP in the presence of TAP and FF. The use of selective aptamers towards CAP leads to a detection limit of  $0.5 \text{ g L}^{-1}$  ( $1.6 \times 10^{-9} \text{ mol L}^{-1}$ ) in buffer solution. Yan et al., 2012 developed a novel electrochemical aptasensor for highly sensitive detection of CAP in honey based on target-induced strand release (TISR). Under optimal conditions, the dynamic linear range is in the range from 1 to  $1000 \times 10^{-9} \text{ mol L}^{-1}$  with the detection limit of  $0.1 \text{ g L}^{-1}$  ( $0.29 \times 10^{-9} \text{ mol L}^{-1}$ ) of CAP.

A few interesting examples of using molecular imprinted polymers (MIP) for detection of phenicols have been described (Thongchai et al., 2010; Suarez-Rodriguez and Diaz-Garcia, 2001). MIPs are fabricated by co-polymerization of suitable functional monomers and cross-linkers in the presence of the target molecule. Removal of the template forms a polymer that selectively recognizes the target analyte. MIPs are usually reusable and exhibit high stability. Mena et al., 2003 used MIP as a selective SPE sorbent prior to the voltammetric determination of CAP. In these studies large volumes of milk sample (17 mL) had to be passed for long time periods (68 min) to reach a detection limit of  $4.5 \text{ g L}^{-1}$  ( $4.7 \times 10^{-8} \text{ mol L}^{-1}$ ).

## ***2-1- Electrochemical Biosensors***

A biosensor is an analytical device combining a biorecognition element with a transducer. The biorecognition element is usually an enzyme, a receptor protein, an antibody/antigen, DNA/RNA, or living cells. A transducer converts the biological reaction or binding into a readable electrical signal. There are many different types of biosensors that can be classified (1) according to the type of biological element used, *e.g.* immunological or enzymatic, or (2) according to the transducer system applied, *e.g.* optical, potentiometric, amperometric, or thermal. For the detection of veterinary drug residues, the most often used biological element are antibodies or ssDNA or RNA aptamers. In addition, the detection of antimicrobial agents in food samples is mostly done based on electrochemical or optical transducing principles. Electrochemical biosensors can be a suitable alternative for the urgent need for rapid, high capacity, highly selective and sensitive screening methods for the detection of antibiotic residues in food (Christ and Bendas, 2011).

### ***2-1-1- Enzymes as bio-recognition element***

Enzymes are widely explored as biorecognition elements in biosensors due to their specificity towards their target. The detection principle is based on measuring the enzymatic product that, depending on its properties, can be sensed by several mechanisms like a change in pH or temperature and optical or amperometric detection. Enzyme based electrochemical biosensors are widely used for applications in food analyses, *e.g.* for the detection of glucose, carbohydrates, pesticides, ethanol, starch and phenols (Kobos, 1987; Yang, 2012). Applying enzymatic biosensors for the detection of antibiotic residues are limited. Rincken and Riik, 2006 developed a lactate oxidase-based amperometric biosensor for the fast determination of

CAP and penicillin residues in milk samples. As there is are significant amounts of bacteria in milk, the respiration process has a great influence on the dynamics of the biosensor signal. The bacterial respiration in air-saturated milk samples is tracked with an oxygen sensor. The decrease of oxygen concentration in the reactor due to the lactate oxidase catalyzed reaction was followed after the elimination of the bacterial respiration. Quantitative analysis of antibiotics was determined by two main characteristic reaction parameters, the total biosensor signal change and the initial maximal slope of signal decrease. The biosensor signal showed the dynamic decrease of oxygen content in milk due to the oxidation of lactate, catalyzed by lactate oxidase and influenced by the presence of antibiotics. As another example, Setford et al.,1999 described  $\beta$ -lactam antibiotic residues in milk based on glucose oxidase with an amperometric transducer. However, there are several limitations in their application. Enzymes always require a co-factor, they are difficult to be purified and in addition they are expensive, and rather unstable. Moreover, they suffer from low sensitivity, the concentration detection of the sensor based on enzymes was only in the ppm ( $\text{mg kg}^{-1}$ ) range.

### ***2-1-2- Antibody as bio-recognition element***

In the field of antibiotic residue monitoring, the most commonly used biological element is the antibody/antigen affinity pair which is widely used in the immunochemical screening of samples. In this case, the biosensor is called an immunosensor. Until now, enzyme-linked immunosorbent assay (ELISA) is the most often used screening method for the determination of phenicols. ELISA is a sensitive, low cost, and reliable method for the detection of veterinary drug residues in different food

samples. In addition, ELISA is very suitable for high-throughput screening purposes. The assay can be in direct format, when antibodies are immobilized on a surface or in an indirect format when an analyte is immobilized.

An immuno voltammetric technique was used for CAP detection in milk (Wei-Wei et al., 2007). The main part of the assay was an indirect ELISA followed by voltammetric measurement after reaction. *p*-nitrophenylphthalate has been used as a substrate which was oxidized to *p*-nitrophenol by alkaline phosphatase as an enzyme label. Then the reaction was stopped and a small-sized electrochemical sensor with a glassy carbon electrode was inserted into the wells of the plate to quantify *p*-nitrophenol by differentiated pulse voltammetry. The method has a sensitivity of  $0.064 \text{ g L}^{-1}$  for CAP in milk.

Recently an amperometric immunosensor for CAP was developed (Kim et al., 2010). The LOD calculated is  $0.045 \text{ g L}^{-1}$ . Modification of a glassy carbon electrode with gold nanoparticles, dendrimers, and cadmium sulfide nanoparticles ensured the high sensitivity of the assay. The immunosensor operated in competitive mode, and the detection was based on electrochemical reduction of hydrogen peroxide catalyzed by hydrazine linked with CAP. The immunosensor assay was examined in beef, chicken, and pork.

Ultra trace analysis of CAP by a real-time, label-free impedimetric immunosensor was described by Chullasat et al., 2011 and based on the change in interfacial resistance and/or capacitance between the electrode surface and analyzed solution once immobilized antibodies react with an antigen. An electrode was modified with a self-assembled thiourea monolayer, gold

nanoparticles, mercapto succinic acid and antibody. The LOD was  $3.2 \times 10^{-8} \text{ g kg}^{-1}$  ( $1.0 \times 10^{-16} \text{ mol L}^{-1}$ ) in buffer and  $1.6 \text{ ng kg}^{-1}$  in shrimps. It is reported that the modified electrode could be reused up to 45 times.

### ***2-1-3- Aptamers as bio-recognition element***

Aptamers are small oligonucleotides consisting of nucleic acids that can bind with high affinity to a given target molecule due to their distinct secondary and tertiary structures. Aptamers are selected from a random pool by an in-vitro process called SELEX and can recognize a specific molecule (Radi, 2011). Aptasensors offer high reproducibility and stability against a wide variety of targets and are quickly arising as suitable candidates for high throughput analytical methods.

Pilehvar et al., 2012 reported on a novel, label-free folding induced aptamer based electrochemical biosensor for the detection of CAP in the presence of its analogues in milk samples (Mehta et al., 2011). The modification of the gold surface with aptamers was characterized by EIS and FT-IR techniques. The thiolated aptamers were immobilized onto a gold electrode surface by means of a self-assembling method. There is a considerable increase in CAP reduction current due to the high affinity binding of the aptamers towards CAP which results an increased electron transfer. To investigate the specificity of the aptasensor toward CAP, experiments were performed in the solution containing TAP and FF which may interfere with the detection of CAP. The developed aptasensor shows a LOD of  $1.6 \times 10^{-9} \text{ mol L}^{-1}$  in buffer and milk.

An electrochemical aptasensor for detection of CAP in honey, based on target-induced strand release (TISR), was introduced by Yan et al., 2012. The CAP aptamer was immobilized on the electrode and then hybridized with the complementary



oligonucleotide (biotinylated detection probe) to form the aptamer/DNA duplex. In the presence of CAP the biotinylated detection probe dissociated from the electrode. Then the binding of streptavidin-alkaline phosphatase (STAP) to the remaining biotinylated detection probe led to an enzyme-amplified electrochemical signal and the signal decreased with increasing CAP concentration (Mehta et al., 2011).

### ***2-2- Electrochemical sensors***

Electrochemical sensors have a wide range of applications in a diversity of domains including environmental monitoring, quality control of food, and clinical analysis. Currently, researchs in this field focus on novel sensing strategies with specific attention to the improvement of specificity, sensitivity, and response time. Providing a high degree of selectivity to electrochemical transducers can be done with chemical or electrochemical modifications. Basically, the modification of an electrode can be immobilization of reagents or electrochemical pretreatment that influence the electrochemical properties of the bare surface. On the other hand, sensitivity of electrochemical sensor is greatly influenced by the applied transduction principle. These electrochemical principles can be categorized as conductivity, potentiometric, amperometric, and voltammetric sensors (Barceló, 2007). For example, simultaneous detection of CAP, TAP and FF by voltammetric methods was reported by Pilehvar et al., 2012. The electrochemical behaviour of CAP in the presence of its derivatives was investigated by CV and SWV. At a gold electrode, CAP results in a sensitive cathodic peak where the potential for CAP reduction is in different range in which TAP and FF reduction happens. This behavior offers the opportunity to introduce a method for

sensing CAP electrochemically in the presence of its derivatives. Calibration graphs were linear in the  $2.5 \times 10^{-6}$ - $7.4 \times 10^{-6}$  mol L<sup>-1</sup> concentration range.

### ***2-2-1- Potentiometric detection***

By potentiometry charged molecules can be detected and the presence of these charged molecules on the surface of the electrode or at the surface of the coating on the electrode leads to a change in potential that can be measured versus a reference electrode (e.g. Ag/AgCl). The equation that describes this principle in an empirical way is the Nickolskii ó Eisenmann equation, which is a derivate of the Nernstian equation:

$$E = E^0 + \frac{59.1\text{mv}}{n} * \log[c_i + K_{i,j}^{\text{pot}} * c_j],$$

Where, E is the recorded potential in mV, n is the charge of the analyte, c<sub>i</sub> the concentration of the analyte, K<sub>i,j</sub><sup>pot</sup> the selectivity coefficient and c<sub>j</sub> the concentration of the interfering ions.

This equation shows us the relation between the intensity of the signal and the concentration of the analyte and compensates for the presence of interfering ions in the solution.

Ganjali et al., 2012 proposed a sensor based on CAP biomimetic molecular imprinted polymer used as a sensing element in a nano-composite carbon paste potentiometric sensor. This molecularly imprinted polymer is used as a coating due to its selectivity towards CAP, created by the unique properties of the molecularly imprinted polymer, and also serves as the

recognition element in order to create an ion-selective electrode. The best results were achieved using a coating with a composition of 5% MWCNT (multi walled carbon nanotubes), 1% NS (nano silica), 20% CAP-MIP (chloramphenicol ó molecularly imprinted polymer), 20% RTIL (room temperature ionic liquid) and 54% graphite powder. This gave a typical nernstian response with a slope of 59.1 mV (+/- 0.4 mV) and a linear range between  $10^{-6}$  mol L<sup>-1</sup> and  $10^{-2}$  mol L<sup>-1</sup>. The detection limit of the proposed sensor is  $1.0 \times 10^{-6}$  mol L<sup>-1</sup> (0.323 mg L<sup>-1</sup>). The response of this sensor was 3.5 to 5 and displayed very good selectivity, response time (18 sec.) and lifetime. Tests on real samples were conducted on CAP in pharmaceutical formulations and showed a good recovery percentage value varying between 97.9-103.4%.

### ***2-2-2- Flow injection Analysis***

Flow injection analysis (FIA) implies the use of a hydrodynamic flow cell where the solution electrochemical sensor is continuously washed by the buffer solution except during measurements when the analyte solution is injected onto the sensor instead. This approach has advantages over batch as it allows repeated independent measurements that can be performed quickly in succession of each other, on the same sensor. FIA coupled with amperometric detection allows us to see a current peak every time an oxidizable or reducible molecule is injected on the electrode. The continuous flow of buffer will remove the oxidized or reduced components, clearing the electrode for the next measurement (Hernandez et al., 2011).

In this manner Liao et al., 2007 proposed a sensor using a disposable wall-jet ring disk carbon electrode. CAP was detected on this sensor by using cyclic voltammetry and amperometry coupled to a FIA setup. Because CAP is irreversibly reduced from

Ph-NO<sub>2</sub> to Ph-NHOH at a potential of -0.6 V (vs. Ag/AgCl), this reduction peak lies very close to the reduction peak of O<sub>2</sub>. Therefore, it is impossible to get accurate readings for CAP in the presence of oxygen. In order to overcome this problem the authors used a FIA setup combined with a screen printed ring disk carbon electrode. In this setup the disk carbon electrode is set at -0.7 V so it irreversibly reduces the Ph-NO<sub>2</sub> to Ph-NHOH. The actual determination of CAP then happens at the ring carbon electrode, which stands at a potential of +0.3 V (vs Ag/AgCl), where Ph-NHOH is oxidized to Ph-NO. This helps to avoid interference from oxygen. For the FIA conditions it was found that the current signals decreased and the repeatability increased as a result of increasing the flow rate. In optimal conditions a linear region was found between 0.1 and 20×10<sup>-6</sup> mol L<sup>-1</sup> for signal current versus concentration with a slope of 0.175 (×10<sup>-6</sup> A/M) and a LOD (S/N = 3) of 74 ×10<sup>-9</sup> mol L<sup>-1</sup> (0.024 mg L<sup>-1</sup>).

Chuanuwatanakul et al., 2008 proposed a sensor based on a boron-doped diamond thin-film electrode. CAP was determined by the use of cyclic voltammetry, hydrodynamic voltammetry and a FIA system coupled to amperometric detection. The proposed analysis with the boron-doped diamond thin film electrode at -0.7 V (vs. Ag/AgCl) was applied to determine CAP in eye drops and spiked milk samples. This method had a linear range between 10<sup>-7</sup> mol L<sup>-1</sup> and 5×10<sup>-5</sup> mol L<sup>-1</sup>, LOD (S/N = 3) of 30 ×10<sup>-9</sup> mol L<sup>-1</sup> (0.010 mg L<sup>-1</sup>), high sensitivity and provided reproducible responses For the FIA conditions a flow rate of 1 mL min<sup>-1</sup> was chosen as a compromise between sensitivity and consumption of the buffer solution.

Codognoto et al., 2010 analyzed the electroanalytical performance of a self-assembled monolayer gold electrode for CAP determination. CAP was analyzed on the gold electrode through the use of cyclic voltammetry, electrochemical impedance spectroscopy and a FIA setup coupled to amperometric detection. The self-assembled monolayer gold electrode was used as a working electrode in an electrochemical cell at -0.75V (vs. Ag/AgCl). CAP was determined in these amperometric cells in an ophthalmic solution and compared to a HPLC method coupled to an UV-detector. The results show that the mean values obtained from the self-assembled monolayer gold electrode did not differ significantly from the values obtained from the UV-detector. In addition the monolayer inhibits the absorption of thiomersal, a preservative commonly used in ophthalmic formulations, granting this sensor an advantage over other gold sensors as it is not inactivated during use. A flow rate of 3 mL min<sup>-1</sup> was chosen for the FIA setup. The detection limit was (S/N = 3) found to be 44 ×10<sup>-6</sup> mol L<sup>-1</sup> (14.218 mg L<sup>-1</sup>) and the linear range of the sensor lay between 0.50×10<sup>-4</sup> mol L<sup>-1</sup> and 10<sup>-3</sup> mol L<sup>-1</sup>.

Norouzi et al., 2010 used the Fast Fourier transformation with continuous cyclic voltammetry at Pt-Au dual microelectrode for the determination of CAP in a FIA system. The dual microelectrode was used in a FIA setup with the flow rate being 7 mL min<sup>-1</sup> combined with an extremely fast scan rate at 70 Vs<sup>-1</sup>. The reason for this very fast sweep rate is the requirement to complete a full cyclic voltammogram before the CAP is washed away by the FIA from the electrode. In this method, the potential wave form consisted of two potential sections for cleaning of the electrode surface, accumulating CAP and a potential ramp, which was continuously applied on the dual disk microelectrodes. Combined with the Fast Fourier transformation in the form of the computer program, that provided an advanced algorithm for integration and filtering, gave this method the

advantage of detecting CAP even in the presence of oxygen and resulted it one of the lowest detection limits of the papers discussed in this section, i.e.  $2.0 \times 10^{-9} \text{ mol L}^{-1}$  ( $0.0006 \text{ mg L}^{-1}$ ) with a linear range over the concentration range  $8.0 \times 10^{-9} \text{ mol L}^{-1}$  to  $2.0 \times 10^{-5} \text{ mol L}^{-1}$  (see table).

As mentioned before Chullasat et al., 2011 performed an ultra-trace analysis of a small molecule (CAP) by a label-free impedimetric immunosensor using a multilayer modified electrode. CAP was determined by a flow injection system combining a  $10 \mu\text{l}$  flow cell with a flow rate of  $100 \mu\text{l min}^{-1}$ . Three types of electrodes were tested under these conditions, the first was a self-assembled thiourea monolayer (SATUM) electrode, the second was generated by modifying the electrode with gold nanoparticles (SATUM/AuNP) in combination with SATUM and the third was modified with the self-assembled monolayer, gold nanoparticles and mercaptosuccinic acid (SATUM/AuNP/MSA). All three electrodes were then modified with the anti-CAP antibody to give them their specific reactivity. This gave the SATUM a linear range of  $1.0$  to  $10 \times 10^{-14} \text{ mol L}^{-1}$ , the SATUM/AuNP a linear range of  $1.0$  to  $10 \times 10^{-15}$  and the SATUM/AuNP/MSA a linear range of  $0.50$  to  $10 \times 10^{-16} \text{ mol L}^{-1}$ . As a result the lowest determination limit was  $0.50 \times 10^{-16} \text{ mol L}^{-1}$  ( $1.616 \times 10^{-11} \text{ mg L}^{-1}$ ) for these types (SATUM/AuNP/MSA) of electrodes.

### ***2-2-3- Nanotechnology***

Nanomaterials (NM) play an important role in sensing and biosensing due to their unique electrical, optical, catalytic or magnetic properties. The high surface-to-volume ratio and dispersion ability of NMs provide a wide adsorptive surface for

target species (Xie et al., 2008). In order to enhance the sensitivity of the sensor, several NMs including multi-walled carbon nanotubes (MWCNTs) and gold nanoparticles (GNPs) were introduced owing to their unique properties (Luo et al., 2001; Li et al., 2009).

Xiao et al., 2007 has fabricated composite film modified glassy carbon electrode based on single-wall carbon nanotube (SWNT), gold nanoparticle (GNP) and ionic liquid. As a result, CAP does not produce discernible peaks on 1-octyl-3-methylimidazolium hexafluorophosphate (OMIMPF<sub>6</sub>)/GCE, bare gold electrode or bare GCE under this condition. On SWNT/GCE, CAP produces a small cathodic peak at 0.66V (vs. SCE). When GNP or OMIMPF<sub>6</sub> is present, the peak increases. On the OMIMPF<sub>6</sub>/GNP/SWNT/GCE, the peak is enhanced further. While the SWNT catalyze the CAP reduction, OMIMPF<sub>6</sub> can extract CAP from the bulk solution and GNP can promote the electron transfer of CAP. Therefore, the combination of OMIMPF<sub>6</sub>/GNP/SWNT provides the best sensitivity. Under the optimized conditions, the peak current is linear to CAP concentration in the range of  $1.0 \times 10^{-8}$  to  $6.0 \times 10^{-6}$  mol L<sup>-1</sup>, and the detection limit was about  $5.0 \times 10^{-9}$  mol L<sup>-1</sup>. The developed sensor shows advantages in terms of repeatability and sensitivity.

#### ***2-2-4- Molecular imprinted polymer (MIP)***

Recently, a few applications of molecularly imprinted polymers (MIP) have been reported. The advantages of using MIPs in electrochemical sensors are related to their ability to selectively bind the target molecules. Therefore a MIP-based sensor should reduce the number of false positives that often occur with other types of sensors.

Alizadeh et al., 2012 introduced a novel voltammetric sensor for the determination of CAP in milk samples. The CAP selective MIP and non-imprinted polymer (NIP) were synthesized and then added to the carbon paste (CP) electrode composition in order to prepare MIP-CP electrodes. The MIP acted as the selective recognition element and pre-concentrator agent for CAP. The differential pulse voltammetry (DPV) signal of a MIP-CP electrode towards CAP is higher than that of the NIP-CP electrodes showing that the MIP-CP electrode can accumulate CAP from the aqueous solution more effectively, compared to the NIP-CP. They also tested the response behavior of some nitro aromatic compounds like metronidazole, para-nitrophenol and nitrobenzene. It can be concluded that in the proposed electrode, the signals of the tested interfering agents are noticeably lower than that of CAP despite the fact that their concentrations are noticeably higher than that of CAP. The analytical usefulness of the prepared electrochemical sensor was demonstrated by applying it to the determination of CAP in milk samples. The sensor showed a linear response range of  $8.0 \times 10^{-9}$ - $1.0 \times 10^{-6}$  mol L<sup>-1</sup> and LOD of  $2.0 \times 10^{-9}$  mol L<sup>-1</sup> (S/N = 3).

Another example of using MIPs for the determination of CAP is reported by Nuo-Wei et al., 2008. In this article, MIPs were prepared for the detection of chloramphenicol succinate (CAP-SC) by a photopolymerization method on screen-printed electrodes and the synthesized films were analyzed using SEM. The authors believe that micropores are formed after the template molecules (CAP-SC) accumulated during the polymerization. A sensor based on MIP for the detection of CAP-SC was developed after the screen printed electrode was connected to an electrochemical analyzer through an electrode slot, and the detection result was recorded by the recorder connected to an electrochemical analyzer. The CV was obtained from the standard CAP-SC solutions recorded. The reduction peak current appeared at 0.3 V (vs. Ag/AgCl) and the absolute value of



the peak current was increased with the concentration of CAP-SC. The developed method also demonstrated a good linear response of reduction peak current to CAP-SC concentration over the  $1 \times 10^{-8}$  to  $1.2 \times 10^{-5}$  mol L<sup>-1</sup> range with a LOD of  $2 \times 10^{-9}$  mol L<sup>-1</sup>. Changes of the reduction peak currents from the N-MIF was not significant compared with the voltammogram obtained from MIF, indicating that the interaction between the MIF and the target molecules changed the electrochemical property of the films on the screen printed electrode (SPE).

Mena et al., 2003 studied the performance of a MIP as a selective solid-phase extraction sorbent, coupled to voltammetric detection, for efficient sample clean up and selective pre-concentration of CAP in ophthalmic solutions and milk. In addition, the effect of the presence of different percentages of methanol in the electrochemical cell containing 0.05 mol L<sup>-1</sup> phosphate buffer (final volume 5.0 mL), on the SW voltammetric response of CAP was checked. When the methanol percentage increased a substantial decrease in the cathodic net peak current was observed. A linear calibration graph was obtained over the  $1.0 \times 10^{-6}$  to  $1.0 \times 10^{-5}$  mol L<sup>-1</sup> (0.32 to 3.2 mg L<sup>-1</sup>) concentration range. A LOD of  $8.8 \times 10^{-7}$  mol L<sup>-1</sup> was calculated for CAP according to the 3 S/N criterion. In order to investigate the selectivity of the sensor, several substances, such as TAP, chloramphenicol diacetate and chloramphenicol base, all with molecular structures similar to CAP. The obtained results demonstrate the usefulness of this methodology. Application of the developed sensor for CAP detection was performed in UHT full-cream and skimmed milk samples.

In addition, the combination of the NMs with MIP offered attractive strategies to enhance the sensitivity and stability of the sensor (Gao et al., 2001; Xie et al., 2006; Banholzer et al., 2008; Okunola et al., 2008). Zhao et al., 2012 introduced a novel electrochemical sensor for the detection of CAP by modifying carboxyl acid functionalized multiwall carbon nanotubes (MWCNT) doped with AuNPs with a MIP film on the surface of a glassy carbon electrode (MIP/MWCNTs-AuNPs/GCE). To characterize the electrochemical properties of the electrodes, cyclic voltammetric experiments were performed. The bare GCE and the *c*-MWNTs- AuNPs/GCE show typical reversible oxidation and reduction peaks, where the redox peak current of *c*-MWNTs- AuNPs/GCE is higher compared to the bare GCE. This phenomenon can be attributed to the improved electron transfer kinetics and the increased adsorption sites of the modified electrode owing to the highly electro active surface area and lower electron transfer resistance of *c*-MWNTs-AuNPs/GCE. Assembling the MIP on the surface, both the imprinted and non-imprinted electrode show a decreased current of  $[\text{Fe}(\text{CN})_6]^{3/4}$  which is due to the blocking effect of the polymerized film. The morphologies of the electrodes before and after MIP polymerization were characterized by TEM and SEM. Both techniques confirm that the AuNPs are uniformly distributed on the surface of the *c*-MWNTs. The author observed that while there is no oxidation peak on the bare GCE, all the modified electrodes exhibit a cathodic peak at approximately 0.6 V, illustrating their improved electron transfers property. Moreover, the effects of accumulation time, the pH value and the concentration of the supporting electrolyte were studied. The peak current increases continuously with the accumulation time in the detection of 10 mg L<sup>-1</sup> CAP on the MIP/MWCNTs-AuNPs/GCE electrode. Dependence of the peak current on the pH value in the range of 4.5-8.5 suggests that only in the range of 4.5-6.5 of PH, the current increases with the pH value. However, the current is stable

between pH 6.567.0 and decreases when the pH value is higher than 7.0. In addition, the results show that the electrolyte concentration has little effect on  $\Delta i$  from 0.05 mol L<sup>-1</sup> to 0.15 mol L<sup>-1</sup>. Therefore, the authors used a universal medium concentration of the electrolyte of 0.1 mol L<sup>-1</sup> phosphate buufer solution (PBS). The applicability of the developed sensor was also investigated using natural seawater from the Black Reef Bay (Dalian, China) and reservoir water from West Mountain Reservoir (Dalian, China) spiked with a known concentration of CAP.

### ***2-2-5- Sensor Pretreatment***

Electrochemical pretreatment was the first method used for activation and improvement of the electrode response. Since the time of its first use, the technique has become widely used to activate electrode performance.

Agüí et al., 2002 reported on the voltammetric determination of CAP in milk at electrochemically activated carbon fiber microelectrodes (CFMEs). Different electrochemical activation pretreatments for CFMEs were checked for CAP. Moreover, different activation times were selected to observe the difference between the voltammetric signals. The CAP net current increased with the activation time up to 5-7 s. Effect of different scan rates were also tested by CV. Besides, the peak potential shifted to more negative values as the scan rate increased. A linear  $i_p$  versus  $v^{1/2}$  plot was found for low scan rates (50-100 mVs<sup>-1</sup>), and corresponds to a diffusion-controlled process. The best CV pretreatment yielded a net current which is much lower than that obtained with SWV activation. The CAP net current obtained was 58% lower in DPV pretreatment when compared with that obtained after the SWV activation. Also, a narrow and symmetrical cathodic peak, with a high net current, appeared when

using the activated fiber microelectrodes. These results were attributed to the increase of the carbon fiber surface area related to its fracture and the appearance of deep fissures. The authors state that different chemical and electrochemical variables have an influence on the activation step, i.e. potential range, number of scans, pH value, type and concentration of electrolyte, as well as the typical parameters of SW. Well-defined CAP cathodic peaks were obtained after electrode activation by cycling at potentials more positive than 2.0 V (vs. Ag/AgCl). Furthermore, a slight decrease of the CAP net current was observed when the pH of this buffer was increased in the 2.0-10.0 range. In addition, the maximum net current responses for CAP were reached when square wave amplitude of 50 mV, a step height of 4 mV, and a frequency of 25 Hz were used for the activation

Chen et al., 2006 reported on a novel approach using a single-use amperometric sensor strip incorporating a three-electrode configuration for convenient, fast, low sample volume and direct detection of nitroaromatic compounds. The voltametric behavior of nitroaromatic compounds at the preanodized screen printed carbon electrode (designated as SPE) was used as working electrode. To prove the broad applicability of the proposed sensors, several model analytes such as CAP, parathion (an organophosphate nerve agent), and 2,4,6-trinitrotoluene (TNT, an explosive) was chosen for study. A well-defined and narrow cathodic peak of these compounds was observed at the SPE. The preanodization treatment makes the reduction peak sharp and hence provides a precise way to identify the substituent effect on nitroaromatic compounds. Characteristic potential shifts are observed for all nitroaromatic compounds, and the shifts depend on the position of the substituent groups. Most

important of all, the single-run approach with the combination of disposable preanodized nafion-coated screen-printed carbon working electrode and portable electrochemical instrument would benefit the on-site monitoring of nitroaromatic compounds.

An electrochemically pretreated glassy carbon electrode (EPGCE) was used by Alemu et al., 2007 for sensitive determination of CAP. Electrochemical pretreatment of glassy carbon electrode was performed by anodic oxidation. The peak current of CAP obtained at EPGCE is 2.2 times greater than that of the bare glassy carbon electrode. A various buffer solutions as supporting electrolytes were tested to check their suitability in the determination of CAP. The most suitable buffer solution was chosen to be acetate buffer. The influence of pH on the peak of CAP was investigated over the range of pH 0.9 to 11.0. The peak current is low at high pH ranges and starts increasing as the pH decreases and reaches a maximum value at pH 5.3. The influence of voltammetric parameters on the current was studied to fix the optimum conditions in the determination of CAP. The peak current increased sharply up to an amplitude of 50 mV and then reached a steady state value. The effect of the potential step on the peak current was also investigated. The plot of the peak current as a function of  $\Delta E_s$  increased sharply at the beginning and continued increasing gently. A potential step of 14 mV was chosen as the optimum value for the analysis. At lower frequencies the current response was very low. At high frequencies the peak current increased almost linearly with increase in  $f$ . However, the shape of the voltammograms became broader as  $f$  increased. The effect of the initial sweep potential on the peak current was also examined. The peak current did not show significant change within the range. Two good linear ranges were obtained between the voltammetric current and CAP concentration. The first was in the linear range  $1.0 \times 10^{-7}$  -  $5 \times 10^{-6}$  mol L<sup>-1</sup> CAP and the second in the linear range  $5 \times 10^{-6}$  to  $7 \times 10^{-5}$  mol L<sup>-1</sup> CAP. For a series of six determinations

of CAP at  $1.00 \times 10^{-5} \text{ mol L}^{-1}$  and  $5.00 \times 10^{-7} \text{ mol L}^{-1}$  levels relative standard deviations of 2.2 % and 3.7 %, respectively were obtained, showing an excellent reproducibility of the EPGCE. When the signal to noise ratio is 3, the LOD was  $6.0 \times 10^{-9} \text{ mol L}^{-1}$ . The developed method did not suffer any interference from preservative agents in the eye drops such as phenyl mercuric nitrate. The detection of CAP eye drops was carried out using the standard addition method in the concentration range that fell within the linear range of CAP concentrations.

Codognoto et al., 2010 prepared monolayers of 2-mercapto-5-methylbenzimidazole (MMB) on a polycrystalline gold electrode via a self-assembly process to produce a self-assembled monolayer. The resulting electrode was investigated by CV and EIS, and applied to the determination of CAP using flow injection analysis along with amperometric detection. The EIS spectrum shows a semi-circle which shows the electrode reaction is purely kinetically controlled, and the charge transfer resistance is expected to increase due to the inhibition of electron transfer by the monolayer on the electrode surface. CV results suggest that the nitro group from the CAP reduces in a first stage to hydroxylamine. At the gold bare electrode the hydroxylamine is adsorbed on the electrode surface and thus in the second scan the oxidation to a nitrous derivative. The absence of this peak in the cyclic voltammogram on the AuMMB-modified electrode shows that the monolayer prevents the adsorption of the hydroxylamine on the thiol modified gold electrode surface. The signal is linear with CAP concentration in the range from  $0.050$  to  $1.000 \times 10^{-6} \text{ mol L}^{-1}$ , and the limit of detection is  $44 \times 10^{-6} \text{ mol L}^{-1}$ .

### ***3-Future directions***

Many studies focus on developing new sensitive analytical devices to reach the lowest possible detection limit for phenicol antibacterial agents in real food samples. However, the real sample analysis needs complicated and time consuming cleaning and preparation steps. Therefore, there is a need to intensify research to find a solution for reducing the sensors performance length. The recent and obvious trend has been in the direction of reduced time and increased sensitivity and performance of sensors. Electrochemical sensors based on novel selective biomolecules for detection of phenicols continue to be reported in the literature. In addition, the use of newly developed nanomaterials has developed a new generation of electrochemical sensors in the field of veterinary drug residue determination in food samples.

#### ***4- Conclusion***

This review has focused on the application of electrochemical (bio)sensors for the detection of phenicolø in food analysis. With increasing use of antibacterial agents in animal treatment, monitoring of residual levels in foodstuffs became more and more important for food safety programs. Various examples of chromatography, QCM, ELISA and electrochemical techniques for the detection of the three members of the phenicolø family have been described, together with their applications in food samples during the last 10 years. However, each technique has its own benefits and limitations but the electrochemical (bio)sensors have undisputed advantages over the classical methods. The electrochemical detection of chloramphenicol (CAP), thiamphenicol (TAP) and florfenicol (FF) offers high sensitivity and selectivity, they are easy to operate and cost-effective which makes this technique promising. Electrochemical (bio)sensors have received great attention for nearly fifty years and

seem to possess great potential for the future. The combination of selective recognition biomolecules including aptamers, enzymes, and antibodies with the high sensitivity of electrochemical detection is promising for monitoring phenicol residues in foodstuff. Potentiometric detection based sensors are suitable for measuring low concentrations and small volumes of phenicol antibiotics, since they have the advantage not to chemically modify a sample. Flow injection systems were used as a separation and pre-concentration step in electrochemical analysis of CAP, TAP and FF in food products and have been summarized in the present overview. Recently, nanotechnology has become more and more used since it offers a solution for integration and high throughput for various electrochemical sensor applications in the field of monitoring consumer products. The unique characteristics of nanomaterials suggest excellent prospects for the interfacing recognition event with signal transduction.

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**Table 1. Legal status of phenicol's**

<b>Compounds</b>	<b>Legal status</b>	<b>Regulation</b>	<b>structure</b>
Chloramphenicol (CAP)	POM*	675/92 1430/94	<inline-graphic>
Florfenicol (FF)	Vetereniray prescription only	2703/94 613/98 2385/99 1322/2001 1181/2002	<inline-graphic>
Thiamphenicol (TAP)	None	895/93 1798/96 1000/98 804/99 1299/2005 1805/2006	<inline-graphic>

\* POM: Prescription only medicine

**Table 2. Alternative methods for phenicol's detection**

Method	Analyte	Matrix	LOD	References
Capillary GC-MS	CAP, TAP, FF	Fish muscels	5 $\mu\text{g kg}^{-1}$	Nagata et al. 1996
GC	CAP, TAP, FF	Shrimp	0.7, 1.4, 1.3 $\text{ng g}^{-1}$	Peening et al. 2000
LC/MS/MS	CAP	shrimp	0.08 $\text{g kg}^{-1}$	Neuhaus et al. 2000
HPLC / PDA GC- (EI)-MS LC- (NESI)-MS/MS	CAP	Pork liver and muscle	25 $\text{ng kg}^{-1}$ 5 $\text{ng kg}^{-1}$ 20 $\text{ng kg}^{-1}$	Li et al. 2002
ELISA GC6MS2 and LC6MS2	CAP	Shrimp	0.1 $\text{g kg}^{-1}$	Impens et al. 2002
LC6 (NESI)-MS/MS	CAP	Chicken meat	0.01 $\text{mg Kg}^{-1}$	Mottier et al.2003
GC-(NCI)-MS LC 6 (NAPCI)-MS/MS	CAP	Equine, porcine, bovine muscle	2 $\text{mg Kg}^{-1}$ 0.02 $\text{mg Kg}^{-1}$	Gantverg et. Al 2003



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LC/MS	CAP, TAP, FF	Farmed aquatic species	0.1, 0.3, 0.1 ng g <sup>-1</sup>	Van De Riet et al. 2003
GC/MS	CAP	Shrimp	0.2 µg kg <sup>-1</sup>	Ramos et al., 2003
QCM	CAP	No	3.2 mg kg <sup>-1</sup>	Park et al., 2004
LC-(ESI)-MS/MS	CAP	Rainbow trout muscle	0.26 mg Kg <sup>-1</sup>	Santos et al. 2005
Chemiluminescent ELISA	CAP	Pork, Beef, chicken, shrimp, milk	3.2 g kg <sup>-1</sup>	Park and Kim, 2006
Qflex kit	CAP	Milk, chicken, honey, shelfish assay	0.02, 0.02, 0.07, 0.07 g kg <sup>-1</sup>	Ferguson et al. 2003
MEKS	CAP, TAP, FF		5.3; 4.3, 4.8 g kg <sup>-1</sup>	Pezza et al., 2006
Chemiluminescent ELISA	CAP	Chicken muscle	6 ng g <sup>-1</sup>	Zhang et al. 2006
GC/MS	CAP, TAP, FF	Pork, poultry, aquatic products	0.03 µg kg <sup>-1</sup>	Li et al. 2006

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SPR	CAP,TAP, FF	Shrimp	0.1 g Kg <sup>-1</sup>	Dumont et al. 2006
LC6ESI6MS6MS	CAP	milk powder	0.09 g kg <sup>-1</sup>	Rodziewucz et al. 2007
GC6MS	CAP	rainbow trouts	0.2 g kg <sup>-1</sup>	Santos et al.
MEKS	CAP, TAP, FF	NO	1.5; 3.2; 7.4 ng g <sup>-1</sup>	Kowalski et al., 2008
LC6ESI-MS/MS	CAP, TAP, FF	Chicken muscle	0.1 g kg <sup>-1</sup>	Zhang et al.2008
LC-(ESI)-MS/MS	CAP	Shrimp	0.06 µg kg <sup>-1</sup>	Tyagi et al. 2008
SPR	CAP	Honey	32 pg g <sup>-1</sup>	Yuan et al. 2009
HPLC-DAD	tetracyclin, sulphonamide, CAP	bovine milk	20 ng g <sup>-1</sup>	Mamani, et al. 2009
HPLC/MS/MS	TAP, FF	Pork, porcine liver, porcine kidney, beef, bovine liver, fish, chicken	1 ng g <sup>-1</sup>	chou et al. 2009

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SPR	CAP	Meat	0.5 ng mL <sup>-1</sup>	Dong et al. 2009
GC-NCI/MS	CAP, TAP, FF	Poultry tissues (muscle,	0.1, 0.5 g Kg <sup>-1</sup>	Shen, et al. 2009
LC-ESI-MS/MS	CAP, FF, TAP	Honey	0.02, 0.02, 0.2 ng g <sup>-1</sup>	Zhao et al. 2009
SPME-LC	CAP	Water	37 ng g <sup>-1</sup>	Aresta et al. 2010
FPIA	CAP	water,milk	10 ng g <sup>-1</sup> , 20 g Kg <sup>-1</sup>	Gasilova et al. 2010
QCM	CAP	Unknown	5 g kg <sup>-1</sup>	Sun et al. 2011
Suspension array technology	tylosin, tetracycline, gentamicin, streptomycin, CAP	milk	25 ng g <sup>-1</sup>	Su et al. 2011
QCM	CAP	Meat, milk, egg, honey	0.2 ng g <sup>-1</sup>	Karasava et al.

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SPR: Surface plasma resonance QCM: Quartz crystal microbalance MEKS: Capillary electrophoresis FPIA: Fluorescence Polarization Immunoassay GC: Gas chromatography MS: Mass spectroscopy ESI: Chemical ionization LC: Liquid chromatography SFE: Solid phase extraction DAD: Diode-Array Detection NCI: Negative chemical ionization NESI:

Negative-Ion Chemical Ionization NACI: Negative atmospheric pressure chemical ionization PDA: Photo diode array ECD:  
Electron capture detector SPME: Solid phase micro extraction.

**Table 3.** Electrochemical (bio)sensor approaches for CAP detection

Type	Method	Analyte	Matrix	LOD	Reference
Bio	Amperometry	CAP	Milk	-	Rinken and Riik, 2006
Bio	Differential pulse voltammetry	CAP	Tris-HCl	0.064 $\mu\text{g L}^{-1}$	Wei-Wei et al., 2007
Bio	Cyclic voltammetry	CAP	PBS-Buffer	-	Tu et al., 2009
Bio & polymer	Cyclic voltammetry-immunosensor	CAP	PBS-Buffer	45 $\text{pg mL}^{-1}$	Kim et al., 2010
Bio	Square wave voltammetry/ Impedance spectroscopy	CAP	PBS-Buffer	0.29 x $10^{-9}$ $\text{mol L}^{-1}$	Yan et al., 2012
Bio	Cyclic voltammetry/ Square wave voltammetr	CAP	Tris-buffer	1.6 x $10^{-9}$ $\text{mol L}^{-1}$	Pilehvar et al., 2012
Polymer (MIP)	Cyclic voltammetry	CAP succinate, CAP, TAP and FF	Perchloric acid buffer	2 x $10^{-9}$ $\text{mol L}^{-1}$	Nuo-Wei et al., 2008
Polymer (MIP)	Cyclic voltammetry / Differential pulse voltammetry	CAP	Phosphoric acid buffer	2 x $10^{-9}$ $\text{mol L}^{-1}$	Alizadeh et al., 2012

<b>Polymer (MIP)</b>	Cyclic voltammetry	CAP	-	$10^{-6} \text{ mol L}^{-1}$	Ganjali, 2012
<b>Polymer (MIP)</b>	Cyclic voltammetry / Differntail pulse voltammetry	CAP	PBS-Buffer	$24 \mu\text{g L}^{-1}$	Zhao et al., 2012
<b>FIA</b>	Cyclic voltammetry	CAP	PBS-Buffer	$74 \times 10^{-9} \text{ mol L}^{-1}$	Liao et al., 2007
<b>FIA</b>	Cyclic voltammetry Hydrodynamic voltammetry	CAP	PBS-Buffer	$30 \times 10^{-9} \text{ mol L}^{-1}$	Chuanuwatanakul et al., 2008
<b>FIA</b>	Cyclic voltammetry	CAP	Acetate buffer	$44 \times 10^{-6} \text{ mol L}^{-1}$	Codognoto et al., 2010
<b>FIA</b>	Cyclic voltammetry	CAP	Phosphoric acid buffer	$2 \times 10^{-9} \text{ mol L}^{-1}$	Norouzi et al., 2010
<b>FIA-Bio</b>	Cyclic voltammetry Impedance spectroscopy	CAP	PBS-Buffer	-	Chullasat et al., 2011
<b>CAP - Amperometry</b>	Square wave voltammetry	CAP	Ammonia Buffer	-	Feng et al., 1998
<b>Amperometry</b>	Cyclic voltammetry Hydrodynamic voltammetry	CAP	PBS-Buffer	$0.50 \mu\text{g mL}^{-1}$	Wang et al., 1999
<b>Amperometry</b>	Square wave voltammetry	CAP	PBS-Buffer	$47 \times 10^{-9} \text{ mol L}^{-1}$	Agüí et al., 2002
<b>Amperometry</b>	Cyclic voltammetry / Square wave voltammetry	CAP	PBS-Buffer	$47 \times 10^{-9} \text{ mol L}^{-1}$	Mena et al., 2003

<b>Amperometry</b>	Cyclic Voltammetry Square wave voltammetry	CAP	Accetate Buffer	$6 \times 10^{-9} \text{ mol L}^{-1}$	Alemu and Hlalele, 2007
<b>Amperometry</b>	Cyclic voltammetry / Square wave voltammetry	CAP / Nitrobenzene / 4-nitrophenol	Phosphate buffer	$0.42 \times 10^{-6} \text{ mol L}^{-1}$	Chen et al., 2006
<b>Amperometry</b>	Cyclic Voltammetry	CAP	Hcl	$0.83 \mu\text{g L}^{-1}$	Chai et al., 2006
<b>Amperometry</b>	Linear sweep voltammetry Cyclic voltammetry	CAP	Phosphate buffer /PBS	$5 \times 10^{-9} \text{ mol L}^{-1}$	Xiao et al., 2007
<b>Amperometry</b>	Cyclic voltammetry	CAP	Tris-buffer	$10^{-6} \text{ mol L}^{-1}$	Pilehvar et al., 2012

PBS  $10^{-2} \text{ mol L}^{-1} \text{ NaH}_2\text{PO}_4$ ,  $10^{-2} \text{ mol L}^{-1} \text{ Na}_2\text{HPO}_4$  and 0.9% NaCl

TRIS  $0.1 \text{ mol L}^{-1} \text{ NaCl}$ ,  $0.02 \text{ mol L}^{-1} \text{ Tris HCl}$ ,  $0.002 \text{ mol L}^{-1} \text{ MgCl}_2$ ,  $0.005 \text{ mol L}^{-1} \text{ KCl}$ ,  $0.001 \text{ mol L}^{-1} \text{ CaCl}_2$