Introduction

The research activity of this PhD thesis was focused on the assembling of amperometric sensors to detect important chemical pollutants in drinking waters, such as ammonia and nitrites. Selected monomers were used to modify electrodes by synthetizing polymer films (via electrochemical polymerization), nanotubule nets (using a "template synthesis" approach) and nanowires (via chemical oxidation).

These polymers were morphologically investigated to evaluate their characteristics suitable to assemble sensors. These last were then analitically characterized in terms of detection limit, sensitivity, reproducibility and stability.

Real samples of drinking and mineral waters were analyzed and recovery studies were carried out.

The first chapters of this thesis describe the main characteristics of chemically modified electrodes and polymers, including the electrochemical techniques used. The experimental section is divided in two different chapters describing the sensors developed for the detection of nitrites and ammonia, respectively.
Chapter I - Chemically modified electrodes

A **Chemically modified electrode** (CME) is an electrode made of a conducting or semiconducting material that is coated with a selected monomolecular, multimolecular, or polymeric film of a chemical modifier and that by means of faradaic (charge-transfer) reactions or interfacial potential differences (no net charge transfer) exhibits chemical, electrochemical, and/or optical properties of the film. Most important characteristics to define a CME are described below.

**Electron transport** is the general term for processes by which electrochemical charge is transported through films of chemicals to electrodes, including electron self-exchange coupled to site diffusion, and conduction by highly conjugated molecular components of the film.

**The Coverage** is the area-normalized quantity, usually given in units of mol/m$^2$, of a designated kind of chemical site in the chemical film. Coverage must be distinguished as to total coverage present versus the coverage that is electrochemically reactive or accessible on some given timescale.

**Biosensor** is a special type of CME or ISE in which a biochemical recognition process is coupled to an appropriate electrochemical transducer. The electrode surface is modified by the attachment of a biocomponent (e.g., enzyme, antigen-antibody, plant or animal tissue, etc.) which functions as the biological recognition element or biochemical receptor [1].
**Chemoreceptor** is a selective receiving site for analyte recognition and reaction. In the case of a biologically derived receptor, the more specific term biochemical receptor or bioreceptor may be used. Chemically modified electrodes (CMEs) have attracted considerable interest over the past two decades as researchers have attempted to exert more direct control over the chemical nature of an electrode. The terminology, definitions and preparation methods of CMEs have been described and classified as well as recommendations provided in a recent IUPAC report [2]. CMEs have found numerous important applications in, e.g., solar energy conversion and storage, selective electroorganic synthesis, molecular electronics, electrochromic display devices, corrosion protection, and electroanalysis. A vast review literature is available on the subject [3-26]. The ability to manipulate the molecular architecture of the bulk matrix of an electrode and its surface in particular has led to a wide range of analytical applications of CMEs and created powerful opportunities for electroanalysis.

Compared with other electrode concepts in electrochemistry, the distinguishing feature of a CME is that a generally quite thin film (from a molecular monolayer to perhaps a few micrometers-thick multilayer) of a selected chemical is bonded to or coated on the electrode surface to endow the electrode with the chemical, electrochemical, optical, electrical, transport, and other desirable properties of the film in a rational, chemically designed manner. The range of
electrode surface properties includes, but is more diverse than, that of ion-selective electrodes (ISEs) which also involve, in their highest forms, rational design of the phase-boundary, partition and transport properties of membranes on or between electrodes. While CMEs can operate both amperometrically (or voltammetrically) and potentiometrically, they are generally used amperometrically, a faradaic (charge transfer) reaction being the basis of experimental measurement or study, whereas ISEs are generally used in potentiometric formats where a phase-boundary potential (interfacial potential difference, ipd) is the measured quantity [27]. Gas-sensing electrodes (e.g., for CO₂, NH₃, NOₓ) are also potentiometrically based [28] although the oxygen electrode, which functions amperometrically, is an exception. Chemically sensitive field effect transistors (CHEMFETs) are basically non-faradaic electrode systems in which electric field variations in the semiconductor gate region control the magnitude of the source-drain current [29]. Enzyme-based electrodes detect the products of a reaction between an immobilized enzyme layer and a reaction substrate in many ways, including both amperometric and potentiometric means. The distinction between CMEs and amperometric enzyme-modified electrodes is thus very narrow, the latter being based on a natural biological catalyst, but also with a rational (bio)molecular electrode design goal in mind.

It is important to keep in mind the concept of film homogeneity. The presence of variations in film properties between the interfaces with the electrode and solution can dramatically affect other properties. While
film homogeneity is not a necessity, without this property a complete description of the behavior of a CME is difficult, if not impossible, to achieve.

1.1 Approaches to modify electrodes:
Electrodes are usually chemically modified by one of four approaches:

(1) **Chemisorption-adsorption** in which the forces involved are the valence forces of the same kind as those operating in the formation of chemical compounds [30]. The chemical film is strongly and, ideally, irreversibly adsorbed (chemisorbed) onto the electrode surface. This approach usually yields monolayer (or less) coverage. Included in this type of modification are the substrate-coupled self-assembled monolayers (SAMs) in which uncorrelated molecules spontaneously chemisorb at specific sites on the surface of the electrode to form a superlattice [31].

(2) **Covalent bonding-linking** agents, such as, e.g., organosilanes or cyanuric chloride, are used to covalently attach from one to several monomolecular layers of the chemical modifier to the electrode surface.

(3) **Polymer film coating** Electron-conductive and nonconductive polymer films are held on the electrode surface by some combination of chemisorption and low solubility in the contacting solution or by physical anchoring in a porous electrode. The polymer film can be organic, organometallic or inorganic; it can already contain the desired chemical modifier or that chemical can be added to the polymer in a second, functionalizing step and can contain the equivalent of a few up to many
thousands of monomolecular layers of the chemical modifier. Included in this form of modification are the substrate-decoupled SAMs in which adsorbate molecules are arranged on the electrode surface independently of any substrate structure [32].

(4) Composite: the chemical modifier is simply mixed with an electrode matrix material, as in the case of an electron-transfer mediator (electrocatalyst) combined with the carbon particles of a carbon paste electrode. Alternatively, intercalation matrices such as certain Langmuir-Blodgett films, zeolites, clays and molecular sieves can be used to contain the modifier.

The research activity of this PhD thesis was focused on study of chemical modified electrodes with polymer coating, as described in experimental section.

CMEs can also contain multiple chemical modifiers, and sometimes these modifiers and/or the electrode substrate may have a particularly designed spatial configuration. That is, a CME may contain one electrocatalyst that reacts with a substrate or acts as a photodonor or acceptor, and a second one to transport charge between the first catalyst and the electrode. Or, the CME may consist of a substrate coated with two different chemical polymers, the second polymer overlaid on the first to form a bilayer of polymer films. These more complex CMEs may be called microstructured electrodes or integrated chemical system electrodes.
Polymer film-coated electrodes may be further subdivided by the process used to apply the film:

(1) Dip-coating: this procedure consists of immersing the electrode material in a solution of the polymer for a period sufficient for spontaneous film formation to occur by adsorption. The film quantity in this procedure may be augmented by withdrawing the electrode from the solution and allowing the film of polymer solution to dry on the electrode.

(2) Solvent evaporation: a droplet of a solution of the polymer is applied to the electrode surface and the solvent is allowed to evaporate. A major advantage of this approach is that the polymer coverage is immediately known from the original polymer solution concentration and droplet volume. (Alternate term: droplet evaporation).

(3) Spin coating: also called spin casting, a droplet of a dilute solution of the polymer is applied to the surface of a rotating electrode. Excess solution is spun off the surface and the remaining thin polymer film is allowed to dry. Multiple layers are applied in the same way until the desired thickness is obtained. This procedure typically produces pinhole-free thin films.

(4) Electrochemical deposition (also called redox deposition): this procedure relies on the variation of polymer solubility with oxidation (and ionic) state, so that film formation will occur, often irreversibly, when a polymer is oxidized or reduced to its less soluble state.

(5) Electrochemical polymerization: a solution of monomer is oxidized or reduced to an activated form that
polymerizes to form a polymer film directly on the electrode surface. This procedure results in few pinholes since polymerization would be accentuated at exposed (pinhole) sites at the electrode surface. Unless the polymer film itself is redox active, electrode passivation occurs and further film growth is prevented.

(6) **Radiofrequency polymerization:** a polymer-filming method in which vapors of the monomer are exposed to a radiofrequency (RF) plasma discharge. The high energetics of the RF discharge may result in chemical damage, thereby producing unknown functionalities and structural modifications to the polymer.

(7) **Cross-linking:** a chemical step designed to couple chemical components of a film on an electrode to impart some desired property to the film such as increased stability, decreased permeability, or altered electron transport characteristics. Cross-linked films are often formed by copolymerization of bifunctional and polyfunctional monomers. Cross-linking may be activated chemically, electrochemically, photolytically, radiolytically, or thermally.

To assemble selective sensors to detect nitrites and ammonia two of these procedure described to modify electrodes, were used. Microstructured films as poly(1,2-1,3-1,4-DAB), poly(pyrrole), poly(o-anisidine), poly(1,8-DAN), were electrochemical synthesized directly on the electrodes surface, while polypyrrole nanowires and polymeric nanotubules were deposited on the electrodes surface by solvent evaporation.
1.2 Phenomena occurring at CMEs

Several phenomena occurring at CMEs are exploited for electroanalytical purposes. These include analyte and/or reagent accumulation, chemical transformation, electrocatalysis, permeability, ionic equilibria, controlled release, and change of mass. They are employed separately or in mutual combinations, simultaneously or, most commonly, in sequence. Principal phenomena occurring at selective chemical modified electrodes for nitrite and ammonia detection were permeability and chemical transformation, respectively.

1.2.1 Chemical transformation

An electro-inactive analyte can be reacted with the appropriate reagent immobilized at the CME to yield an electroactive product suitable for electrochemical determination. Electrode selectivity and sensitivity towards certain functional groups can be improved in this way. For instance, Pt electrodes were modified by adsorption of an allylamine layer for determination of ferrocenecarboxaldehyde [28]. Electro-oxidation of the imine product of condensation of the carbonyl analyte and the immobilized amine gave rise to the detection signal. Alternatively, primary amines were determined by using a carbon paste electrode (CPE) modified with quaternized poly(vinylpyridine) having an anion functionalized aromatic aldehyde as a counter ion. The amines were reacted at the modified CPE and the electro-oxidation current of the resulting imine was used as the detection signal [29]. Also, the modifying effect of an analyte on
the electrode-immobilized reagent can be exploited for analytical purposes. Accordingly, in a rather unusual application, Cs\(^+\) was determined indirectly, in the presence of the Na\(^+\) excess. This determination was based on the electrochemical response of iron sites within the selective cation-exchange \([\text{NiFe(CN)}_6]^-/\text{[NiFe(CN)}_6]^{2-}\) matrix modifying an Ni electrode [33]. The mechanism of this selective detection consists in microstructure changes in the modifier lattice, induced by minute amounts of the analyte.

1.2.2. **Permeability**

Permeability is a general term describing discriminative transport through a membrane coating [34] that controls the access of analyte and interfering substances to the electrode surface. An electrode coated with the permeable membrane is accessible to the target analyte while interfering substances are rejected or prevented from reaching the electrode surface. Thus, the electrode selectivity is improved [35,36]. Permeability can be controlled either by thermodynamics (in terms of partition) or by the transport rate (governed by diffusion within the film). Under dynamic conditions, electrodes coated with a permeable film can reveal selectivity.

Mechanisms of the permeability transport are based on differences in properties, such as charge, size, shape, polarity or chirality of the analyte and interfering substances. For example, a cation-exchange polymer film coating an electrode is a membrane barrier to anions from solution while cations can freely partition into it.
Obviously, the charge selectivity of an anion-exchange polymer film is opposite. A polyanionic perfluorosulfonated ionomer, i.e., Nafion®, and poly(estersulfonic acid) are examples of widely used cation-exchange permselective membranes while Tosflex® [37], an anion-exchanger analogue of Nafion®, is an example of an anion-exchange membrane. Cellulose acetate and poly(carbonate) are typical examples of size-exclusion membranes. The electrode selectivity can be additionally enhanced by designing multilayer or mixed membranes that combine the transport properties of the individual membrane. A polycrystalline Pt electrode modified both with Nafion® and cellulose acetate, and used for direct oxidative determination of nitric oxide, illustrates combined anion- and size-exclusion discriminative properties of the modifying film [38,39]. The sieving properties of size-exclusion films can also impart higher electrode stability due to prevention of surface fouling by undesired precipitation or adsorption. An electrode modified with a SAM of long alkyl chain molecules having terminal acid/base or ionic groups can enhance or suppress selected redox reactions depending on the charge of the terminal group. For instance, SAMs of HS(CH₂)₆COOH on the Au electrode discriminated against ascorbates in the voltammetric determination of dopamine [40]. The permeability of the membranes can be combined with their other properties, such as electrocatalysis or biocatalysis. For instance, a charge mediator and/or redox enzyme can be attached or entrapped in the membrane. Induced charge-exclusion properties of a CME are exploited for potential-controlled gating of ions.
For instance, anion transport can be switched on and off selectively by electro-oxidation and electroreduction, respectively, of a poly(pyrrole) film electrodeposited on a porous Au electrode separating two electrolyte solutions. Indirect ion-exchange immunoassay at a Nafion® modified electrode consists of accumulation, by ion exchange, of a cationic product of enzymatic redox transformation in solution of an anionic substrate, followed by voltammetric determination of the accumulated product [41,42].
References


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Chapter II: Polymers

A polymer is a substance composed of molecules with large molecular mass composed of repeating structural units, or monomers, connected by covalent chemical bonds. Henri Braconnot did start in 1811 his pioneer work in derivative cellulose compounds, perhaps the earliest important work in polymer science. The term polymer was coined in 1833 by Jöns Jakob Berzelius. The development of vulcanization later in the nineteenth century improved the durability of the natural polymer rubber, signifying the first popularized semi-synthetic polymer. The first wholly synthetic polymer, Bakelite, was introduced in 1909. Despite significant advances in synthesis and characterization of polymers, a proper understanding of polymer molecular structure did not come until the 1920s. Before that, scientists believed that polymers were clusters of small molecules (called colloids), without definite molecular weights, held together by an unknown force, a concept known as association theory. In 1922, Hermann Staudinger proposed that polymers consisted of long chains of atoms held together by covalent bonds, an idea which did not gain wide acceptance for over a decade, and for which Staudinger was ultimately awarded the Nobel Prize. An important contribution to synthetic polymer science was given by the Italian chemist Giulio Natta and Karl Ziegler who won the Nobel Prize in Chemistry in 1963 for the development of the Ziegler-Natta catalyst. In the intervening century, synthetic
polymer materials such as Nylon, polyethylene, Teflon, and silicone have formed the basis for a burgeoning polymer industry. Synthetic polymers today find application in nearly every industry and area of life. Polymers are widely used as adhesives and lubricants, as well as structural components for products ranging from children's toys to aircraft. Polymers such as poly(methyl methacrylate) find application as photoresist materials used in semiconductor manufacturing for use in high-performance microprocessors. Future applications include flexible polymer-based substrates for electronic displays and improved time-released and targeted drug delivery.

In 1907, Leo Baekeland created the first completely synthetic polymer, Bakelite, by reacting phenol and formaldehyde at precisely controlled temperature and pressure. Subsequent work by Wallace Carothers in the 1920s demonstrated that polymers could be synthesized rationally from their constituent monomers. The intervening years have shown significant developments in rational polymer synthesis. Most commercially important polymers today are entirely synthetic and produced in high volume, on appropriately scaled organic synthetic techniques [43].

Laboratory synthetic methods are generally divided into two categories, condensation polymerization and addition polymerization. However, some newer methods such as plasma polymerization do not fit neatly into either category. In chemical compounds, polymerization occurs via a variety of reaction mechanisms which vary in complexity due to functional groups present in reacting
compounds and their inherent steric effects explained by VSEPR Theory. In more straightforward polymerization, alkenes, which are relatively stable due to σ bonding between carbon atoms form polymers through relatively simple radical reactions; conversely, more complex reactions such as those that involve substitution at the carbonyl atom require more complex synthesis due to the way in which reacting molecules polymerize.

As alkenes can be formed in somewhat straightforward reaction mechanisms, they form useful compounds such as polyethylene and polyvinyl chloride (PVC) when undergoing radical reactions, which are produced in high tonnages each year due to their usefulness in manufacturing processes of commercial products, such as piping, insulation and packaging. Polymers such as PVC are generally referred to as "singular" polymers as they consist of repeated long chains or structures of the same monomer unit, whereas polymers that consist of more than one molecule are referred to as "co-polymer".

Other monomer units, such as formaldehyde hydrates or simple aldehydes, are able to polymerize themselves at quite low temperatures (>80°C) to form trimers; molecules consisting of 3 monomer units which can cyclize to form ring cyclic structures, or undergo further reactions to form tetrarmer, or 4 monomer-unit compounds. Further compounds either being referred to as oligomers in smaller molecules. Generally, because formaldehyde is an exceptionally reactive electrophile it allows nucleophilic addition of hemiacetal intermediates, which
are generally short lived and relatively unstable "mid stage" compounds which react with other molecules present to form more stable polymeric compounds. Addition polymerization involves the linking together of molecules incorporating double or triple chemical bonds. These unsaturated monomers (the identical molecules which make up the polymers) have extra internal bonds which are able to break and link up with other monomers to form the repeating chain. Addition polymerization is involved in the manufacture of polymers such as polyethylene, polypropylene and polyvinyl chloride (PVC). A special case of addition polymerization leads to living polymerization. Condensation polymerization occurs when monomers bond together through condensation reactions. Typically these reactions can be achieved through reacting molecules incorporating alcohol, amine or carboxylic acid (or other carboxyl derivative) functional groups. When an amine reacts with a carboxylic acid an amide or peptide bond is formed, with the release of water (hence condensation polymerization.) This is the process through which amino acids link up to form proteins, as well as how kevlar is formed. The chain growth-step growth system categorizes polymers based on their mechanism. While most polymers will fall into their similar category from the addition-condensation method of categorization, there are a few exceptions. Chain growth polymers are defined as polymers formed by the reaction of monomer with a reactive center. These polymers grow to high molecular weight at a very fast rate. It is important to note that the overall conversion rates between chain and step growth polymers are similar, but
that high molecular weight polymers are formed in addition reactions much more quickly than with step polymerizations. Addition polymerization involves the breaking of double or triple bonds, which are used to link monomers into chains. In the polymerization of ethane, its π bond is broken and these two electrons rearrange to create a new propagating center like the one that attacked it. The form this propagating center takes depends on the specific type of addition mechanism. There are several mechanisms through which this can be initiated. The free radical mechanism was one of the first methods to be used. Free radicals are very reactive atoms or molecules which have unpaired electrons. Taking the polymerization of ethene as an example, the free radical mechanism can be divided into three stages: initiation, propagation and termination. Initiation is the creation of free radicals necessary for propagation. The radicals can be created from radical initiators, such as organic peroxide molecules, molecules containing an O=O single bond, by reacting oxygen with ethylene. The products formed are unstable and easily break down into two radicals. In an ethene monomer, one electron pair is held securely between the two carbons in a sigma bond. The other is more loosely held in a π bond. The free radical uses one electron from the π bond to form a more stable bond with the carbon atom. The other electron returns to the second carbon atom, turning the whole molecule into another radical. Propagation is the rapid reaction of this radicalized ethene molecule with another ethene monomer, and the subsequent repetition to create the repeating chain. Termination occurs when a radical
reacts in a way that prevents further propagation. The most common method of termination is by coupling where two radical species react with each other forming a single molecule. Another, less common method of termination is disproportionation where two radicals meet, but instead of coupling, they exchange a proton, which gives two terminated chains, one saturated and the other with a terminal double bond. Free radical addition polymerization of ethylene must take place at high temperatures and pressures, approximately 300°C and 2000 atm. While most other free radical polymerizations do not require such extreme temperatures and pressures, they do tend to lack control. One effect of this lack of control is a high degree of branching. Also, as termination occurs randomly, when two chains collide, it is impossible to control the length of individual chains. A newer method of polymerization similar to free radical, but allowing more control involves the Ziegler-Natta catalyst especially with respect to polymer branching. Other forms of addition polymerization include cationic addition polymerization and anionic addition polymerization. While not used to a large extent in industry yet due to stringent reaction conditions such as lack of water and oxygen, these methods provide ways to polymerize some monomers that cannot be polymerized by free radical methods such as polypropylene. Cationic and anionic mechanisms are also more ideally suited for living polymerizations, although free radical living polymerizations have also been developed. Step growth polymers are defined as polymers formed by the stepwise reaction between functional groups of monomers. Most step
growth polymers are also classified as condensation polymers, but not all step growth polymers (like polyurethanes formed from isocyanate and alcohol bifunctional monomers) release condensates. Step growth polymers increase in molecular weight at a very slow rate at lower conversions and only reach moderately high molecular weights at very high conversion (i.e. >95%).

To alleviate inconsistencies in these naming methods, adjusted definitions for condensation and addition polymers have been developed. A condensation polymer is defined as a polymer that involves elimination of small molecules during its synthesis, or contains functional groups as part of its backbone chain, or its repeat unit does not contain all the atoms present in the hypothetical monomer to which it can be degraded [44].

2.1 Polymer structure and properties
Types of polymer 'properties' can be broadly divided into several categories based upon scale. At the nano-micro scale are properties that directly describe the chain itself. These can be thought of as polymer structure. At an intermediate mesoscopic level are properties that describe the morphology of the polymer matrix in space. At the macroscopic level are properties that describe the bulk behavior of the polymer.

2.1.1 Structure
The structural properties of a polymer relate to the physical arrangement of monomers along the backbone of the chain. Structure has a strong influence on the other
properties of a polymer. For example, a linear chain polymer may be soluble or insoluble in water depending on whether it is composed of polar monomers (such as ethylene oxide) or nonpolar monomers (such as styrene). On the other hand, two samples of natural rubber may exhibit different durability even though their molecules comprise the same monomers.

Monomer identity
The identity of the monomers comprising the polymer is generally the first and most important attribute of a polymer. Polymer nomenclature is generally based upon the type of monomers comprising the polymer. Polymers that contain only a single type of monomer are known as homopolymers, while polymers containing a mixture of monomers are known as copolymers. Poly(styrene), for example, is composed only of styrene monomers, and is therefore is classified as a homopolymer. Ethylene-vinyl acetate, on the other hand, contains more than one variety of monomer and is thus a copolymer. Some biological polymers are composed of a variety of different but structurally related monomers, such as polynucleotides composed of nucleotide subunits. A polymer molecule containing ionizable subunits is known as a polyelectrolyte. An ionomer is a subclass of polyelectrolyte with a low fraction of ionizable subunits.

Chain linearity
The simplest form of polymer molecule is a straight chain or linear polymer, composed of a single main chain. The
flexibility of an unbranched chain polymer is characterized by its persistence length. A branched polymer molecule is composed of a main chain with one or more substituent side chains or branches. Special types of branched polymers include star polymers, comb polymers, and brush polymers. If the polymer contains a side chain that has a different composition or configuration than the main chain, the polymer is called a graft or grafted polymer. A cross-link suggests a branch point from which four or more distinct chains emanate. A polymer molecule with a high degree of crosslinking is referred to as a polymer network. Sufficiently high crosslink concentrations may lead to the formation of an 'infinite network', also known as a 'gel', in which networks of chains are of unlimited extend - there is essentially all chains have linked into one molecule.

Chain size
Polymer bulk properties may be strongly dependent on the size of the polymer chain. Like any molecule, a polymer molecule's size may be described in terms of molecular weight or mass. In polymers, however, the molecular mass may be expressed in terms of degree of polymerization, essentially the number of monomer units which comprise the polymer. For synthetic polymers, the molecular weight is expressed statistically to describe the distribution of molecular weights in the sample. This is because of the fact that almost all industrial processes produce a distribution of polymer chain sizes. Examples of such statistics include the number average molecular weight
and weight average molecular weight. The ratio of these two values is the polydispersity index, commonly used to express the "width" of the molecular weight. The space occupied by a polymer molecule is generally expressed in terms of radius of gyration or excluded volume.

Monomer arrangement in copolymers
Monomers within a copolymer may be organized along the backbone in a variety of ways.
- Alternating copolymers possess regularly alternating monomer residues
- Periodic copolymers have monomer residue types arranged in a repeating sequence
- Random copolymers have a random sequence of monomer residue types
- Statistical copolymers have monomer residues arranged according to a known statistical rule
- Block copolymers have two or more homopolymer subunits linked by covalent bonds.

2.1.2 Chemical properties of polymers
The attractive forces between polymer chains play a large part in determining a polymer's properties. Because polymer chains are so long, these interchain forces are amplified far beyond the attractions between conventional molecules. Different side groups on the polymer can lend the polymer to ionic bonding or hydrogen bonding between its own chains. These stronger forces typically result in higher tensile strength and melting points. The intermolecular forces in polymers can be affected by dipoles in the monomer units. Polymers containing amide
or carbonyl groups can form hydrogen bonds between adjacent chains; the partially positively charged hydrogen atoms in N-H groups of one chain are strongly attracted to the partially negatively charged oxygen atoms in C=O groups on another. These strong hydrogen bonds, for example, result in the high tensile strength and melting point of polymers containing urethane or urea linkages. Polyesters have dipole-dipole bonding between the oxygen atoms in C=O groups and the hydrogen atoms in H-C groups. Dipole bonding is not as strong as hydrogen bonding, so a polyester's melting point and strength are lower than Kevlar's (Twaron), but polyesters have greater flexibility. Ethene, however, has no permanent dipole. The attractive forces between polyethylene chains arise from weak van der Waals forces. Molecules can be thought of as being surrounded by a cloud of negative electrons. As two polymer chains approach, their electron clouds repel one another. This has the effect of lowering the electron density on one side of a polymer chain, creating a slight positive dipole on this side. This charge is enough to actually attract the second polymer chain. Van der Waals forces are quite weak, however, so polyethene can have a lower melting temperature compared to other polymers.

2.2 Polymer characterization
The characterization of a polymer requires several parameters which need to be specified. This is because a polymer actually consists of a statistical distribution of chains of varying lengths, and each chain consists of monomer residues which affect its properties. A variety
of techniques are used to determine the properties of polymers. Techniques such as wide angle X-ray scattering, small angle X-ray scattering, and small angle neutron scattering are used to determine the crystalline structure of polymers. Gel permeation chromatography is used to determine the number average molecular weight, weight average molecular weight, and polydispersity. FTIR, Raman and NMR can be used to determine composition. Thermal properties such as the glass transition temperature and melting point can be determined by differential scanning calorimetry and dynamic mechanical analysis. Pyrolysis followed by analysis of the fragments is one more technique for determining the possible structure of the polymer [45].

2.3 Polymer degradation
Polymer degradation is a change in the properties - tensile strength, colour, shape, etc - of a polymer or polymer based product under the influence of one or more environmental factors such as heat, light or chemicals. It is often due to the hydrolysis of the bonds connecting the polymer chain, which in turn leads to a decrease in the molecular mass of the polymer. These changes may be undesirable, such as changes during use, or desirable, as in biodegradation or deliberately lowering the molecular mass of a polymer. Such changes occur primarily because of the effect of these factors on the chemical composition of the polymer. The degradation of polymers to form smaller molecules may proceed by random scission or specific scission. The degradation of polyethylene occurs by random scission - that is by a random breakage
of the linkages (bonds) that hold the atoms of the polymer together. When heated above 450°C it degrades to form a mixture of hydrocarbons. Other polymers – like polyalphamethylstyrene – undergo 'specific' chain scission with breakage occurring only at the ends. They literally unzip or depolymerize to become the constituent monomer. In a finished product such a change is to be prevented or delayed. However the degradation process can be useful from the view points of understanding the structure of a polymer or recycling/reusing the polymer waste to prevent or reduce environmental pollution. Polylactic acid and Polyglycolic acid, for example, are two polymers that are useful for their ability to degrade under aqueous conditions. A copolymer of these polymers is used for biomedical applications such as hydrolysable stitches that degrade over time after they are applied to a wound. These materials can also be used for plastics that will degrade over time after they are used and will therefore not remain as litter. These are two example of the so called "biopolymers". Biopolymers (also called renewable polymers) are produced from biomass for use in the packaging industry. Biomass comes from crops such as sugar beet, potatoes or wheat: when used to produce biopolymers, these are classified as non food crops. These can be converted in the following pathways:

Sugar beet > Glyconic acid > Polyclonic acid
Starch > (fermentation) > Lactic acid > Polylactic acid (PLA)
Many types of packaging can be made from biopolymers: food trays, blown starch pellets for shipping fragile goods, thin films for wrapping. Biopolymers are renewable, sustainable, and can be carbon neutral. Biopolymers are renewable, because they are made from plant materials which can be grown year on year indefinitely. These plant materials come from agricultural non food crops. Therefore, the use of biopolymers would create a sustainable industry. In contrast, the feedstocks for polymers derived from petrochemicals will eventually run out. In addition, biopolymers have the potential to cut carbon emissions and reduce CO2 quantities in the atmosphere: this is because the CO2 released when they degrade can be reabsorbed by crops grown to replace them: this makes them close to carbon neutral. Some biopolymers are biodegradable: they are broken down into CO2 and water by microorganisms. In addition, some of these biodegradable biopolymers are compostable: they can be put into an industrial composting process and will break down by 90% within 6 months. Biopolymers that do this can be marked with a 'compostable' symbol, under European Standard EN 13432 (2000). Packaging marked with this symbol can be put into industrial composting processes and will break down within 6 months (or less). An example of a compostable polymer is PLA film under 20μm thick: films which are thicker than that do not qualify as compostable, even though they are biodegradable.
2.4 Polymers from aromatic diamines
Since discovered in the late 19th century increasing research interest has focused on the electrically conductive nature of the aromatic polymer family such as polyaniline (PAN), aniline (AN) with its derivative copolymers, polyaminopiridine and its copolymers, polyaminoquinoline, polymethilquinoline, polypyrrole (PPY) and its copolymers and polytiophene. In recent years the polymer family is considered as being composed of organic conductive materials with very promising properties such as good environmental stability, moderately high conductivity upon doping suitable ions and higher gas separation efficiencies. Furthermore, the polymers exhibit widely potential applications in rechargeable battery, electrochromic display, electrocatalyst, antistatic and anticorrosive materials, electrode materials sensor, detector and gas separation membranes. There are extensive studies in the literature concerning the synthesis, characterization and application of conductive polymers of this family. Among the techniques reported for the synthesis of the polymers from aromatic diamines, chemically oxidative polymerization and electrochemically oxidative polymerizations have been scarcely used. A principal advantage of chemically oxidative polymerization concerns the possibility of mass production of powdered products at a reasonable cost. The process is slightly complicated and the polymerization time is much longer than the electropolymerization and photooxidative polymerization. One of the great advantages of electropolymerization is
that almost all aromatic diamines are applicable for polymerization. The oxidant is not needed, but a suitable potential or current must be applied through the monomer solution for a successful electropolymerization. This polymer film can be easily produced on a metallic object of any desired shape, and a uniform doping of film can be simply accomplished.

An important application of aromatic diamine polymers that has only begun to emerge since 2000 is the amperometric detection of electroinactive anions in solution. Amperometric detection is a useful and important testing method with advantages of rapid response, low detection limit, low cost and ease of fabrication and miniaturization. Conducting polymer-modified electrodes have been applied in the amperometric detection of many electroinactive ions, based on the fact that oxidation of the polymer involves the incorporation of an anionic species to counterbalance the positive sites generated in the oxidative process. A complicated equilibrium between protons and anions should be established in order to compensate for the charge on the protonated groups by the charge of coadsorbed anions [46]. This characteristic was studied to assemble a selective amperometric sensor to detect ammonia in drinking water as discussed in experimental section.

There are a few investigations on the permselectivity through electropolymerized films of various aromatic diamines polymers. Film thickness is one of the most important factors affecting the permselectivity characteristics. An extra benzene ring, as in naphthalene, would confer greater hydrofobicity on the
films formed, leading to a lower degree of salvation, closer packing of the macromolecular chains, reduced diffusion of the solvated interference species through the films and finally lower permeability. The fabrication of a controllable permselectivity electrode is of great significant in conjunction with amperometric sensor [45]. This characteristic was studied to assemble a selective amperometric sensor to detect nitrites in drinking water as discussed in experimental section.

2.5 Nanopolymers
The scientific work on the study of nanoscopic world started at the end of the fifties, ideally inspired by the Richard Feynman’s talk in 1959 at the California Institute of Technology (Caltech). Fundamentally, Feynman asserted that ”we can not manipulate the atoms one at a time only because nobody studied the problem sufficiently; in fact physic principles that hinder this possibility were not know”.

Nanotechnologies are devoted to the manipulation and manufacture of materials and devices on the nanometric dimensions. The materials structured on nanometric or subnanometric scale show new properties and functionalities that can be modulated by selective control of morphology, dimensions and assembling of particles. There are two principal reasons for qualitative differences in material behaviour at the nanoscale (traditionally defined as less than 100 nanometres). First, quantum mechanical effects come into play at very small dimensions and lead to new physics and chemistry.
Second, a defining feature at the nanoscale is the very large surface-to-volume ratio of these structures. This means that no atom is very far from a surface or interface, and the behaviour of atoms at these higher-energy sites affects significantly the properties of the material. Two very different paths exist to realize nanostructured materials: the top-down strategy of miniaturizing current technologies and the bottom-up strategy of building ever-more-complex molecular devices atom by atom.

Top-down approaches are good for producing structures with long-range order and for making macroscopic connections, while bottom-up approaches are best suited for assembly and establishing short-range order at nanoscale dimensions [46]. Nanotechnology may allow to manufacture lighter, stronger, and programmable materials that require less energy to produce than conventional materials, that produce less waste than with conventional manufacturing, and that promise greater fuel efficiency in land transportation, ships, aircraft, and space vehicles. Nanocoatings for both opaque and translucent surfaces may render them resistant to corrosion, scratches, and radiation. Nanoscale electronic, magnetic, and mechanical devices and systems with unprecedented levels of information processing may be fabricated, as may chemical, photochemical, and biological sensors for protection, health care, manufacturing, and the environment; new photoelectric materials that will enable the manufacture of cost-efficient solar-energy panels; and molecular-semiconductor hybrid devices that may become engines for the next revolution in the information
age. The potential for improvements in health, safety, quality of life, and conservation of the environment are vast. Sensors are central to almost all modern control systems. For example, multiple sensors are used in automobiles for such diverse tasks as engine management, emission control, security, safety, comfort, vehicle monitoring, and diagnostics. While such traditional applications for physical sensing generally rely on microscale sensing devices, the advent of nanoscale materials and structures has led to new electronic, photonic, and magnetic nanosensors, sometimes known as “smart dust” [47,48]. Because of their small size, nanosensors exhibit unprecedented speed and sensitivity, extending in some cases down to the detection of single molecules. For example, nanowires made of carbon nanotubes, silicon, or other semiconductor materials exhibit exceptional sensitivity to chemical species or biological agents. Electrical current through nanowires can be altered by having molecules attached to their surface that locally perturb their electronic band structure. By means of nanowire surfaces coated with sensor molecules that selectively attach particular species, charge-induced changes in current can be used to detect the presence of those species. This same strategy is adopted for many classes of sensing systems [49-53].
Chapter III - Electrochemical techniques

3.1 Cyclic Voltammetry
The most widely used voltammetric technique is cyclic voltammetry (CV). In CV the potential is linearly swept between two limiting potentials. At each potential value the passage of the current due to a redox reaction is measured. CV is considered as the most reliable and efficient techniques to study redox systems and is usually performed as a qualitative experiment to obtain electrochemical information.
Cyclic voltammetry is often the first technique used in an electroanalytical study. In particular, it offers a rapid location of redox potentials of the electroactive species, and a convenient evaluation of the effect of media upon the redox process.
The CV is usually performed in a solution containing a redox species or using an electrode modified with a redox system. When the potential is swept in the positive direction the reduced species will be oxidised starting close to the half-wave potential (E1/2) of the redox couple and an anodic current results (ipa). When the potential sweeps back to negative values, the species starts being reduced resulting in a cathodic current (ipc) which has its maximum near the half-wave potential. These two parameters in the case of a reversible redox couple follow the Randles Sevich equation and their values are given by:

\[ ip = (2.69 \times 10^{-5}) n^{3/2} A C D^{1/2} v^{1/2} \]  
(eq a)
where $n$ is the number of electrons, $A$ is the electrode area (in cm$^2$), $C$ is the concentration in mol/cm$^3$, $D$ is the diffusion coefficient (in cm$^2$/s) and $v$ is the scan rate (in V/s). According to this equation, the current is directly proportional to the concentration of the electroactive substance and increases with the square root of the scan rate. When a reversible couple is taken in consideration, the ratio between the anodic and cathodic peaks (at the same conditions) should be equal to 1. If the electron transfer between the electrode and the redox couple is fast enough, the system is in constant equilibrium and obeys the Nernst equation and so the distance between the two potential peaks should be equal to $0.059/n$ V, where $n$ is the number of electrons exchanged during the electrochemical reaction.

This technique is extremely useful, CV is usually used only for acquiring qualitative information about electrochemical reactions and other are the methods which offer more accuracy and a better sensitivity.

3.2 Amperometric methods
Amperometric measurements are usually performed in a three electrode set-up where the potential of the working electrode is maintained by a potentiostat and is relative to a reference electrode (usually Ag/AgCl or saturated calomel electrode) and the current flowing between the working and a counter (auxiliary) electrode is measured.

3.2.1 Batch stirred amperometry
This set-up is very simple, reliable and allows fast measurements. The working electrode is immersed in a
stirred solution. The electroactive species is forced to reach the electrode surface and a very rapid equilibrium between the diffusion of the species to the electrode and the redox reaction is achieved. This results in a signal which starts from a background point (background current) and reaches a final value (limiting current), that is maintained constant by the stirring of the solution, which allows the continuous diffusion of the electroactive species at the electrode surface. The signal is usually due to the adding of the electroactive species in the solution. The signal obtained is constant because the bulk concentration of the analyte does not change in the solution since the redox reaction at the electrode surface involves only a little amount of analyte.

For this reason, it is also possible to add the same species several times in order to obtain a calibration curve and in order to calculate the sensitivity, linear range and detection limit of the sensor.

The sensitivity is usually calculated by dividing the current value obtained with a certain concentration of analyte by the value of this concentration and is often given as current signal/concentration (A/M). Sometimes, in order to make this value more "universal", the sensitivity is divided by the area of the electrode (A/M cm²).

Detection limit, in the case of batch stirred amperometry is usually considered as the concentration of analyte giving a signal that is three times higher than the noise signal (detection limit = S/N=3) of the background current.
3.2.2 Chronoamperometry
Chronoamperometry is an electrochemical technique in which the potential of the working electrode is stepped, and the resulting current from faradaic processes occurring at the electrode (caused by the potential step) is monitored as a function of time. It’s a differential technique: it’s sensitivier and faster than classical amperometry.

3.3 Flow injection analysis (FIA)
This technique is based on the use of a peristaltic pump which drives a carrier solution (buffer) to the electrode surface with a constant flow rate (ml/min). The electrode is inserted in a cell with a fixed volume and the inlet of the flow could come perpendicular to the electrode surface (wall-jet cell) or longitudinally to the electrode (thin-layer cell). When the signal obtained with the buffer is stable, the sample is directly injected into the carrier stream by an injection valve. The signal obtained will have the shape of a peak, since the sample is fastly removed from the electrode surface by the buffer carrier solution. The current will be a function of the loop volume and of the flow rate. The peak current will be proportional to the analyte concentration [54].
Chapter IV – Detection of nitrites in drinking water using chemically modified electrodes with polymeric films and nanotubule nets.

Abstract

Platinum electrodes were modified by electropolymerized films and polymer nanotubule nets and were applied to nitrite detection in drinking water. Several analytical parameters were investigated such as: different monomers (1,2- 1,3- 1,4-DAB, pyrrole, o-anisidine, 1,8-DAN), permeability toward nitrites and other interferences, permselectivity toward nitrites, operational and long-term stability. The best performances were obtained with a Poly(1,3-DAB) film and the assembled sensor was characterized morphologically by scanning electron microscope and electrochemically by cyclic voltammetry and amperometry coupled to flow injection analysis (FIA), in terms of linear range of concentration (10-1000 µM), limit of detection (2 µM), reproducibility (RSD%: 0.4) and linear regression (y/µA=1.14 x/µM + 2.6). Finally real samples (tap and mineral drinking water) were analyzed by adding standard nitrite solutions and the recovery was evaluated showing no matrix effect on sensor performances.
1 Introduction

The quantitative determination of nitrite concentration is of rapidly increasing interest, especially for the monitoring of drinking water quality and in the food industry when water is often used as a raw material. Nitrite ions are a precursor in the formation of nitrosamines which have been shown to be carcinogenic [1]. Moreover, nitrites are routinely added to meat products as a preservative against food poisoning microorganisms such as Clostridium botulinum [2]. Furthermore when nitrites are present at high concentration in blood, they can react with the iron (III) of the haemoglobin, forming methemoglobin which has no oxygen carrying ability; this disease is called methemoglobinemia or “Blue Baby Syndrome” [3]. For these reasons, nitrite’s presence in drinking water has to be limited and in Italy legal limits are fixed at a value of 2 µM [4].

Many methods have been reported [5,6] for quantitative determination of nitrite including chromatography, potentiometry and fluorimetry, but nitrites are traditionally determined spectrophotometrically by their reaction with sulphanilamide and N-naphthylenediamine (Griess reaction). Nitrites are also electroactive species that are readily oxidized at platinum electrodes polarized at +900 mV vs Ag/AgCl. Since the potential for nitrite oxidation is high, many other electroactive compounds present in complex media can interfere in the nitrite analysis. Permselective coatings offer the
promise of bringing higher selectivity and stability to electrochemical devices. This is accomplished by exclusion from the surface of unwanted matrix constituents, while allowing transport of the target analyte. Different avenues to control the access to the surface, based on different transport mechanism, have been proposed. These include the use of charged-exclusion coating [7], hydrophobic lipid [8] or alkilthiols layers [9] or size-exclusion polymeric films [10]. Such anti-interference membrane barriers offer an effective separation step in situ on the surfaces and hence protect the surface against adsorption of large macromolecules or minimize overlapping signal from undesired electroactive interference. Size-exclusion properties are attributed to the morphology of electropolymerized films [11].

In this thesis working platinum electrodes were covered by different electropolimerized barriers which have size exclusion properties and which have been synthetized by two different techniques: polymeric films traditionally [12] obtained by cyclic voltammetry and polymeric nanotubule nets obtained by “template synthesis” approach [13]. Permeability toward nitrites and interferences and the correspondent permselectivity were evaluated. For the first time, the influence of the polymer structure on permeability was explained by morphological studies. The best performances were obtained using Poly(1,3-DAB) film and Poly(1,2-DAB) nanotubule nets in terms of permselectivity and the resulting sensors were characterized to detect nitrites by amperometry in flow injection analysis (FIA). Best analytical performances were obtained modifying the platinum electrode by
traditional electropolymerized film and operational and long term stability, were studied. Real samples (drinking mineral and tap water) were analyzed and recovery studies were performed, showing the absence of matrix effects.

2 Experimental

2.1 Reagents and solutions

1,2-diaminobenzene (1,2-DAB), 1,3-diaminobenzene (1,3-DAB), 1,4-diaminobenzene (1,4-DAB), NaNO₂, Na₂SO₃, Phenol and NaClO₄ were purchased from Sigma Chemical Co. (St. Louis, MO); 1,8-diaminonaphtalene (1,8-DAN), o-anisidine, pyrrole were from Fluka (Buchs, Switzerland); CH₃COOH, NaH₂PO₄, CH₃COONa, HCl, HClO₄, H₂SO₄, Ascorbic Acid (AA) and CH₂Cl₂ were from Carlo Erba (Milan, Italy). Pt foils (thickness 0.025 mm) were purchased from Sigma-Aldrich and polycarbonate nanoporous particle track-etched
membranes (PC nanoPTMs) having a thickness of 10 µm, a pore density of $1 \times 10^8$ pores/cm$^2$ and pore diameters ranging from 15 to 100 nm, were obtained from Nucleopore (Italy).

### 2.2 Apparatus

An AUTOLAB PGstat/12 potentiostat/galvanostat was used for voltammetric studies and electropolymerizations. The amperometric measurements were carried out with a 641 VA-Detector (Metrohm, Switzerland) and currents were recorded using a Linseis L6512 recorder (Linseis, Selb, Germany). A (Pt-Ag | AgCl | 3M; model 805/CPG/6, from AMEL, Milan, Italy) was used as combined auxiliary/reference electrode, and a platinum electrode (model 492/Pt/3, AMEL, Milan, Italy) represented the working electrode.

For FIA measurements combined with electrochemical detection, we used a wall-jet cell (model 656, Metrohm, Herisau, Switzerland), a peristaltic pump Minipuls 3 (Gilson, France) with a six-way injection valve (model 5020) and a closed loop of 250 µl (Rheodyne, USA) and PTFE connection tubes (i.d. 0.5 mm from Supelco, Bellofonte, CA).

Morphologic studies were carried out at room temperature using a Field Emission Scanning Electron Microscope (model S-4000, Hitachi, Japan) having an accelerating voltage of 20 keV.
2.3 Procedure

2.3.a) Electrode polishing
The platinum electrode surfaces were polished with alumina powder ($\text{Al}_2\text{O}_3$, Buehler, Evanston, IL) having different particle size: 1, 0.3 and 0.05 $\mu\text{m}$ before use. After rinsing with distilled water, the electrodes were pre-treated by potential cycling in 0.5 M $\text{H}_2\text{SO}_4$ from $-0.2$ to $+1.2$ vs Ag/AgCl at a scan rate of 20 mV/s, until no changes were observed in the cyclic voltammograms [12].

2.3.b) Synthesis of polymeric films
Traditional polymeric films were synthetized directly on the electrode surface by cyclic voltammetry [14]. All the monomers, except the substituted naphthalene derivative, were used at a concentration of 5 mM and dissolved in 0.1 M phosphate buffer solution at pH 6.5 [15]. Solutions were deoxygenated with $\text{N}_2$ for 15 min just before the electropolymerization experiment. The potential was continuously cycled from 0 to + 0.8 V (vs Ag/AgCl) for 20 scans at a scan rate of 2 mV/s for 1,2-DAB, 1,3-DAB, 1,4-DAB, pyrrole and o-anisidine. 1,8-DAN (5 mM) was electropolymerised in HCl 1 M. This higher acidity was required for monomer solubilization presumably because of its higher hydrophobicity. The potential was continuously cycled from −0.15 to + 1.3 V (vs Ag/AgCl) for 20 scans at a scan rate of 2 mV/s.
2.3.c) Synthesis of polymeric nanotube nets

Polymeric nanotube were produced by a “template synthesis” [13] approach. A metallic Pt foil (1 cm²) has been used as working electrode. The polycarbonate nanoporous particle track-etched membranes were used as a template membrane for the synthesis of polymer nanostructured nets and they were assembled as working electrode in a conventional one-compartment cell on a platinum foil. The electropolymerization was performed, at room temperature using platinum as counter electrode, and an Ag/AgCl as reference electrode. All the monomers were used at a concentration of 5 mM and dissolved in 0.01 M HClO₄ + 0.1 M NaClO₄, as supporting electrolyte. Solutions were deoxygenated with N₂ for 15 minutes just before the electropolymerization experiment. Different potentials were applied for 250 s during the chronocoulometric experiment, depending on the monomer: 0.6 V for the three diaminobenzene isomers, 0.75 V for 1,8-DAN, 0.8 V for pyrrole and o-anisidine. Then, the template was dissolved by dichloromethane and the polymer nanotubules were placed directly on the platinum electrode surface, by drop coating. For the preliminary investigations 50 nm pore of polycarbonate (PC) template membrane, were used for all the monomers. Then the pore diameter was varied in order to evaluate the size exclusion effect of the nanostructured polymeric film on nitrite ion permeability.
2.3.d) Study of permeability and permselectivity

The permeability toward nitrites and several common interferences such as ascorbic acid, sulfites and phenols, was evaluated by cyclic voltammetry [14]. The potential was cycled between 0.2 V and 1.2 V at scan rate of 20 mV/s. All these analytes were used at a concentration of 40 mM, in 0.1 M acetate buffer pH 4 and the oxidation current peaks of nitrite and interferences were compared.

2.3.e) FIA procedure

For amperometric measurements in a flow injection analysis (FIA) the optimised parameters were found to be: flow-rate 0.6 ml/min, applied potential + 0.9 V vs Ag/AgCl, and 0.1 M acetate buffer, pH 4 as carrier solution. Standard nitrite solutions were prepared in 0.1 M acetate buffer pH 4 and then injected into a 250 µl loop, using a six-way injection valve.
2.3.f) Operational stability study
Study of operational stability was carried out by cyclic voltammetry. The potential was cycled between 0.2 V and 1 V at scan rate of 50 mV/s. 200 scans were performed in the same day. Nitrite was used at concentration of 40 mM, in 0.1 M acetate buffer, pH 4 and the peak currents were recorded.

2.3.g) Long term stability study
A long term stability study was carried out by cyclic voltammetry. The potential was cycled between 0.2 V and 1 V at scan rate of 50 mV/s. All the analytes were used at concentration of 40 mM, in 0.1 M acetate buffer, pH 4 and the peak current were recorded. Each day measurements were performed using fresh solutions. When not in use, the sensor was kept dried at room temperature.

2.3.h) Recovery studies
Recovery studies were carried out by amperometry in batch mode using 0.5 M acetate buffer pH 5 to minimize pH changes in real sample analysis. Buffer (2 ml) was diluted directly in drinking water (8 ml) and spiked by 20-50 μM of nitrites.
3 Results and discussion

3.1 Study of permeability

The goal of this thesis work is to investigate various polymeric barriers that could be used to assemble a sensor that will be highly permeable to nitrite but that excludes as much as possible potential interference. For this purpose a study about permeability toward nitrites and some common interferences, was performed. Three target interferents which can be oxidized at the same working potential, were selected: ascorbic acid (AA), phenol (PhOH) and sulfite. AA represents the most common interferent for applications of biosensors in clinic and food analysis [16]; Phenols are toxic substances frequently occurring in the aquatic environments, being generated by petroleum and petrochemical industries. Phenols are considered as priority pollutants to control, since they are harmful to organisms at low concentrations [17,18]. Finally, sulfite is a small anion comparable to nitrite and it is commonly used in the food and pharmaceutical industries as preservative and antioxidant. Sulfite and its oxidation products are pollutants and sulfiting agents, in particular, have an allergenic effect on hypersensitive individuals [19]. Film permeability was evaluated measuring the peak current by cyclic voltammetry for each analyte. Then the Permeability (P%) was calculated using the equation 1, as described in [12]:

\[
P(\%) = \left( \frac{I_{\text{sample}}}{I_{\text{blank}}} \right) \times 100
\]
where \( P\% = \frac{I_{\text{film}}}{I_{\text{bare}}} \times 100 \)  (1)

Table 1 shows the results obtained for the traditional polymeric films. Among them poly(1,3-DAB) had a good \( P\% \) for nitrite while also shows good rejection of interferents. The results of permeability obtained for platinum electrodes modified by nanostructured polymeric net are reported on Table 2. Almost all of the films exhibit high permeability toward nitrite, but also toward interferents. A better behaviour was obtained using poly(1,2-DAB) nanotubule net, which represented also a compromise between good permeability for nitrite with a significant repulsion of interferents.

**Table 1**

Permeability of traditional polymeric films

<table>
<thead>
<tr>
<th>Monomers used for the synthesis of polymeric films</th>
<th>( P% ) ( \text{NO}_2^- )</th>
<th>( P% ) ( \text{SO}_3^{2-} )</th>
<th>( P% ) PhOH</th>
<th>( P% ) AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-DAB</td>
<td>14.5</td>
<td>91.3</td>
<td>41.9</td>
<td>7.24</td>
</tr>
<tr>
<td>1,3-DAB</td>
<td>25.6</td>
<td>2.42</td>
<td>3.13</td>
<td>0.28</td>
</tr>
<tr>
<td>1,4-DAB</td>
<td>0.910</td>
<td>0.0520</td>
<td>1.22</td>
<td>0.183</td>
</tr>
<tr>
<td>1,8-DAN</td>
<td>0.620</td>
<td>1.63</td>
<td>29.4</td>
<td>0.490</td>
</tr>
<tr>
<td>o-anisidine</td>
<td>3.02</td>
<td>3.60</td>
<td>21.5</td>
<td>0.220</td>
</tr>
<tr>
<td>pyrrol</td>
<td>6.06</td>
<td>0.762</td>
<td>0.524</td>
<td>5.84</td>
</tr>
</tbody>
</table>
Table 2

Permeability of polymeric nanotubule nets

<table>
<thead>
<tr>
<th>Monomers used for the synthesis of polymeric nanotubules nets</th>
<th>P % NO₂⁻</th>
<th>P % SO₃²⁻</th>
<th>P % PhOH</th>
<th>P % AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-DAB</td>
<td>4.05</td>
<td>0.790</td>
<td>4.28</td>
<td>0.390</td>
</tr>
<tr>
<td>1,3-DAB</td>
<td>34.3</td>
<td>28.3</td>
<td>50.4</td>
<td>25.2</td>
</tr>
<tr>
<td>1,4-DAB</td>
<td>65.7</td>
<td>59.1</td>
<td>83.3</td>
<td>20.9</td>
</tr>
<tr>
<td>1,8-DAN</td>
<td>17.1</td>
<td>17.7</td>
<td>82.1</td>
<td>14.7</td>
</tr>
<tr>
<td>o-anisidine</td>
<td>88.8</td>
<td>65.1</td>
<td>93.9</td>
<td>66.8</td>
</tr>
<tr>
<td>pyrrol</td>
<td>19.8</td>
<td>11.7</td>
<td>8.5</td>
<td>19.2</td>
</tr>
</tbody>
</table>

3.2 Study of permselectivity

Since a film that comparably reduces the response to an analyte and potential interferents offers no advantages over a bare platinum electrode, the determination of the permselectivity represents an important factor for evaluation of the sensor performance. It is defined as the ratio between nitrite permeability and interferent permeability, as reported in the equation 2:

\[ P = \frac{P\% \text{ nitrites}}{P\% \text{ interferences}} \]
3.2.a) Traditional polymeric films

Fig. 1 shows results obtained for traditional polymeric films. Poly(1,3-DAB) represents the best polymeric layer in terms of high permeability for nitrite and permselectivity toward all the investigated interferents. In particular the permselectivity of this polymeric film could be explained in terms of its relatively high degree of hydrophobicity, where the oxidation of both amino groups resulted in an extensively cross-linked and less permeable polymer matrix. In addition the discrimination against electroactive species by charge repulsion represents another means of achieving selectivity, although it is very well known that all the polymeric films except the poly(1,2-DAB) significantly reject acetaminophen, a neutral molecule[20]. In fact, poly(1,3-DAB) showed lower permeability toward negatively charged molecules and the neutral one (PhOH), confirming that in this case, an additional electrostatic repulsion mechanism could act combined to the hydrophobicity and the polymeric chain structure effects, increasing the analytical performances in terms of permselectivity. According to literature [14] poly(1,8-DAN), resulted more hydrophobic than the three isomers of diaminobenzene demonstrating that there is a lower degree of solvatation, a closer packing of the polymer chains, and hence a lower permeability towards all the molecules considered in this work, even for nitrite. Considering the ratio between the hydrophobicity and the polymer
chain structures, poly(1,2-DAB), poly(1,4-DAB), poly(o-anisidine) and poly(pyrrole) have only one amino or hydroxyl group involved in the polymer formation and the resulting films are relatively hydrophilic [14]. For this reason a low degree of cross-linking between the polymer chains could be reasonable of a comparable permeability toward nitrite and interferences demonstrating no good performances in terms of permselectivity.
Fig.1
Permselectivity of traditional polymeric films

a) P% NO$_2^-$ / P% AA

b) P% NO$_2^-$ / P% SO$_3^{2-}$

c) P% NO$_2^-$ / P% PhOH
3.2.b) Polymeric nanotubule net

Fig. 2 shows the results obtained synthesizing polymeric nanostructures using polycarbonate membrane having 50 nm-pore size. The best performances in terms of permselectivity were obtained using poly(1,2-DAB) nanotubule net. The higher permselectivity values are probably related to denser distribution of these structures that is directly related to an easier process of electropolymerization. Infact small pore sizes of template (nanometer cut off) play an important role during the monomer diffusion process making easily 1,2-DAB electropolymerization, because only one amino group is involved. This effect is strictly related to poly(1,2-DAB) molecular structure if compared to the other polymers having complex molecular architectures both in terms of the different substituent positions on the aromatic ring (poly(1,3-DAB) and poly(1,4-DAB)) and a major number of the aromatic rings in the structure (poly(1,8-DAN)). For this purpose, to confirm this hypothesis related to the permselectivity performances of the poly(1,2-DAB) nanotubules and their nanostructured morphology, an additional study was performed using different pore diameters of polycarbonate membrane during the poly(1,2-DAB) nanotubules growth. The best analytical responses were obtained with nanotubules having 30 nm of diameter (see Table 3 and 4) considering a compromise between a good permeability and a good permselectivity, respectively. This nanometer cut off minimizes the major permeability toward interferences, typical of the largest pore diameter of the template membrane, giving an easier
access for the polymer diffusion into the template pores compared to the smaller pore diameter template.
Fig. 2
Permselectivity of polymeric nanotubule nets

a) P% NO$_2^-$ / P% AA

b) P% NO$_2^-$ / P% SO$_3^{2-}$

c) P% NO$_2^-$ / P% PhOH
3.3 Morphological characterization

The morphology of the electropolimerized films was investigated using a field emission type scanning electron microscope (FE-SEM). Fig. 3a shows poly(1,3-DAB) film synthesized directly on a platinum foil having a thickness of 0.025 mm and it reveals the presence of the typical globular structure of the electrosynthesized polymers [21]. In any case this nodular arrangement produces a dense and overlapped polymeric film (Fig. 3b) which is probably responsible of the high permeability for nitrite and suitable permselectivity toward all the investigated interferences.

Poly(1,2-DAB) nanotubule nets synthesized by the template method dissolving the membrane in dichloromethane, showed a typical filled cylindrical morphology [22]. This coating provides a larger surface area per unit volume than the electrodeposited polymeric films. So more porous layers were obtained and this is in agreement with high permeability values calculated for these nets.
Fig. 3: Poly[1,3-DAB] film grown in 0.1 M phosphate buffer pH 6.5 by cyclic voltammetry (0 – 0.8 V vs Ag|AgCl; 2 mV/s; 10 scans) WE: Pt foil (0.025 mm of thickness)

a)

b)
3.4 FIA procedure

Having identified the best microstructured and nanostructured polymeric films synthesized on the probe surface, they were analytically characterized in a FIA analysis system for the amperometric detection of nitrite. Calibration curves for nitrite were generated using the two different electrodes in the system and on the basis of these results parameters are summarized in table 4. The microstructured poly(1,3-DAB) modified platinum electrode shows a good linearity over the range 10-1000 \( \mu \text{M} \), a linear regression equation of \( \frac{I}{\mu \text{A}} = 1.14 \frac{x}{\mu \text{M}} + 2.6 \), and a detection limit (LOD=3\( \sigma \)) of 2 \( \mu \text{M} \).

On the other hand the best nanostructured sensor, assembled using poly(1,2-DAB) nets, showed good linearity over the range 10-1000 \( \mu \text{M} \), a linear regression equation of \( \frac{I}{\mu \text{A}} = 0.20 \frac{x}{\mu \text{M}} + 2.8 \) and a detection limit of 2 \( \mu \text{M} \).

Table 4

Study of different pore size PC membrane effect on the permselectivity of poly(1,2-DAB) nanotubules nets

<table>
<thead>
<tr>
<th>Pore size diameter (nm)</th>
<th>( \frac{\text{P NO}_2^- / \text{P AA}}{} )</th>
<th>( \frac{\text{P NO}_2^- / \text{P SO}_4^{2-}}{} )</th>
<th>( \frac{\text{P NO}_2^- / \text{P PhOH}}{} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.88</td>
<td>3.57</td>
<td>1.12</td>
</tr>
<tr>
<td>30</td>
<td>10.4</td>
<td>0.95</td>
<td>0.68</td>
</tr>
<tr>
<td>50</td>
<td>7.61</td>
<td>3.30</td>
<td>1.46</td>
</tr>
<tr>
<td>100</td>
<td>1.55</td>
<td>2.24</td>
<td>1.28</td>
</tr>
</tbody>
</table>
3.5 Stability studies

A platinum electrode modified by poly(1,3-DAB) traditional film that showed the best analytical parameters was studied for stability. Operational stability was evaluated by performing 200 measurements in a day. All peak current values were referred to the initial value. Fig. 4 shows that the sensor loses 50% of signal over 100 measurements. It is interesting to note that for the first time, an operational stability study demonstrated reasonable sensor behavior, working continuously during the same day.

Long term stability was also evaluated storing the sensor dried at room temperature when not in use. Fig 5 shows the sensor response toward nitrite and interferents expressed in terms of I%, which is defined in the following equation:

\[ I\% = \frac{I_n}{I_a} \times 100 \]  

(3)

where \( I_a \) was the peak current value of nitrite obtained on the first day of measurement, and \( I_n \) was the peak current value recorded after \( n \) days (measuring a solution of 40 mM of nitrite every day). Under these operational conditions, the nitrite sensor it turns out to be stable, since it lost 70% of the initial signal during one month. Considering that a previous study concerning the long-term stability was not reported before in literature, these data could be considered very interesting to
evaluate the life time of these sensors under extreme conditions.

Fig. 4

Operational stability
Fig. 5

Long term stability
3.6 Recovery study

Also nitrite recovery data were obtained using a poly (1,3-DAB) film modified platinum electrode. Standard solutions of nitrites were added to different drinking water samples (tap water and two different mineral waters, in which no nitrites were present) to obtain final concentrations of 20 and 50 mM, respectively. These recovery data, reported in Table 5, showed the absence of matrix effect.

Table 5
Recovery study performed adding standard solutions of nitrites to different water samples.
The nitrite sensor was a platinum electrode modified by microstructured poly(1,3-DAB) film

<table>
<thead>
<tr>
<th>Water sample</th>
<th>Nitrites added (µM)</th>
<th>Nitrites found before spiking (µM)</th>
<th>Expected value (µM)</th>
<th>Measured value (µM)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>13</td>
<td>65</td>
</tr>
<tr>
<td>Tap water</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>48</td>
<td>96</td>
</tr>
<tr>
<td>Mineral water “Perla”</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>Mineral water “Perla”</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>48</td>
<td>96</td>
</tr>
<tr>
<td>Mineral water “Sant’Agata”</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td>Mineral water “Sant’Agata”</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>44</td>
<td>88</td>
</tr>
</tbody>
</table>
4 Conclusion

Platinum electrodes were modified by polymeric barriers synthesized by different techniques to have a selective sensor for nitrite detection. The best resulting sensor was obtained using poly(1,3-DAB) traditional film and it had the sensitivity required for implementation of Italian legal limits for nitrite and there was no demonstrable matrix effect when it was applied for measurements of nitrite in tap and mineral water. The long term stability of the sensor needs to be improved in order to use this sensor as a tool for continuous monitoring.
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Publishers, Inc, pp. 91-92 (Chapter 3)


Chapter V - Detection of ammonia in drinking water using chemically modified electrodes by polypyrrole films and nanowires

Abstract

Highly oriented pyrolitic graphite (HOPG) electrodes were modified with conductive polypyrrole in two different forms: microstructured electrosynthesized films and nanowires obtained by chemical oxidation. Morphological characterizations were carried out and both sensors were investigated analytically for ammonia determination in water. Satisfactory results as linear concentration range (10-200 μM), linear regression equation \( \frac{y}{\mu A} = 1.71 \times \mu M + 4.32 \), reproducibility (R.S.D. % = 3 \( n=3 \)) and limit of detection (LOD=30 μM) were obtained for electrodes assembled with the electrosynthesized film. The LOD is improved by coating HOPG electrodes with polypyrrole nanowires, resulting in a value of 5 μM. Other analytical parameters were determined (linear range of concentration (10-200 μM), linear regression equation \( \frac{y}{\mu A} = 2.36 \times \mu M - 1.36 \), reproducibility (R.S.D. % = 3 \( n=3 \))). Finally real drinking water samples were analyzed and the recovery study showed that there was no matrix effect on the sensor performances.

1. Introduction
Ammonia is part of the natural nitrogen cycle; it arises from the biological decomposition of nitrogen compounds and from the extensive use of fertilisers and pesticides in the agricultural sector. The determination of ammonia is very important for monitoring leakage from refrigeration systems, industrial hygiene, clinical diagnosis, and environmental protection. The most usual ammonia determination is performed by spectrophotometric methods linked to use of the Berthelot reaction [1]. In addition, ion chromatography can be used for ammonium detection, but to eliminate interferences by other ionic species it has to be coupled with time consuming sample preparation procedures [2]. Therefore there is a growing interest in alternative methods for rapid, easy and efficient determination of ammonia. An interesting possibility involves using an electrochemical procedure. Ammonium determination can be performed by potentiometric ion selective electrodes [3,4,5,6], also used in combination with ammonia forming enzymes [7]. Among all the analytical methods for ammonia determination, amperometric measurements using chemically modified electrodes (CME’s) are widely reported and are based on CuBr films [8], polyvinylpyrrolidone [9], polyaniline-poly(acrylonitrile-butadiene-styrene) [10] and polyaniline chemistry [11,12]. Gaseous ammonia is known to reduce the oxidized form of the conducting polymer polypyrrole (PPy) [13]. Trojanowicz et al. [14] described an amperometric ammonia sensor for the determination of dissolved ammonia in aqueous samples, where PPy is electrochemically deposited on a platinum electrode. The amperometric response is attributed to the reversible doping-undoping of the polymer matrix in the presence or absence of ammonia. When the PPy electrode comes in contact with an aqueous solution of ammonia, the ammonia
(according to the undoping-doping model) reduces PPy. If a potentiostat is used to keep the potential of the PPy electrode at a constant positive value, the PPy film is immediately reoxidized (doped) to its original redox state [15]. Following this scheme, this work proposes an amperometric sensor, based on an highly oriented pyrolitic graphite electrode (HOPG) modified with conducting polypyrrole, for the detection of ammonia in drinking water. Two different approaches are described here: microstructured polypyrrole films electrochemically synthesized on the electrode surface and polypyrrole nanowires chemically synthesized and deposited by casting on the HOPG electrode. Both of these chemical sensors were analytically characterized in terms of limit of detection, linear range of concentration, RSD%, sensitivity and stability. In addition, “synthetic water” is used to test the effect of interferents and finally real drinking water samples were analyzed.

2. Experimental

2.1 Reagents and solutions
Al2O3 was purchased from Buehler, Evanston, IL; Pyrrole, Fe(CN6)3-, FeCl3 and H3BO3 were purchased from Fluka (Buchs, Switzerland); NaCl, NaOH, HCl and acetonitrile were purchased from Carlo Erba (Milan, Italy); NH4Cl, MgSO4, CaCl2, KNO3, Na2CO3 and PVA were purchased from Sigma-Aldrich (St. Louis, USA); SDS was purchased from Merck (Darmstadt, Germany). All these reagents were of analytical grade and they were used without any further purification. Distilled water was obtained by a Multi-Q system.
2.2 Apparatus

An AUTOLAB PGstat/12 potentiostat/galvanostat was used for oxidative electropolymerizations. Amperometric measurements were carried out with a 641 VA-Detector (Metrohm, Switzerland) and currents were recorded using a Linseis L6512 recorder (Linseis, Selb, Germany). A Pt-Ag|AgCl|KCl(3M) (model 805/CPG/6, from AMEL, Milan, Italy) was used as combined auxiliary/reference electrode, and a highly oriented pyrolic graphite (model 492/PG/3, AMEL, Milan, Italy) represented the working electrode. Morphological studies were carried out at room temperature using a Field Emission Scanning Electron Microscope (model S-4000, Hitachi, Japan) having an accelerating voltage of 20 keV.

2.3 Procedure

2.3.a) Electrode polishing

The pyrolic graphite electrode surfaces were polished with alumina powder (having different particle size: 1, 0.3 and 0.05 μm) before use. After rinsing with distilled water, the electrodes were sonicated for 15 minutes in a solution 1:1 water:ethanol.

2.3.b) Synthesis of microstructured polymeric films

Traditional polymeric films were synthesized directly on the electrode surface by chronoamperometry, as widely described in literature[14]. For this purpose, the HOPG electrode was
immersed in a solution containing 0.1 M of pyrrole and 0.14 M of NaCl, as supporting electrolyte. A potential of +0.80 V vs Ag/AgCl was applied for 3 minutes.

2.3.c) Chemical synthesis of polypyrrole nanowires
100 ml of a solution containing the monomer (0.18 M pyrrole) and a surfactant as template (0.18 M SDS) were mixed with 100 ml of a solution containing an oxidant (0.26 M FeCl3) and a stabilizer (3% PVA). The final solution was kept at 0°C for 24 h and then the resulting polypyrrole precipitate was vacuum-filtered and washed copiously with distilled water, methanol and acetone for several times. Finally, it was dried overnight in oven, at 37°C [16].

2.3.d) Amperometric study
According to the literature [14] an applied potential of +0.30 V (vs Ag/AgCl) is sufficiently low to obtain some undoping of the polymer and the undoping is additionally favored by the interaction with ammonia. Measurements were realized, in batch mode, using a 50 mM borate buffer pH 10, containing 0.14 M NaCl. The buffer pH value was fixed at 10 to obtain a large amount of ammonia in solution (pKb= 9.25). In addition, the introduction of chloride in the sample solution resulted in a doping process, which was observed as a current signal. The introduction of chloride into the buffer solution contributed to eliminate this effect.

2.3.e) Synthetic water
Synthetic water was obtained by the addition of several cations and anions normally found in drinking water, to
evaluate interference effects on the amperometric NH3 response; it is an aqueous solution containing 1.1 mM of MgSO4, CaCl2, KNO3, Na2CO3.

2.3.f) Stability studies
Operational stability was evaluated performing 100 continuous measurements in a 50 µM ammonium chloride standard solution on the same working day. The long term stability was evaluated measuring a 50 µM ammonium chloride standard solution, every day for two weeks. Electrodes when not in use, were stored dried.

2.3.g) Recovery studies
Recovery studies were carried out in amperometric batch mode using 0.5 M borate buffer + 1.4 M NaCl, pH 10, to minimize pH changes in real sample analysis. Buffer (2 ml) was diluted directly in drinking water (8 ml) spiked by ammonium chloride solution to have a final concentration of 20 and 50 µM.

3. Results and discussion

3.1 Microstructured polypyrrole modified electrode characterization
The polypyrrole morphology obtained from the electrochemical synthesis was investigated using a field emission type scanning electron microscope (FE-SEM). Fig. 1 shows a clearly visible polymeric film grown by chronoamperometry with globular structures characterizing the surface morphology of the layer. Such nodular systems are typical features of the
conducting polymers obtained by electrochemical polymerization. Moreover it can be observed that the polymeric film completely covers the electrode surface, resulting in an homogenous and very compact layer [17, 18].

The PPy layer on the HOPG electrode was produced under potentiostatic conditions at + 0.80 V vs Ag/AgCl from a solution containing 0.14 M sodium chloride and 0.1 M pyrrole, as described in the experimental section. For a very short time a transparent yellow film, corresponding to the neutral state of the polymer, is formed and this is followed after several seconds by the formation initially of a dark blue and then a brownish-black layer of oxidized polymer. A 3 min polymerization time proved to give CME’s having optimum amperometric response for ammonia. The response was improved by the use of freshly prepared monomer solutions to deposit the film. The electropolymerization of pyrrole without stirring, when compared to that done with stirring led to ten-fold higher sensitivity of the resulting probe for the amperometric determination of ammonia. This can be attributed to the formation of a more ordered structure of the polymer under diffusion-controlled conditions [14]. During the electropolymerizations of pyrrole, the positive charges of the polymer backbone are balanced by doping anions. Both the structural and electrochemical properties of the polymer depend substantially on the dopant anion. Several experiments were performed, using Cl−, ClO4− and PO43− as counter anions, aiming to stabilize the radical cation during the electropolymerization [19]. In this work chloride was used as dopant, because better results were obtained: it was also included in the buffer to minimize interferent effects, which become relevant if the real sample is drinking water. For two
different reasons the sensitivity of the amperometric detection of ammonia is affected by the pH of the buffer used: a change in pH shifts the protonation equilibrium of ammonia in the solution but it was also reported that the PPy chain is pH sensitive and undergoes a deprotonation process with a pKa around 9-11. For this reason, different buffers with different pHs were studied and a borate buffer pH 10, was selected to obtain higher current signals [20].

The electrochemically-active area was evaluated using chronoamperometry in a solution of 1mM Fe(CN6)3-, a compound which shows good electrochemistry at bare and modified HOPG electrodes. The slope of the linear region of the I vs t-1/2 plot in the fast scan region yields the product 

\[ I_d = nFAC^0D^{1/2}v^{1/2} \]  

(1) 

Where \( C^0 = 1 \text{mM} \) and \( D = 7.9 \times 10^{-10} \text{cm}^2 \text{s}^{-1} \) are the concentration and diffusion coefficient respectively of Fe(CN6)3- while the other parameters have their usual meanings. The apparent geometric area of the HOPG electrode is 0.070 cm2, while the electrochemical area thus estimated is 0.090 cm2.
3.2 Polypyrrole nanowires modified electrode characterization

The properties of the modifying materials depend not only of the chemical composition, but also on their morphologies. On account of their small size, nanoscale materials possess unique properties including electrical conductivity, higher signal/noise ratio and the possibility to be functionalized, so as to make them suitable for a wide range of applications, such as sensor chips, biosensors, nano-arrays, nanomotors [21]. The synthesis of nanoscale materials has attracted great interest in the past ten years; conducting polymers have also been intensively studied for their one-dimensional conjugated structures and adjustable conductivity [22]. Polypyrrole nanofibers, nanotubes and nanowires can be synthesized by an electrochemical polymerization approach using a template method [23]. This method allows to modulate the final polymer properties, such as shape, length, diameter using different
electrochemical techniques. Normally polycarbonate or alumina membranes having nanometer pores were used as templates to grow the polymeric nanostructures. The polymers grow into the pores of the template membrane resulting in a cylindrical shape. In this work polypyrrole nanowires were synthesized by chemical oxidation, because it is simpler and cheaper to produce large quantities of nanomaterial. Using this method, FeCl₃ was employed as oxidant and PVA as stabilizer while SDS was chosen as surfactant. Polypyrrole nanowires were used to modify HOPG electrodes by casting 6 µl of a 1 mg/ml PPy nanowires dispersion using acetonitrile as solvent. Fig. 2 shows FE-SEM images of the polypyrrole nanowires synthesized by chemical oxidation. It can be noted the characteristic topography of the chemically polymerized conducting polymer: presence of fibrillar structures that confer an interesting coral-like morphology to the deposit. From the SEM pictures it is possible to estimate the nanowire dimensions; in particular the lengths range from 1 to 2 µm whereas the diameters are comprised between 100-150 nm. It is well known that experimental conditions influence the morphology of the polymer: nanowires can be obtained using a 0.07 M and adding an emulsion agent such as PVA 3% [16]. After casting, the electrode can be used to measure ammonium solution by the amperometric method. The electrochemically-active area was evaluated by chromamperometric experiments, as described above for the microstructured PPy film modified electrode (eq. 1) and resulted in a larger active surface area (0.820 cm²), relative to the apparent geometric area, as expected when employing nanomaterials.
3.3 Analytical results

In Table 1 calibration equations and analytical parameters are reported for both of the modified electrodes. Measurements were carried out initially in the selected buffer to study ideal conditions and then were repeated diluting a concentrated buffer solution with synthetic water, to evaluate the matrix effect. Good results were obtained in terms of limit of detection, which was found to be comparable to that fixed by Italian law for ammonia (30 μM for drinking water) [24]. The best signal/noise ratio that was obtained using polypyrrole nanowire-modified electrodes that also gave a lower detection limit, was relative to results obtained with microstructured polypyrrole-modified electrodes. The results obtained using synthetic water were used to calculate recovery values when real drinking water samples were analyzed.
Analytical parameters obtained by the amperometry calibration curves performed at HOPG modified electrodes

HOPG electrode
modified by
Measurements performed in Linear range

<table>
<thead>
<tr>
<th></th>
<th>(M) Slope</th>
<th>(M) a R.S.D. %</th>
<th>(slop) Bias</th>
<th>(A) a R.S.D. %</th>
<th>(bias) LODb</th>
<th>(M) Sensitivity</th>
<th>(A/M-1cm-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microstructured polypyrrole film</td>
<td>50 mM borate buffer pH 10 + 0.14 M NaCl</td>
<td>10 - 200</td>
<td>1.71 3</td>
<td>4.32 2</td>
<td>30 24.4</td>
<td>4.32</td>
<td>2</td>
</tr>
<tr>
<td>Polypyrrole nanowires</td>
<td>50 mM borate buffer pH 10 + 0.14 M NaCl</td>
<td>10 - 200</td>
<td>2.36 3</td>
<td>-1.36 4</td>
<td>5 33.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microstructured polypyrrole film</td>
<td>0.5 M borate buffer pH 10 + 1.4 M NaCl (dilute by synthetic water)</td>
<td>10 - 100</td>
<td>5.40 2</td>
<td>3.52 1</td>
<td>24 77.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polypyrrole nanowires</td>
<td>0.5 M borate buffer pH 10 + 1.4 M NaCl (dilute by synthetic water)</td>
<td>10 - 100</td>
<td>16.6 4</td>
<td>99.8 3</td>
<td>10 237</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Applied potential: + 300 mV vs Ag/AgCl
a n=3
b LOD= 3S/N
3.4 Stability study
The operational stability was evaluated by performing 100 measurements in a day. Results are expressed in terms of I%, which is defined in the following equation:

$$I% = \left( \frac{I_n}{I_a} \right) \times 100$$  \hspace{1cm} (eq. 2)

Where Ia was the current value obtained on the first measurement, and In was the current value recorded after n measurements (measuring a solution of 50 μm of ammonium). Fig. 3 shows that a microstructured polypyrrole film-modified HOPG electrode loses 50% of signal over 80 measurements; a polypyrrole nanowires modified HOPG electrode is less stable, losing 50% of signal only after 30 measurements.

Fig. 3
Operation stability of the modified HOPG electrodes

Long term stability was also evaluated by storing the microstructured dried sensors at room temperature when not in use. Nanostructured sensors were stable for 1 day. Fig. 4 shows the microstructured sensor response toward ammonium in terms of I%, calculated as in (eq. 2) where Ia was the current value obtained on the first day of measurements, and In was the current value recorded after n days (measuring a solution of 50 μm of ammonium). Under these operational conditions, this sensor turns to be relatively stable because it lost 70% of the initial signal during a week.

Fig. 4
3.5 Real sample analysis

Both electrodes were used to analyze real samples. "Synthetic water" was used to evaluate the matrix effect by the addition of standard solutions of ammonium. Finally, real drinking water samples were analyzed using calibration curves obtained with "synthetic water". Recovery values are shown in Table 2 and ranged from 92 to 107%. These results confirm that there was a negligible matrix effect occurred in real samples using our developed sensors.

Table 2.
Recovery study performed adding standard solution of ammonium to different drinking water samples

<table>
<thead>
<tr>
<th>HOPG electrode</th>
<th>Modified by</th>
<th>Samples</th>
<th>NH4+ added (μM)</th>
<th>NH4+ found before spiking (μM)</th>
<th>Expected value (μM)</th>
<th>Measured value (μM)</th>
<th>R.S.D. (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microstructured polypyrrole film</td>
<td>Mineral water “Santa Croce”</td>
<td>50</td>
<td>20</td>
<td>70</td>
<td>75</td>
<td>0.8</td>
<td>107</td>
<td></td>
</tr>
</tbody>
</table>
Microstructured polypyrrole film  Mineral water "Sorgente" 50
8  58  57  1.2  98
Polypyrrole nanowires Mineral water "Santa Croce" 50  22
72  70  2.8  97
Polypyrrole nanowires Mineral water "Sorgente" 50  11  61
56  3.2  92

4. Conclusions

Two sensors for ammonia detection in drinking water were developed using either conducting polypyrrole films or polypyrrole nanowires. The first prototype sensor is assembled by synthesizing the microstructured polymer directly on the surface of an HOPG electrode. Satisfactory results in terms of sensitivity and stability are obtained, but a high LOD was observed. To improve this parameter, the HOPG electrode was then modified by polypyrrole nanowires. It is hypothesized that the more compact structure of microstructured film, compared with that of nanowires, acts as a barrier for the analyte. Effectively, better results were obtained with nanowires also because of the higher surface, electrical conductivity and signal/noise ratio. Finally both sensors could be successfully used to detect NH3 in real samples. The polypyrrole nanowire-modified HOPG electrode showed higher sensitivity, while the microstructured one was more stable and thus more suitable for use in automated systems.
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Chapter V – Detection of ammonia in drinking water using chemically modified electrodes by polypyrrole films and nanowires

Abstract

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1. Introduction

Ammonia is part of the natural nitrogen cycle; it arises from the biological decomposition of nitrogen compounds and from the extensive use of fertilisers and pesticides in the agricultural sector. The determination of ammonia is very important for monitoring leakage from refrigeration systems, industrial hygiene, clinical diagnosis, and environmental protection. The most usual ammonia determination is performed by spectrophotometric methods linked to use of the Berthelot reaction [1]. In addition, ion chromatography can be used for ammonium detection, but to eliminate interferences by other ionic species it has to be coupled with time consuming sample preparation procedures [2]. Therefore there is a growing interest in alternative methods for rapid, easy and efficient determination of ammonia. An interesting possibility involves using an electrochemical procedure. Ammonium determination can be performed by potentiometric ion selective electrodes [3,4,5,6], also used in combination with ammonia forming enzymes [7]. Among all the analytical methods for ammonia determination, amperometric measurements using chemically modified electrodes (CME’s) are widely reported and are based on CuBr films [8], polyvinylpyrrolidone [9], polyaniline-poly(acrylonitrile-butadiene-styrene) [10] and polyaniline chemistry [11,12]. Gaseous ammonia is known to reduce the oxidized form of the conducting polymer polypyrrole (PPy) [13]. Trojanowicz et al. [14] described an amperometric ammonia sensor for the determination of
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2. Experimental

2.1 Reagents and solutions

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2.3.a) Electrode polishing
The pyrolitic graphite electrode surfaces were polished with alumina powder (having different particle size: 1, 0.3 and 0.05 µm) before use. After rinsing with distilled water, the electrodes were sonicated for 15 minutes in a solution 1:1 water:ethanol.

2.3.b) Synthesis of microstructured polymeric films
Traditional polymeric films were synthesized directly on the electrode surface by chronoamperometry, as widely described in literature[14]. For this purpose, the HOPG electrode was immersed in a solution containing 0.1 M of pyrrole and 0.14 M of NaCl, as supporting electrolyte. A potential of + 0.80 V vs Ag/AgCl was applied for 3 minutes.

2.3.c) Chemical synthesis of polypyrrole nanowires
100 ml of a solution containing the monomer (0.18 M pyrrole) and a surfactant as template (0.18 M SDS) were mixed with 100 ml of a solution containing an oxidant (0.26 M FeCl₃) and a stabilizer (3% PVA). The final solution was kept at 0°C for 24 h and then the resulting polypyrrole precipitate was vacuum-filtered and washed copiously with distilled water, methanol and acetone for several times. Finally, it was dried overnight in oven, at 37°C [16].
2.3.d) Amperometric study
According to the literature [14] an applied potential of +0.30 V (vs Ag/AgCl) is sufficiently low to obtain some undoping of the polymer and the undoping is additionally favored by the interaction with ammonia. Measurements were realized, in batch mode, using a 50 mM borate buffer pH 10, containing 0.14 M NaCl. The buffer pH value was fixed at 10 to obtain a large amount of ammonia in solution (pKb = 9.25). In addition, the introduction of chloride in the sample solution resulted in a doping process, which was observed as a current signal. The introduction of chloride into the buffer solution contributed to eliminate this effect.

2.3.e) Synthetic water
Synthetic water was obtained by the addition of several cations and anions normally found in drinking water, to evaluate interference effects on the amperometric NH₃ response; it is an aqueous solution containing 1.1 mM of MgSO₄, CaCl₂, KNO₃, Na₂CO₃.

2.3.f) Stability studies
Operational stability was evaluated performing 100 continuous measurements in a 50 µM ammonium chloride standard solution on the same working day. The long term stability was evaluated measuring a 50 µM ammonium chloride standard solution, every day for two weeks. Electrodes when not in use, were stored dried.
2.3.g) Recovery studies

Recovery studies were carried out in amperometric batch mode using 0.5 M borate buffer + 1.4 M NaCl, pH 10, to minimize pH changes in real sample analysis. Buffer (2 ml) was diluted directly in drinking water (8 ml) spiked by ammonium chloride solution to have a final concentration of 20 and 50 µM.

3. Results and discussion

3.1 Microstructured polypyrrole modified electrode characterization

The polypyrrole morphology obtained from the electrochemical synthesis was investigated using a field emission type scanning electron microscope (FE-SEM). Fig. 1 shows a clearly visible polymeric film grown by chronoamperometry with globular structures characterizing the surface morphology of the layer. Such nodular systems are typical features of the conducting polymers obtained by electrochemical polymerization. Moreover it can be observed that the polymeric film completely covers the electrode surface, resulting in an homogenous and very compact layer [17,18].

The PPy layer on the HOPG electrode was produced under potentiostatic conditions at + 0.80 V vs Ag/AgCl from a solution containing 0.14 M sodium chloride and 0.1 M pyrrole, as described in the experimental section. For a very short time a transparent yellow film, corresponding to the neutral state of the polymer, is formed and this is followed after several seconds by the formation initially of a dark blue and then a brownish-black layer.
of oxidized polymer. A 3 min polymerization time proved to give CME’s having optimum amperometric response for ammonia. The response was improved by the use of freshly prepared monomer solutions to deposit the film. The electropolymerization of pyrrole without stirring, when compared to that done with stirring led to ten-fold higher sensitivity of the resulting probe for the amperometric determination of ammonia. This can be attributed to the formation of a more ordered structure of the polymer under diffusion-controlled conditions [14]. During the electropolymerizations of pyrrole, the positive charges of the polymer backbone are balanced by doping anions. Both the structural and electrochemical properties of the polymer depend substantially on the dopant anion. Several experiments were performed, using Cl\(^-\), ClO\(_4\)^- and PO\(_4\)^3- as counter anions, aiming to stabilize the radical cation during the electropolymerization [19]. In this work chloride was used as dopant, because better results were obtained: it was also included in the buffer to minimize interferent effects, which become relevant if the real sample is drinking water. For two different reasons the sensitivity of the amperometric detection of ammonia is affected by the pH of the buffer used: a change in pH shifts the protonation equilibrium of ammonia in the solution but it was also reported that the PPy chain is pH sensitive and undergoes a deprotonation process with a pK\(_a\) around 9-11. For this reason, different buffers with different pHs were studied and a borate buffer pH 10, was selected to obtain higher current signals [20].
The electrochemically-active area was evaluated using chronoamperometry in a solution of 1mM Fe(CN$_6$)$_3^-$, a compound which shows good electrochemistry at bare and modified HOPG electrodes. The slope of the linear region of the I vs t$^{-1/2}$ plot in the fast scan region yields the product

\[ nFAC°D^{1/2}v^{1/2} \]

according to the Cottrell equation:

\[ I_d = nFAC°D^{1/2} \frac{2}{(\pi)^{1/2}} \]

Where \( C° = 1\text{mM} \) and \( D = 7.9\times10^{-10} \text{ cm}^2 \text{ s}^{-1} \) are the concentration and diffusion coefficient respectively of Fe(CN$_6$)$_3^-$ while the other parameters have their usual meanings. The apparent geometric area of the HOPG electrode is 0.070 cm$^2$, while the electrochemical area thus estimated is 0.090 cm$^2$. 

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Fig. 1
Polypyrrole microstructured film grown by chronoamperometry.
(E = 0.80 V vs Ag/AgCl, for 180 s) from a solution containing 0.1 M of pyrrole and 0.14 M of NaCl.
WE: GC foil

3.2 Polypyrrole nanowires modified electrode characterization
The properties of the modifying materials depend not only of the chemical composition, but also on their morphologies. On account of their small size, nanoscale materials possess unique properties including electrical conductivity, higher signal/noise ratio and the
possibility to be functionalized, so as to make them suitable for a wide range of applications, such as sensor chips, biosensors, nano-arrays, nanomotors [21]. The synthesis of nanoscale materials has attracted great interest in the past ten years; conducting polymers have also been intensively studied for their one-dimensional conjugated structures and adjustable conductivity [22]. Polypyrrole nanofibers, nanotubes and nanowires can be synthesized by an electrochemical polymerization approach using a template method [23]. This method allows to modulate the final polymer properties, such as shape, length, diameter using different electrochemical techniques. Normally polycarbonate or alumina membranes having nanometer pores were used as templates to grow the polymeric nanostructures. The polymers grow into the pores of the template membrane resulting in a cylindrical shape. In this work polypyrrole nanowires were synthesized by chemical oxidation, because it is simpler and cheaper to produce large quantities of nanomaterial. Using this method, FeCl$_3$ was employed as oxidant and PVA as stabilizer while SDS was chosen as surfactant. Polypyrrole nanowires were used to modify HOPG electrodes by casting 6 µl of a 1 mg/ml PPy nanowires dispersion using acetonitrile as solvent. Fig. 2 shows FE-SEM images of the polypyrrole nanowires synthesized by chemical oxidation. It can be noted the characteristic topography of the chemically polymerized conducting polymer: presence of fibrillar structures that confer an interesting coral-like morphology to the deposit. From the SEM pictures it is possible to estimate the nanowire dimensions; in particular the lengths range from 1 to 2
µm whereas the diameters are comprised between 100-150 nm. It is well known that experimental conditions influence the morphology of the polymer: nanowires can be obtained using a 0.07 M and adding an emulsion agent such as PVA 3% [16]. After casting, the electrode can be used to measure ammonium solution by the amperometric method. The electrochemically-active area was evaluated by chronamperometric experiments, as described above for the microstructured PPy film modified electrode (eq. 1) and resulted in a larger active surface area (0.820 cm²), relative to the apparent geometric area, as expected when employing nanomaterials.
Fig. 2.
Polypyrrole nanowires obtained by chemical oxidation in solution
(0.18 M SDS, 0.18 M pyrrole, 0.26 M FeCl₃, PVA 3%; kept at 0°C for 24h)
3.3 Analytical results

In Table 1 calibration equations and analytical parameters are reported for both of the modified electrodes. Measurements were carried out initially in the selected buffer to study ideal conditions and then were repeated diluting a concentrated buffer solution with synthetic water, to evaluate the matrix effect. Good results were obtained in terms of limit of detection, which was found to be comparable to that fixed by Italian law for ammonia (30 µM for drinking water) [24]. The best signal/noise ratio that was obtained using polypyrrole nanowire-modified electrodes that also gave a lower detection limit, was relative to results obtained with microstructured polypyrrole-modified electrodes. The results obtained using synthetic water were used to calculate recovery values when real drinking water samples were analyzed.
Table 1

Analytical parameters obtained by the amperometry calibration curves performed at HOPG modified electrodes

<table>
<thead>
<tr>
<th>HOPG electrode modified by</th>
<th>Measurements performed in</th>
<th>Linear range (µM)</th>
<th>Slope (µM)</th>
<th>R.S.D. % (slope)</th>
<th>Bias (µA)</th>
<th>R.S.D. % (bias)</th>
<th>LODb (µM)</th>
<th>Sensitivity (µAµM⁻¹cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microstructured polypyrrole film</td>
<td>50 mM borate buffer pH 10 + 0.14 M NaCl</td>
<td>10 - 200</td>
<td>1.71</td>
<td>3</td>
<td>4.32</td>
<td>2</td>
<td>30</td>
<td>24.4</td>
</tr>
<tr>
<td>Polypyrrole nanowires</td>
<td>50 mM borate buffer pH 10 + 0.14 M NaCl</td>
<td>10 - 200</td>
<td>2.36</td>
<td>3</td>
<td>-1.3</td>
<td>6</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Microstructured polypyrrole film</td>
<td>0.5 M borate buffer pH 10 + 1.4 M NaCl (dilute by synthetic water)</td>
<td>10 - 100</td>
<td>5.40</td>
<td>2</td>
<td>3.52</td>
<td>1</td>
<td>24</td>
<td>77.1</td>
</tr>
<tr>
<td>Polypyrrole nanowires</td>
<td>0.5 M borate buffer pH 10 + 1.4 M NaCl (dilute by synthetic water)</td>
<td>10 - 100</td>
<td>16.6</td>
<td>4</td>
<td>99.8</td>
<td>3</td>
<td>10</td>
<td>237</td>
</tr>
</tbody>
</table>

Applied potential: + 300 mV vs Ag/AgCl

\( a \) \( n=3 \)

\( b \) LOD= 3S/N
3.4 Stability study

The operational stability was evaluated by performing 100 measurements in a day. Results are expressed in terms of I%, which is defined in the following equation:

\[ I\% = \left( \frac{I_n}{I_a} \right) \times 100 \quad \text{(eq. 2)} \]

Where \( I_a \) was the current value obtained on the first measurement, and \( I_n \) was the current value recorded after \( n \) measurements (measuring a solution of 50 \( \mu \text{m} \) of ammonium). Fig. 3 shows that a microstructured polypyrrole film-modified HOPG electrode loses 50% of signal over 80 measurements; a polypyrrole nanowires modified HOPG electrode is less stable, losing 50% of signal only after 30 measurements.

Fig. 3
Operation stability of the modified HOPG electrodes
Long term stability was also evaluated by storing the microstructured dried sensors at room temperature when not in use. Nanostructured sensors were stable for 1 day. Fig. 4 shows the microstructured sensor response toward ammonium in terms of $I\%$, calculated as in (eq. 2) where $I_a$ was the current value obtained on the first day of measurements, and $I_n$ was the current value recorded after $n$ days (measuring a solution of 50 µm of ammonium). Under these operational conditions, this sensor turns to be relatively stable because it lost 70% of the initial signal during a week.

Fig. 4
Long term stability of the microstructured polypyrrole film modified HOPG electrodes
3.5 Real sample analysis

Both electrodes were used to analyze real samples. “Synthetic water” was used to evaluate the matrix effect by the addition of standard solutions of ammonium. Finally, real drinking water samples were analyzed using calibration curves obtained with “synthetic water”. Recovery values are shown in Table 2 and ranged from 92 to 107 %. These results confirm that there was a negligible matrix effect occurred in real samples using our developed sensors.

Table 2.
Recovery study performed adding standard solution of ammonium to different drinking water samples

<table>
<thead>
<tr>
<th>HOPG electrode Modified by</th>
<th>Samples</th>
<th>$\text{NH}_4^+$ added (µM)</th>
<th>$\text{NH}_4^+$ found before spiking (µM)</th>
<th>Expected value (µM)</th>
<th>Measured value (µM)</th>
<th>R.S.D. % (n=3)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microstructured polypyrrole film</td>
<td>Mineral water “Santa Croce”</td>
<td>50</td>
<td>20</td>
<td>70</td>
<td>75</td>
<td>0.8</td>
<td>107</td>
</tr>
<tr>
<td>Microstructured polypyrrole film</td>
<td>Mineral water “Sorgente”</td>
<td>50</td>
<td>8</td>
<td>58</td>
<td>57</td>
<td>1.2</td>
<td>98</td>
</tr>
<tr>
<td>Polypyrrole nanowires</td>
<td>Mineral water “Santa Croce”</td>
<td>50</td>
<td>22</td>
<td>72</td>
<td>70</td>
<td>2.8</td>
<td>97</td>
</tr>
<tr>
<td>Polypyrrole nanowires</td>
<td>Mineral water “Sorgente”</td>
<td>50</td>
<td>11</td>
<td>61</td>
<td>56</td>
<td>3.2</td>
<td>92</td>
</tr>
</tbody>
</table>
4. Conclusions

Two sensors for ammonia detection in drinking water were developed using either conducting polypyrrole films or polypyrrole nanowires. The first prototype sensor is assembled by synthesizing the microstructured polymer directly on the surface of an HOPG electrode. Satisfactory results in terms of sensitivity and stability are obtained, but a high LOD was observed. To improve this parameter, the HOPG electrode was then modified by polypyrrole nanowires. It is hypothesized that the more compact structure of microstructured film, compared with that of nanowires, acts as a barrier for the analyte. Effectively, better results were obtained with nanowires also because of the higher surface, electrical conductivity and signal/noise ratio. Finally both sensors could be successfully used to detect NH$_3$ in real samples. The polypyrrole nanowire-modified HOPG electrode showed higher sensitivity, while the microstructured one was more stable and thus more suitable for use in automated systems.
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Sviluppo di un sistema automatico per la determinazione elettrochimica di nitriti e nitrati nelle acque

2. V. Biagiotti, F. Valentini, A. Curulli, M. Badea, D. Moscone, G. Palleschi
New strategies to assemble selective and sensitive sensors for the detection of nitrites
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3. V. Biagiotti, M. Bugio, F. Valentini, A. Amine, C. Lete, G. Palleschi
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10. V. Biagiotti, F. Valentini, A. Amine, D. Moscone, G. Palleschi
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14. V. Biagiotti, F. Valentini, D. Moscone, G. Palleschi
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15. V. Biagiotti, F. Valentini, D. Moscone, G. Palleschi
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(Articles):
Synthesis and characterization of polymeric films and nanotubule nets used to assemble selective sensors for nitrite detection in drinking water
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17. V. Biagiotti, F. Valentini, A. Amine, E. Tamburri, M.L. Terranova, D. Moscone, G. Palleschi
Synthesis and characterization of a microstructured polypyrrole films and a polypyrrole nanowire modified electrodes for amperometric detection of ammonia in drinking water
Submitted

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