1. Introduction

The basic principle of detection of a biosensor is based on the specific interaction between the analyte of interest and the recognition element. As a result of this specific interaction, changes are produced one or several physical-chemical properties (pH, electron transference, heat transfer, change of potential or mass, variation of optical properties, etc.). These changes are detected and can be measured by a specific transductor (Thévenot et al., 2001; Wang, 2004). Electrochemical biosensors are based on the electrochemical species consumed and/or generated during a biochemical interaction process of a biological active substance and analyte. Electrochemical biosensors, especially the amperometric, have an important position among the biosensors. Since 2000s until the completion of this review, intensive research activity has been devoted to the development of amperometric biosensors. The statistic in ScienceDirect search showed 6950 items found for publication made with pub-date > 1999 and keywords Electrochemical biosensor. Of which about 38% with application to detect ethanol, 47% for glucose and 15% to phenolic compound.

The electrochemical biosensor usually consists of three phases: receptors phase, transducer phase and a signal amplifier phase. The receptor phase incorporates a biological or biomimetic recognition element (nucleic acid, enzyme, anti-body, tissue, organelles or whole cells). The most important phase in an amperometry biosensor is the receptor biomolecule by the selectivity of the device to a given analyte or condition. The transducer is the conductive phase, which converts a biochemical signal into a reading or measurement. And the amplifier is the computerized apparatus/software using to boosts/readout of signal.

The role of the transducer in a biosensor is to generate a measurable signal of the analyte interacts with the biological molecule associated with the transducer surface. So, in the case of the optical transducer, it generates a signal measured as a light intensity proportional to the concentration of analyte in the sample; this may be an inverse relationship. The composite-films of the surface optical biosensor has an important role in the process in which changes in optical characteristics such as absorption, rotation, refractive index, bio/chemiluminescence, and fluorescence are related to the analyte concentration (Koncki et al., 2001). Martin (2002) showed how the fiber optical immunosensors based on long-period gratings that have limited sensitivity at the refractive index of ordinary aqueous solutions (~1.33). And using composite such as films of titanium dioxide, for example, can raise the local refractive index of the sensor (~1.42), thus increasing sensitivity. Titanium dioxide is
commercially available and has been extensively used in the paint industry because of its ability to scatter visible light efficiently.

Electrochemical transducers generate a current or voltage in proportion to the analyte being measured as result of the electron transference; again this may be an inverse relationship. There are numerous components to any biosensor configuration. Through the years, a great many combinations have been proposed and demonstrated, although far fewer have been commercial successes. Figure 1 presents the two most popular platforms for electrochemical biosensor including: carbon paste electrode for discrete or continuous measurements and disposable screen-printed electrode (SPE) for low-cost and single-use field applications.

![Electrochemical composite biosensor for analyte detection](image)

**Fig. 1.** Electrochemical composite biosensor for analyte detection

In the literature different procedures have been developed to manufacture electrochemical transducers. These conducting composite materials could be considered to be random assemblies of minielectrodes, in which “edge effects” contribute significantly to the Faradic current. Several transducer composite show electrochemical advantages over those built using a single conductive material (platinum, gold, silver, carbon, mercury, graphite, etc.). The conductive parts of the arrays have different sizes and shapes, and it is this randomness that prevents theoretical models based on homogeneously distributed discs, to be used to describe the composite electrode behaviour (Barsan et al., 2009). Using different types of carbon, entrapped in a suitable, normally polymeric, binder, various composite electrodes have been extensively used in electroanalytical measurements.

The desirable characteristics of composite-biosensors usually cited are: i) high versatility in shape and size; ii) lower cost; iii) easy fabrication; iv) higher signal-to-noise ratio; v) surface regeneration; vi) provide suitable mechanic and conducting characteristics, vii) possibility to incorporate other components in the bulk of composite (mediators, cofactors or other biomolecules) to enhance selectivity or sensitivity, and viii) a long-term stability and lifetime. Conducting composites are interesting alternatives for the construction of electrochemical biosensors. The capability of integrating various materials is one of their main advantages. Thus, the composites used for the construction of biosensors, that have been proposed by several researchers and are used efficiently in different types of analytical determinations of
various electroactive species, showing the robustness and sensitivity (Kress-Rogers & Brimelow, 2003; Ahammad et al., 2009; Lojou & Bianco, 2006; Bojorge et al., 2009). Several materials have been used to construct electrochemical composite biosensor. Among them, carbon-based matrices are the most applied due to costs and their electronic conductivity. Aspects related with the protocols for bio-molecules immobilization on the composite support have been widely proposed for many researchers (Caramori & Fernandes, 2004; Ikeda et al., 2002; Shan et al., 2007; Mateo et al., 2000). Difficulties still pose problems such as being reproducible, simple to use, and stable immobilization of the biological component. For example, to obtain a fast response time and a reliable reading a thin layer of immobilized bio-molecule is desirable and shelf life and operational stability demand a high value of immobilized enzymatic activity. Adsorption to adequate surfaces including a metal electrode layer yields relatively unstable systems (Mateo et al., 2006).

The immobilization of bio-molecules on composite surface, the composite supports should be designed to permit a minimum desorption of native proteins, which is possible through the preparation and selection of arrays that generate for a very strong ionic adsorption of enzymes. In this aspect, Montes et al., 2006 shows as the surface of the enzyme penicillin G acylase was chemically modified under controlled conditions: chemical amination of the protein surface of carboxylic groups (using soluble carbodiimide and ethylenediamine) and chemical succinylation (using succinic anhydride) of amino groups. The full chemical modification produced some negative effects on enzyme stability and activity, although partial modification (mainly succinylation) presented negligible effects on both enzyme features. The chemical amination of the protein surface permitted the immobilization of the enzyme on carboxymethyl and dextran sulphate - coated support, while the chemical succinylation permitted the enzyme immobilization on DEAE-agarose and polyethylenimine coated supports. Immobilization was very strong on these supports, mainly in the polymeric ones, and dependent of the degree of modification, although the enzymes still can be desorbed or inactivated after incubation under drastic conditions.

The immobilization on ionic polymeric beds allows a significant increase in enzyme stability against the inactivation and inhibitory effects of organic solvents, very likely by the promotion of a certain partition of the organic solvent out of the enzyme environment. These results suggest that the enrichment of the surface of proteins with ionic groups may be a good strategy to take advantage of the immobilization of enzymes via ionic exchange on ionic polymeric beds. For example, one should mention the work of Hentze & Antonietti (2002) that describe conventional and modern techniques of porous organic polymers synthesis. A great variety of polymer architectures and functions can be gained by foaming, phase separation, imprinting or templating approaches. Several applications of porous polymers are discussed, focusing on biotechnological and biomedical applications, such as chromatography, protein synthesis, drug carrier systems, tissue engineering and others. This work describes several approaches for the construction of device and rigid-composites application for detection health and environmental target analyte, such as ethanol and phenol. The first approach is based on a matrix of graphite-epoxy as electrochemical transducer where the immobilization of HRP enzyme for detection of phenol. The second approach is based on matrixes of graphite-epoxy and graphite-Teflon, which are incorporated with HRP/TYR enzymes for the detection of phenol. These bio-composites offer several potential advantages over more traditional to the electrodes based on a modified surface phase driving. The ability to integrate different materials into one is its main advantage, besides the improved electrochemical properties. The different properties
of these materials are discussed and compared. The third approach is based on composite Graphite / Epoxy / Pani. Here the polyaniline polymer (PANI) was selected because of its ease of preparation and economic good environmental stability, and conductivity that occur after doping. Several arrays of composite were prepared with different fractions of PANI and were characterized by their morphological, electrical and mechanical. Ahuja et al. (2007) reviewed the state of the art of the use conducting polymers to construct biosensors, studying the different aspects of biomolecules immobilization techniques. Electrochemical biosensors based on polyaniline immobilized with different recognizing biomolecules like as oxide-reduction enzymes, nucleic acids, antibodies, were just constructed and reported (Wei & Ivaska, 2006; Dhand et al., 2011). The term polyaniline is employed to refer a class of polymers with repeated units of ‘ring-N’ and the base form is [Ping et al., 1997]:

\[
\text{[\begin{array}{c}
\text{H} \\
\text{N} \\
\text{y} \\
\text{1-y} \\
\end{array}\}}
\]

The value of y in (I) can varied from unity, the completely reduced polymer with amine form for all nitrogen atoms, to zero, which is the completely oxidized form with imines form for all nitrogen atoms. The degree of protonation of the polymeric base depends on its oxidation state and on the pH of the environment. The terms leucoemeraldine (LE), emeraldine (EB) and perniganiline refer to the different oxidation states of the polymer where \(y = 1, 0.5 \) and \(0\), respectively. The emeraldine half-oxidised and protonated form is the conductive polymer (Abdiryim et al., 2005).

2. Materials composite

Composite biosensors are made from two or more constituent materials, conductive material and nonconducting-binding material, with significantly different physical or chemical properties which remain separate and distinct at the macroscopic or microscopic scale within the finished structure. The conductive materials involve the use of nature different of carbon-based matrices, such as: glassy carbon, graphite and nano-carbon and or also by mix of a polymer with electrical conductivity property. They are of highest purity grade and used as delivered from commercial sources (Aldrich, Sigma-Aldrich, Fluka). The main purpose of the conducting phase in the composite is to supply the electrical conductivity needed for the conduction of the electrical biosignal. Another material consists of agglutinating agents (insulator materials), such as: epoxy resins, silicone, polyurethane, metacrylate resin, Teflon, etc. The main purpose of agglutinating agents is mechanical consistence assuring the durability and resistance to the electrolytic working medium. The conductivity is defined by the connectivity of the conducting particles in the midst of the polymer. When a bicomposite is prepared, it is imperative that its calibration curve is studied to define the composition that guarantees a proper electrical conductivity without losing the mechanical and physical rigidity of the bicomposite. Electrochemical composites can be classified according to how the phases are distributed within the composite matrix. For example, a conducting composite electrode surface can be prepared as an ordered array or as a random arrangement (ensemble) of conducting regions separated by an insulator. The random composite mixtures are classified according to the distribution of the conductor within the composite matrix. If the conductor particles are distributed randomly within the
composite matrix the composite is said to be of the dispersed type. If the conductor extends throughout the composite in a random fashion with regions of pure insulator and pure conductor that do not intermix, the composite is said to be of the consolidated type (Ates & Sarac, 2009).

2.1 Carbon
Carbon is an ideal electrode substrate due to its wide anodic potential range, low residual current, chemical inertness, low cost, fast response time, and ease for fabrication in different configuration and size and suitability for various sensing and detection applications (Wang, 2001). Carbon electrodes allow scans to more negative potentials than platinum or gold, as well as good anodic potential windows. Carbon is observed in several variants such as, glassy carbon, graphite, fullerene, graphene and carbon nanotubes (Ates & Sarac, 2009).

2.2 Graphite
Graphite is a dark gray, soft and porous material with adsorption capacity and is naturally abundant and highly conductive fillers (with an electrical conductivity of $10^4$ S/cm at ambient temperature) for conducting polymer composites (Du et al., 2004) and electrical resistivity is $50 \mu \Omega \cdot m$ (Sengupta et al., 2010) and its density is $1.95-2.3$ g/cm$^3$. In graphite, the carbon atoms are only bonded in two dimensions. Bond angle in graphite is $120^\circ$. Each carbon atom in graphite is sp$^2$ hybridized and with a distance between basal planes of 3.35 Å. Three out of four valence electrons of each carbon atom are used in bond formation with three other carbon atoms while the fourth electron of each carbon atom forms delocalized π-bonds which spreads uniformly over all carbon atoms. The carbon atoms are arranged hexagonally in a planar condensed ring. Also, the layers are stacked parallel to each other, with the atoms within the rings bonded covalently, whereas the layers are loosely bonded together by Van der Waal’s forces. The anisotropic nature of graphite is the result of the two types of bonding acting in different crystallographic directions. The ability of graphite to form a solid film lubricant may be attributed to these two contrasting chemical bonds. The weak Van der Waal’s forces govern the bonding between the individual layers and also there are no bonds between the layers, the layers can easily slip off one to another, making it an ideal lubricant, and resulting in a reduced coefficient of friction and, hence, wear, and by this same reason, the graphite is a good material for pencils - layers come off and get left on the paper as you write.

On the other hand, given the good electro-catalytic properties, the graphite has been used as an electrode material in the construction of disposable amperometric biosensors for the detection of various analytes such as ethanol, phenol and glucose, and many others (Kirgöz et al., 2006; Llopis et al., 2005; Mailley et al., 2003). By this, the aim of this study was to show the potential use of graphite in biosensor, based on the direct electronic transfers between the enzyme and mediator conducting salt, which are contained in a polymeric matrix of epoxy resin and graphite powder or Teflon and graphite powder. These devices combine the advantages of the biosensors based on solid composites and the electro-catalytic properties of an organic conducting salt, such as PANI.

2.3 Glassy carbon
Another common form of carbon electrode material is the glassy carbon (GC), which is relatively expensive and difficult to build. Glassy carbon also called vitreous carbon, is an advanced material of pure carbon combining glassy and ceramic properties with these of
graphite. GC is a mechanically hard material, negligible porosity. The higher electrical resistance (0.48 and 0.09 vs. 0.28 and 0.18 $\Omega$·cm at 325 and 725 ºC, respectively) and its linear temperature dependence in the annealed sample are attributed to formation of graphite crystalline nuclei in the previously homogeneous and amorphous glassy carbon (Barykin et al., 1976). GC is a class of non-graphitizing carbon that is widely used as an electrode material in electrochemistry and for high-temperature crucibles. It is prepared by subjecting the organic precursors to a series of heat treatments at temperatures up to 3000 ºC (Kinoshita et al., 1988; Jenkins et al., 1972, 1976). Unlike many non-graphitizing carbons, it is impermeable to gases and chemically extremely inert, especially when prepared at very high temperatures. It has been demonstrated that the rates of oxidation of glassy carbon in oxygen, carbon dioxide, or water vapour are lower than those of any other carbon (Harris, 2004). It is also highly resistant to attack by acids. Thus, whereas normal graphite is reduced to a powder by a mixture of concentrated sulphuric and nitric acids at room temperature, glassy carbon is unaffected by such treatment, even after several months. The structure of glassy carbon has been the subject of research since it was first produced in the early 1960s. However, the properties which make glassy carbon so valuable in these applications are poorly understood, since its detailed atomic structure is not known. A model for the structure of glassy carbon put forward many years ago has gained wide acceptance, but appears to suffer from serious shortcomings. In particular, it fails to account for the chemical inertness of the carbon, and for its high proportion of closed porosity (Harris, 2004).

2.4 Carbon paste

The carbon paste (CP) is used for construction of carbon paste electrodes (CPE’s) for voltammetric determination, which are made usually of high purity graphite powder or another type of carbon, as carbon nanotubes, dispersed in a non-conductive mineral oil such as Nujol or silicone oil to form a paste. Common types of carbon pastes are soft and non-compact, and have to be kept in special bodies. A holder for carbon pastes can be realized as a well drilled into a short Teflon rod (Peng et al., 1993) a glass tube (Pei et al., 1991) or a polyethylene syringe (Švancara et al., 2005) filled with a paste, which is electrically contacted via a conducting wire. Exhaustive reviews on CP-based sensors have been published the last two decades, where various types of biosensors (e.g., those for amino acids, ethanol, fructose, galactose, glucose, glycerol, lactate, xanthine, etc.) based on related oxidases and dehydrogenases, whole cells, and plant tissues are reviewed. A major advantage of CP-based biosensors are very low background current and are the feasibility of bulk modification of the electrode material with biocatalyst as well as with other components essential for their effective functioning. Renewable or disposable surfaces so that each measurement can be performed on the new surface and not be affected by the residuals from the previous measurement (Bard & Rubinstein, 1996). The constructions of CP biosensor are very simple; however, there is one aspect which makes them not very convenient for practical use and this is the necessity of refilling the carbon paste in experiments requiring a regular removal of the electrode surface layer. Another smart construction circumventing this time-consuming procedure were proposed by Švancara et al., 2005 and Kalcher et al., 2009 who proposed piston-driven electrode holders where the desired amount of the used paste could simply be extruded from the electrode body and smoothed away or cut off.
Another advantage of the CP is that has no need for the sample to have a high electrical conductivity because the conduction is mainly accomplished by the graphite. The currents obtained are within a suitable range due to the small sample amounts. This means that the signal resolution is improved. The use of CP composite biosensor helps in investigating substances in small sample amount and the electrochemical reactions proceed at the surface of the paste electrode. At which, an ion transfers between the solid sample and the electrolyte solution is possible.

The properties of the CP depend on the specific components employed the manner of preparation and maintenance. The properties of CP-based biosensor can be partially improved by the incorporation of additives into the paste, e.g., polyethylenimine, acetylenic polymers, polyaniline, chitosan, glutamate, cationic antibiotics, silica and carbon nanotubes to prevent the leakage of mediator, covalent binding of the mediator to a polymer matrix (Mailley et al., 2003; Anik & Çevik, 2009; Tingry et al., 2006). Recently a novel carbon paste ion selective electrode for determination of trace amount of holmium was prepared by Ganjali M. R. et al., 2009. The authors used multi-walled carbon nanotubes and nanosilica for improvement of a holmium carbon paste sensor response. The approaches to improve the properties of CP mentioned above have certain limitations and deficiencies. On one hand, it is still unclear how additives might affect the biosensor performance at the molecular level, thus making the search for suitable additives for each specific biosensor rather difficult and more random than systematic. On the other hand, covalently bound mediators exhibit modified electrochemical properties and reduced mobility, which affect the reaction rate with enzymes.

The main disadvantages of the CP are the fragile surface or weak mechanical properties due to their creamy texture, which can easily lead to disintegration of the system; and the solubility of the pasting liquid in organic solvents, which often show voltammograms with a higher irreversibility than in cases where no organic binders are used. Therefore, several carbon composite electrodes based on carbon nanotubes and a solid matrix have been proposed. Dues to its insulating nature efficiency in the presence of a solid matrix decreases electrode reaction rates more than a pasting liquid does unless an active carbon surface is exposed (Bard, 1996; Kalcher et al., 2006).

2.5 Carbon nanotubes

Carbon nanotubes (CNTs) represent one of the best examples of novel nanostructures derived by bottom-up chemical synthesis approaches. CNTs are molecular-scale tubes of graphitic carbon with outstanding properties. They are among the stiffest and strongest fibres known, and have remarkable electronic properties and many other unique characteristics. CNTs has received a great deal of attention as an electrode material, because these have good electrocatalytic properties (Wang, 2005). Merkoçi, 2006 showed an interesting review paper on CNTs in analytical sciences covers the full calendar from their discovery in 1991 until 2005 and treat analytical aspects of interest in the coupling of CNTs to enzymes, DNA, proteins and, finally, the use of CNTs for several applications such as in chromatography, sensors and biosensors, nanoprobes, etc. Commercial applications have been rather slow to develop, however, primarily because of the high production costs of the best quality nanotubes. For these reasons they have attracted huge academic and industrial interest, with several articles on nanotubes being published the lasts years (Ruoff & Lorents, 1995; Gouveia-Caridade et al., 2008; Yadav et al., 2011).
Carbon nanotubes have received considerable attention in the field of electrochemical sensing, due to their unique structural 1D nature (Javey, 2009), for instance, unique tubular nanostructure, due to their superior mechanical (Schadler et al., 1998) and electronic and chemical properties (Saito et al., 1998), large specific surface, excellent conductivity, superior to 100 Scm\(^{-1}\) (Yao et al., 2000), modifiable sidewall, good biocompatibility, and so on. Solubilization and biological functionalization of carbon nanotubes have greatly increased the usage of carbon nanotubes in biomedical applications such as biosensors and nanoprobases (Teker, 2008).

The structure of CNTs is like a sheet of graphite rolled up into a tube of diameter 1-10 nm, and hence forms hollow tubules of a single layer of carbon atoms (Wang, 2005). CNTs include both singlewalled (SWNT or SWCNT) and multiwalled (MWNTS) structures (Baughman et al., 2002). CNTs have very high surface-to-volume ratios and, therefore, promise depending on the direction of hexagons, nanotubes can be classified as either zigzag, armchair or chiral. When scientists make nanotubes, they tend to get a mixture of several types due to different types of nanotubes have different properties. Today, a major challenge in nanoscience is finding a way to make just one type of nanotube. In this aspect, an interesting study made by Safarova et al., 2007, who shows how parameters of SWCNTs, specially a diameter and length of one nanotube or a bundle of nanotubes and a number of nanotubes in the bundle, can be determinated using the techniques as Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM).

### 2.6 Rigid carbon composites

According to Céspedes & Alegret (2000), rigid carbon composites are ideal for the construction of electrochemical sensors. The plastic nature of these materials makes them modifiable, permitting the incorporation of fillers before they are cured. A large number of biological materials can be immobilised by blending them with these composites to form new biocomposite materials, showing robustness and sensitivity. These biocomposites not only act as reservoirs of the biological material but may also contain catalysts, mediators and cofactors that improve the response of the resulting electrochemical biosensors. The carbon-based matrices are the most applied due to their high conductivity, whereas epoxy resins, silicone, polyurethane, metacrylate resin, Teflon, etc, can be employed as agglutinating agents (insulator materials). The main characteristic of these composites is their rigidity, resulting in a high mechanical stability over time. This type of composite biosensor offers additional important advantages: the sensing surface can be renewed by a simple polishing procedure. The proximity of the redox centers of the biological material and the conducting sites on the sensing surface favours the transfer of electrons between electroactive species. So, for example, a universal affinity platform for electrochemical genosensing can be easily achieved by modifying the graphite-epoxy composite with avidin to obtain an avidin biocomposite, where biotinylated DNA can be rapidly single-point attached (Pividori et al., 2005). Thus, this often allows the regeneration of the biological component without using co-substrates and mediators, it can be customized easily according to biocatalytical and/or electrochemical requirements; it presents low background currents, favoring a high signal-to-noise ratio, and lower detection limits and it offers a new active layer after removal of the outer surface, extending the lifetime of such electrodes.
2.6.1 Graphite-epoxy composite
Graphite and epoxy resins are employed to construct rigid composites that can be used in aqueous or nonaqueous media. Serra et al., 2005 showed a comparison study of the behavior of different rigid composite matrices for the construction of amperometric tyrosinase biosensors, widely used for the detection of phenolics compounds. So, they showed that the great advantage of Graphite-epoxy composite (GEC) composite over carbon paste composite is their tolerance to organic solvents, due to a better reproducibility of the amperometric measurements both with and without regeneration of the electrode surface by polishing. Kirgöz et al., 2006 showed the modification of a GEC electrode with bacterial cells to detection of xenobiotic in waste water samples. Pseudomonas putida DSM 50026 was used as a biological component and the measurement was based on the respiratory activity of the cells. This study the combination of microorganisms with GEC composite electrodes provided economic and practical disposable biosensors.

The physical and chemical effects of water on graphite/epoxy composite were investigated in an interesting work of Zhou and Luke (1995). This paper demonstrates by diffusion data that the time for the onset of non-Fickian behavior is inversely related to the increase in exposure temperature. So, if a relatively low temperature of exposure there is no dissolution of the surface of the material or physical damage and the behaviour profile of weight gain is Fickian. Pointing out that Fick's laws are differential equations that describe the flow of a substance and the concentration versus time and position. And with the temperature increase, cracks, voids, surface peeling and dissolution occur. An explanation for this effect is that the cracks retain water which contributes to the behavior of absorption higher than the theoretical Fickian diffusion curve or the epoxy resins used in manufacturing GEC are capable of undergoing a significant and irreversible thermal oxidative degradation at high temperatures after submission or within a narrow range of its maximum temperature of superior service. When exposed to temperatures high enough to cause degradation of resin, these materials experience a drop in glass transition temperature which effectively increases the room-temperature mechanical strength properties of the composite. Below a certain threshold of exposure, these compounds are visually and microscopically damaged, embrittlement and cracking of the surface causes a loss in resistance the impact of the material. Therefore, composite GEC exposed to conditions of overheating may suffer irreversible damage and catastrophic in a very short time period.

From the viewpoint of the effects of electrochemical of the GEC electrode have an anion response close to the Nernstian behavior, 1-3 potential salt and are more sensitive to OH\(^{-}\)\). This response of the transducer is due to an electroreduction of the adsorbed oxygen on the graphite surface in such way that an increase in the electrode potential takes place due to a change in the oxidation state (Rodriguez-Huerta et al., 2006).

2.7 Carbon/PANI or derivates composites
Li & Shi (2011), published a review about the electrochemical applications of composites prepared with chemically converted graphenes (CCGs) and conducting polymers as polyaniline (PANI), polypirrole (PPy), polythiophene (PTh), to construct sensors and, consequently, biosensors. The electrochemical activity and sensing of the composites prepared with SPANI (acid doped PANI) and CGC were improved and tested with cyclic voltammetric studies. The electropolymerization of polyaniline on the carbon surface was discussed and described by Ates and Sarac (2009) in order to improve the proprieties of the
conducting polymers and the application to construct sensors, biosensors, capacitors and batteries. Gómez et al., 2011, reported a chemical precipitation technique to synthesized graphene-polyaniline nanocomposite for electrodes and electroanalysis, and the morphology was characterized by using scanning electron microscopy, transmission electron microscopy, Fourier transform infrared spectroscopy and Raman and cyclic voltammetry. Tung et al., 2011, prepared a nanocomposite with selenious acid, doped polyaniline and graphite nanoplatelet (GNP), and characterized by using SEM, FTIR, X-ray diffraction, and electrical conductivity measurement. A crystalline structure was shown and the electrical conductivity increases with the increase of the GNP content although lower than the HCl doped one. Kim and Park (2011), used multi-walled carbon nanotube and grapheme to prepare a nanocomposite with polyaniline (M-GR/PANI) and higher sensitive CVs were observed when compared with a graphite-polyaniline composite CV. A bienzymatic biosensor was designed for glucose oxidase and horseradish peroxidase based on covalent attachment onto carboxylic-derived multi-walled carbon nanotube for the deposition electroactive polyaniline by Sheng and Zheng (2009), and the linear range for glucose detection was 0.05 mM – 8.0 mM. Zhan et al., 2009, proposed a new strategy for a highly sensitive amperometric biosensor immobilizing tyrosinase on the surface of the polyaniline-ionic liquid-carbon nanofiber composite for phenols detection. A linear range from $4.0 \times 10^{-10}$ to $2.1 \times 10^{-6}$ mM was obtained for catechol detection.

3. Methodology for preparation of composite transducers

In this section, the protocols for construction to make the maximum use of composites-transducers are described. Especially the behavior of graphite to transfer electronic device promoted the electrochemical sensor. Rigid matrices based on epoxy/graphite, and the use of thermoplastic resins such as Teflon are very useful tools in building composite electrode for its flexibility in shape and size, allowing easy adaptation to a variety of electrode configurations (conventional flow-through, screenprinted, etc.).

3.1 Preparation of the carbon paste electrodes

Carbon paste electrodes are the most popular electrodes in electroanalytical chemistry and bioelectrochemistry due to that exhibit low background current and are easily prepared. The types of carbon pastes are soft and non-compact, and have to be kept in special bodies. A holder for carbon pastes can be realized as a well drilled into a short Teflon rod, a glass tube or a polyethylene syringe filled with a paste, which is electrically contacted via a conducting wire. Carbon pastes usually employed for the fabrication of CPE are usually prepared as follow: (i) Carbon paste are prepared by mixing graphite powder with Nujol oil or paraffin oil in a mortar until it was uniformly wetted using a graphite/Nujol. The ratio varies for each biosensor proposed in the literature: of 4/1 w/w; 50:50 % m/m; 75:25% m/m. These ratios are employed as it provides convenient analytical properties. (ii) The enzymes are incorporated in the carbon paste using an enzymatic ratio up to 10% w/w. (iii) Sometimes, redoxmediators substances such as quinones and ferrocenes, are incorporated to facilitate electron transfer between the electrode and enzyme employed with ratios of mediators ranging from 1 to 6% w/w. (iv) After blending, the pastes were packed into a Teflon electrode holder (geometric surface area of about 0.07 to 3 cm$^2$) with electrical wire. (v) The electrode surface was smoothed on a paper to produce a reproducible working surface.
The use of CNTs also has recently been reported to build nanotubes paste electrode (MWCNTPE). Patrascu et al., 2011 showed the use of MWCNTPEs as voltammetric sensors to selectively detect dopamine in the presence of serotonin. Which, it was prepared by thoroughly hand-mixing the multi-wall carbon nanotubes powder with paraffin oil (60:40 w/w) in a mortar. The paste is packed into 1.0 mL polyethylene disposable syringes with a copper wire being used for electrical contact. The surfaces of the electrodes were smoothed by polishing with emery paper.

### 3.2 Preparation of GEC composites

The epoxy composites filled with graphite are prepared by solution intercalation method, whereby graphite is added to the mixtures of epoxy resin and curing agent. The materials were mechanically stirred, in order for the epoxy resin to intercalate inside the conductive fillers, especially into the graphite interlayers and pores. After the conductive fillers were mixed with epoxy resins, solvents presents in the mixture are evaporated with continuous stirring. The polymer matrix system consists of epoxy resin and a cure agent, and generally all the components are commercial products, and are used as received, without purification.

Epoxy resins, aromatic or aliphatic, have similar ether linkages as that of the Nafion® membranes commonly used in fuel cells. So it is reasonable to select epoxy resins as the polymer matrix in the composite bipolar plates. One of the most popularly used in composites is formed by curing diglycidal ether of bisphenol A (DGEBA). It was used under its commercial designation (Dow Chemical Company; DER 332) with triethyltetramine (TETA). The basic reaction involves an amine reacting with the epoxy ring. Resulting in simple chain extension and cross-linking. The former reaction generates a new secondary amine that can also react with DGEBA. This is often referred to as branching. The reaction also produces a hydroxyl group that has long been considered as being ideally located to aid (catalyze) the branching reaction.

The criterion of the selection between the type epoxy resin and the cure agent observed in the majority of the publishing paper is in order to adjust the viscosity of the solution during mixing and provide flexibility to the cured products. However, usually the curing processes of these materials need high temperatures, curing at 150°C, 80% of epoxide groups were converted (Merad et al., 2007; Laza et al., 2005), that would be fatal for the biological component. So that, should be reduced the curing temperature and prolong the curing time. These precautions can be extended to other chemical modifiers added to the biocomposite such as redoxmediators, catalysts, etc. that are quite sensitive to wide temperature variations.

Rigid phenyl rings are particularly useful to provide resins with improved heat and thermal resistance and char formation. Undesirably, too rigid backbone structures also drastically reduce the processability of a resin due to viscosity increase, and produce a relatively still but brittle material.

The table 1 show different ratios of the composition of rigid composites based in GEC resins, which isn’t standard. So, when to same resin is used, the curing conditions can vary and should be optimised depending on the nature of the fillers present in the biocomposite. For example, a study proposed by Fatibello et al., 2007 show the effect of graphite powder varying from 30 to 80% (m/m), epoxy resin from 5 to 60% (m/m), and tissue from 5 to 20% (m/m) on the biosensor response using 0.025 mol L⁻¹ catechol solution at pH 7. The best composition obtained was in function of the remarkable robustness and sensitivity of the sensor.

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Table 1. Compositions of Graphite/Epoxy composites Biosensor.

<table>
<thead>
<tr>
<th>Graphite</th>
<th>epoxy</th>
<th>Hardener</th>
<th>Biomolecule</th>
<th>Other</th>
<th>Time cure</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 part wt</td>
<td>1 part wt</td>
<td>a part wt</td>
<td>-</td>
<td>-</td>
<td>55 °C for 2 h</td>
<td>Wang J. et al., 1989</td>
</tr>
<tr>
<td>4 part wt</td>
<td>16 part wt</td>
<td>-</td>
<td>15 mg GOD/g composite</td>
<td>one part wt</td>
<td>35 – 40°C, for a week</td>
<td>Céspede et al., 1993</td>
</tr>
<tr>
<td>1 part wt</td>
<td>4 parts wt</td>
<td>-</td>
<td>20mg HRP /g graphite</td>
<td>-</td>
<td>40°C for 4 days</td>
<td>Morales et al., 1996</td>
</tr>
<tr>
<td>18%</td>
<td>71%</td>
<td>-</td>
<td>2%</td>
<td>9%</td>
<td>-</td>
<td>Martorell et al., 1997</td>
</tr>
<tr>
<td>1 part wt</td>
<td>4 parts wt</td>
<td>-</td>
<td>g 0.9% (w/w) RlgG</td>
<td>-</td>
<td>40 °C for 1 week</td>
<td>Santandreu et al., 1997</td>
</tr>
<tr>
<td>10%</td>
<td>40%</td>
<td>40%</td>
<td>5%</td>
<td>5%</td>
<td>72 h at 28 °C</td>
<td>Dutra et al., 2000</td>
</tr>
<tr>
<td>1 : 1 (graphite: epoxy-hardener)</td>
<td>1 part</td>
<td>0.4 part</td>
<td>0.15 g</td>
<td>-</td>
<td>60°C for 24 h</td>
<td>Puig-Lleixà et al., 2001</td>
</tr>
<tr>
<td>1 part wt</td>
<td>4 part wt</td>
<td>-</td>
<td>150 μl</td>
<td>-</td>
<td>40 °C for 1 week</td>
<td>Pividori et al., 2003</td>
</tr>
<tr>
<td>9.5%</td>
<td>76%</td>
<td>-</td>
<td>5% GOD</td>
<td>9.5%</td>
<td>40 °C for 1 week</td>
<td>Llopis et al., 2005</td>
</tr>
<tr>
<td>75%</td>
<td>10%</td>
<td>-</td>
<td>15%</td>
<td>-</td>
<td>-</td>
<td>Lupetti et al., 2006</td>
</tr>
<tr>
<td>100 mg</td>
<td>60%</td>
<td>40%</td>
<td>10mg IgG-HRP</td>
<td>-</td>
<td>25°C for 96 h</td>
<td>Bojorge et al., 2007</td>
</tr>
<tr>
<td>100 mg</td>
<td>70%</td>
<td>30%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Pauliukaite et al. 2009</td>
</tr>
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</table>

3.3 Preparation of graphite/teflon composites

The same as epoxy resin, Teflon (polytetrafluoroethylene, PTFE) is other the nonconducting binder used to construct rigid composite transducers with electrochemical sensing applications (Peña et al., 2001; Wang & Musameh, 2003). The main advantage of the incorporation of enzymes into graphite-Teflon composite matrixes is the compatibility of these electrodes with organic or predominantly organic solvents.

The most popular procedure is summarized as: Teflon powder is dissolved in hexane by gentle mixing. Then, the hexane is evaporated under vacuum. Hexane is used to precipitate any asphaltenes presents. Then, an appropriate amount of graphite powder is added to the Teflon and mixed with the enzyme in a mortar for a time to incubate. Finally, the mixture is pressed in into pellets, by means of a Carver pellet press at 10,000 kg cm$^{-2}$ for 10 min. From this mother pellet, several cylindrical portions of each pellet are bored, and each portion is press-fitted into a Teflon holder. Electrical contact is made through a stainless steel screw (Carralero et al., 2006).
4. Experimental section

One of the most important steps of building a biosensor is to immobilize the biomolecules. A successful matrix-transducer-composite should immobilize or integrate biomolecules stably at a transducer surface and efficiently maintain the functionality of the biomolecules, while providing accessibility towards the target analyte and an intimate contact with the transducer surface.

The bases immobilization proposed here are: Enzymes are hydrophobically adsorbed on the supports of high ionic strength. There is a covalent “intermolecular” reaction between the adsorbed absorbed protein and the media. The immobilized protein is incubated at alkaline pH to increase multipoint covalent attachment to stabilize the enzyme. The hydrophobic surface of hydrophylized support is the reaction of other groups of amino acids in order to reduce unfavourable interactions enzyme-support hydrophobic. This strategy produced a significant increase in stability, for example, immobilization of enzymes such as HRP, TYR and AOX, which has been extensively used in determination of analyte-target different such as ethanol and phenol and, in comparison with the stability achieved using conventional protocols. The development of a good biocompatible matrix for the immobilization of biomolecules is very crucial to improve the analytical performance of biosensor. A total of four electrochemical sensors were made using different composites: carbon paste, GEC and Teflon/Graphite, to which different enzymes were added to the bulk mixture (see Figure 2). The composition of the composites were selected among successful combinations in the literature (Bojorge et al., 2007; Carralero et al., 2006), and according to preliminary exploratory experiments in our laboratory.

Fig. 2. Rigid-Composite electrodes based on graphite used in the array. a) Initial assembly with electrical contact in a Teflon-tube; b) preparation of the composite paste mixture: an excess of the mixture is placed on the tip of the electrode body during the curing time; c) final aspect after curing and polishing, which is carefully performed to ensure a smooth and flat surface area of 0.015 ± 0.005 cm$^2$. 

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4.1 Preparations of graphite composites biosensors

4.1.1 Graphite-epoxy/peroxidase-tyrosinase composite
The bi-enzymatic composite was initially prepared by homogenizing the mass from 0.150 to 0.200 g of graphite powder (Fluka, Cat. No. 50870), previously treated with H$_2$O$_2$ [Bojorge et al., 2007] and a specific mass of 0.050g of Tyrosinase (Sigma) and HRP (Toyobo-Brazil) in a mortar for at least 20 min. After that, epoxy resin at a ratio of 1: 4 wt/wt was subsequently added to this mixture in a mortar and mixed for at least 20 min to produce the final biocomposite paste, that was packed (1000 mg) into the tip of depth of 3mm of a cylindrical Teflon sleeve body (1.5 mm I.D) and a copper wire was used to provide the external electric contact. All composites were prepared at room temperature.

4.1.2 Graphite/teflon-peroxidase-tyrosinase composite electrode
Composite enzyme electrodes were fabricated in the form of cylindrical pellets, as follows. Graphite (Fluka, Cat. No. 50870), 0.10 g horseradish peroxidase (E.C. 1.11.1.7, 270 I.U. mg$^{-1}$; Toyobo-Brazil), 0.014 g and mushroom Tyrosinase (EC.1.14.18.1, Sigma T3824, Sigma-Aldrich Inc.), were accurately weighed and thoroughly mixed by mechanic stirring for 1 h in a 0.4mL suspension of a 0.1 mol L$^{-1}$ phosphate buffer solution of pH 7.2 at 4 ºC. Next, put it desiccator at room temperature to evaporate the water. Next, Teflon powder (Aldrich) was added in at proportion of 70% wt. and mixed thoroughly by hand. The mixture was pressed into pellets by using a Carver pellets press at 10000kg cm$^{-2}$ for 10min. The diameter of these pellets was 1.3 cm and their thickness 0.4 cm, approximately. Several 3.0-mm diameter cylindrical portions of the pellet were bored, and each portion was press-fitted into a Teflon tube. Electrical contact was made through a stainless steel flat-tip screw.

4.1.3 Graphite – PANI/epoxy –peroxidase composite electrode
The powder graphite received a previous treatment with oxygen peroxide and dried in desiccators. The chemical modified graphite was mixed with the emeraldine polyaniline (Sigma) in a proportion of 30:70 (percentage, w/w) and epoxy resin added (60:40, w/w). The paste was used to fill the cylindrical Teflon sleeve body (1.5 mm ID) electrode and rest 2 hours at 60ºC to dry. After that, the composite was well polished to immobilize the horseradish peroxidase (Toyobo-Brazil) with glutaraldehyde (2.5%, v/v) as the agent for the covalent linkage.

4.1.4 Graphite-Polyaniline/epoxy-Bi-enzymatic composite electrode
The chemically modified graphite-emeraldine polyaniline composite (30:70, w/w) was mixed with horseradish peroxidase (Toyobo of Brazil) and alcohol oxidase (E.C.1.1.3.13, Sigma-Aldrich Inc.) adjusting the corrects UI/mg composite values. After that, the epoxy resin was added to the composite and the moisture rested for a few minutes at environment temperature for aggregation. The bi-enzymatic immobilized paste was used to fill the cylindrical Teflon electrode (Serra et al., 2003).

4.1.5 Graphite paste - peroxidase electrode
0.3 g graphite powder was added to the equivalent of 0.2 g of Nujol mineral oil (density = 0.836 g/mL) and then was added 0.01 g of HRP dispersing it slowly for about 10 minutes until a homogeneous paste. The resulting paste is inserted into a pipe end polymer (made from the body of a commercial syringe) into the tip of depth of 3mm of a cylindrical Teflon
sleeve body (1.5 mm I.D) and a copper wire was used as electrical contact. The surface is gently polished with A4 paper or tissue paper to achieve a smooth flat surface. Rinsed the surface of the electrode with deionized water.

4.2 Electrical resistivity measurements
Electrical resistivity measurements of the all composites pellets based on graphite and were performed according to the ASTM D 257 standard testing method with a Keithley 6517A electrometer (Cleveland, OH, USA) as the source. From these measurements and the geometric dimensions of the sample composites electrodes, both the volume resistivity and surface electrical insulating materials and can be calculated as well as the corresponding conductivities.

4.3 Morphology
Morphology studies of all composites pellets were carried out with SEM. All the composites pellets were mounted directly onto aluminum specimen stubs with two-sided adhesive carbon tabs (Pelco, Redding, CA) and coated with gold for approximately 45 s at 20 IA and 75 mTorr. Samples were analyzed in a JEOL JSM-6460 emission scanning electron microscope dotted with detector electron back-scattered diffraction pattern and system integrated analysis station software used to interface with the SEM and EDS detector operating at an accelerating voltage of 30 KV. Representative micrographs were chosen to illustrate each composite’s pellet.

4.4 Electrochemical technique and procedure
The electrodes were cycled for 3–5 times in distilled water in order to get stable voltammetric responses before performing the measurements with analyte samples. Cyclic Voltammetry measurements were taken using a potentiostat Autolab/PGSTAT12 (Ecochemie, Netherlands, http://www.ecochemie.nl/). The following parameters were fixed: First potential: −1.0 V; Second Potential: 1.0 V; Scan rate: 0.1 V·s⁻¹ and Step potential: 0.00244 V. All experiments were carried out without any oxygen removal from the sample and with no physical surface regeneration of the working electrodes after each measure. In order to obtain reliable measurements, and to prevent the accumulative effect of impurities on the working electrode surfaces, an electrochemical cleaning stage was considered between measures. This stage was performed by applying a conditioning potential of +1.5 V for 30 s after each experiment, in a cell containing 5 ml of distilled water.

4.5 Results and discussion
4.5.1 Conductivity characteristics
Chemical compositions of the materials of different composites obtained, the variation of the electrical conductivities are presented in Figure 3. To single filler epoxy composite as a function of the graphite loading concentration the electrical conductivity of graphite/epoxy resin increase gradually with increasing graphite filler. This phenomenon shows that the graphite acts as the transfer medium for electrons hence the electrical conductivity of the composite would be increased. However, the electrical conductivity of the graphite composite is still low, reaching only 0.0078 S/cm at loading concentration of 80 w/w %. This suggests that composites filled with graphite only are not suitable for the achievement of high electrical conductivity. A low resistivity is desirable when high currents are flowing
through the electrode body in order to avoid the iR drop of the applied potential. However, when the composite are constituted of graphite/Teflon the performance of conductivity is different. The electrode resistivity was lower when working with high ratios of graphite, but an inverse tendency for mechanical strength was observed. This means that when graphite is fully intercalated with Teflon, the material is a rather poor electrical conductor, so, the change in Teflon composition fraction could create variations in conductivity probably due to an induces defects in the graphite structure with the intercalation with Teflon. In relation to the conductivity of the PANI-Graphite/epoxy composite (GEC-PANI), it can be see that the conductivity of composites increased with increasing graphite content. The conductivity found for neat polyaniline was $0.00249 \text{ Scm}^{-1}$. However after addition of 1, 2 and 3 w/w % of graphite the electrical conductivity of composites increased slightly and for pellets prepared using 10-30 w/w % of graphite the electrical conductivity of composites increased significantly showing a maximum rate conductivity of $28 \mu\text{Scm}^{-1}$ for GEC-PANI 30 w/w % sample as shown in Figure 3, indicating that, for the electrical properties, the PANI-Graphite/epoxy 30 w/w% present the better compromise between filler and polymeric matrix.

![Fig. 3. Electrical conductivity of composites in variation graphite composition](image)

4.5.2 SEM analysis

The SEM is a very good technique for investigating the morphology of composites based on graphite, due to provide information about the structure, size and distribution of the graphite particles in the matrix of the composite. Figure 4 shows scanning electron micrographs of carbon paste, GEC, Graphite-PTFE and GCE-PANI composites pre-treated as described in the experimental section. As can be seen, carbon paste composite made of graphite powder mixed and Nujol oil (Figure 4a) the electrode surface has a rough surface morphology and is heterogeneous with lighter areas, associated with the Nujol oil, and darker areas corresponding to the graphite conducting micro-structures. Figure 4b shows the SEM of GEC surface, which is less rough than CP. However, cluster appears also, which it appears agglomerated in random areas. This is due to the graphite particles randomly
distributed and randomly oriented in the epoxy resin. Another important characteristic of the graphite-epoxy composite is the rather flat fracture surface indicating the nature of brittle fracture. Figure 4c show SEM image of the worn surface for Graphite-Teflon, SEM examinations of Graphite-Teflon show slightly ribbed i.e., less creation and development of the cracks occurred on the surfaces. This can be seen with the naked eye during the preparation, whose surface is brighter and sharper with Teflon than epoxy, because developing a uniform transfer layer on the composite-surface. Figure 4d shows SEM image for graphite-PANI-epoxy that revealed also the uniform morphology of the structures in which the outer layers were PANI and the inner layer consisted of graphite. Such a uniform morphology is desirable because it enables a material with high ionic conductivity to achieve fast charge/discharge rates. In the present study, however, the morphology of PANI was strictly granular and it coalesced, making the surface rough with no uniformity.

Fig. 4. Electronic microscopic images of composites: a) GP (Graphite Paste) electrode; b) GEC (graphite epoxy composite) electrode; c) GEC (Graphite Teflon composite) electrode and d) GEC-PANI. All electrode surfaces were polished as explained in the text. The same accelerated voltage (20 KV) and resolution (5µm) were used.

4.5.3 Electrochemical properties

Figure 5 shows the cyclic voltammetric behaviors of different composites based on graphite with potential sweep rates of 100 and 50 mV/s between -0.1 and 1 V in solution containing 1 mM K₄[Fe(CN)₆] and were recorded on the five cycle of a repetitive potential sweep
program. As it can be seen, the voltammograms that show the well-defined redox peaks is the GEC, which it is related to the intrinsic characterization of graphite. However, for the voltammogram of the PANI-graphite composites show a nearly rectangular-shaped voltammogram, typical of capacitive systems. This characteristic is attributed mainly to the enhancement of the conductivity due to the graphite particles added to the polyaniline chains. The anodic peak, occurred at potential of ~ 0.2 V, is connected to doping of chloride anions indicating transition of leucoemeraldine form of PANI to emeraldine salt. Further increase in potential, above ~ 0.50 V, refers to transition of emeraldine salt to pernigraniline salt. Therefore, it can be observed that the electrochemical kinetics of redox transition of PANi-graphite composites has been performed.

![Cyclic voltammograms for 10 mM potassium ferricyanide employing the composites sensors: a) using GP composite, b) using Graphite-Teflon composite, c) Graphite-Epoxy composite, d) Polyaniline Graphite-Epoxy composite. Scan rate 100 mV/s.](image)

In agreement with this, the reaction kinetics of the electroactive species in concentrated solutions may be slowed due to the slow mobility of the molecules. In the Figure 5d, the degradation of the PANI-pernigraniline salt electrode almost was not observed for that potential region. The possible explanation could be to the fact that some forms of PANI are potential dependent and pH dependent [Tawde et al., 2001] or the fractal dimensions dependent [Ghanbari et al., 2006] evaluated from the cyclic voltammetry. Hence, at such low pH, extent of the degradation products was negligible and practically had no influence on charge/discharge characteristics of the PANI electrode during initial cyclization. However, this work is still in progress, in further studies it would be beneficial to investigate the
influence of pH and anions on degradation of PANI during the cyclization. Though, attention must be paid to each measurement made with a composite film as accurate reproducibility of the arrays and polymeric composites is very difficult. The results indicated that the electrochemical methods could be used as a simple tool for analyzing the structure of conducting polymers and their composites.

5. Conclusion

The electrochemical performance of the composite electrodes is strongly affected by the presence of the insulator matrix of the polymeric binder, which coats the graphite particles, at the same time increasing electrode resistivity which is influenced by the distance between the particles. Since it is known that a low rate of evaporation of the solvents provides the smoothest surfaces, the material was dried at low temperatures of $\approx 20$ to 30°C. Different morphologies are expected for different composite compositions. We can conclude that the nature of the surface film plays a key role for the electrode stability or to capacity decrease always related to an increase of the electrode itself impedance.

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Composite materials, often shortened to composites, are engineered or naturally occurring materials made from two or more constituent materials with significantly different physical or chemical properties which remain separate and distinct at the macroscopic or microscopic scale within the finished structure. The aim of this book is to provide comprehensive reference and text on composite materials and structures. This book will cover aspects of design, production, manufacturing, exploitation and maintenance of composite materials. The scope of the book covers scientific, technological and practical concepts concerning research, development and realization of composites.

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