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Fabrication of Biosensors Using Vinyl Polymer-grafted Carbon Nanotubes

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1. Introduction

A biosensor is commonly defined as a device incorporating a bioreceptor connected to a transducer, which converts an observed response into a measurable signal proportional to analyte concentration which then is conveyed to a detector (Eggins, 1996). As demonstrated in Fig. 1, a biosensor consists of a bio-element and a sensor-element. A specific bio-element, including enzyme, antibody, microorganism, cell, and DNA, recognizes a specific analyte, and a sensor element transduces the change in the biomolecules into an electrical signal. Biosensors can be classified either by their bioreceptor or their transducer. Biosensors are known as enzymatic biosensors (enzymes), genosensors (DNAs), immunosensors (antibodies), etc. depending on the bioreceptors used. Biosensors can also be divided into several categories based on the transduction process, such as electrochemical, optical, piezoelectric, and thermal/calorimetric. Among these, electrochemical biosensors are the most widespread, numerous and successfully commercialized devices of biomolecular electronics (Dzyadevych et al., 2008).

Much literature on carbon nanotube (CNT)-based biosensors has been published over the past several years because CNTs have the following advantages: (1) small size with large surface area, (2) high sensitivity, (3) fast response time, (4) enhanced electron transfer and (5) easy protein immobilization on CNT-modified electrodes, coupled with the fact that several methods have been developed (J. Wang & Musameh, 2003a; J. Wang et al., 2003b; Y. Saito et al., 1993). These properties make CNTs ideal for use in electrochemical biosensors and nanoscale electronic devices. Such potential applications would greatly benefit from CNTs in promoting the electron-transfer reaction of biomolecules, including catecholamine neurotransmitters (J. Wang et al., 2002a), cytochrome *c* (J. Wang et al., 2002b), ascorbic acid (Z. H. Wang et al., 2002), NADH (Musameh et al., 2002), and hydrazine compounds (Zhao et al., 2002). The insolubility of CNTs in most solvents is a major barrier for developing such CNT-based biosensing devices. Therefore, surface modification is necessary for CNT materials to be biocompatible and to improve solubility in common solvents and selective binding capability to biotargets.

There are two main approaches for surface modification of CNTs: a non-covalent wrapping or adsorption and covalent chemical tethering. The non-covalent approach includes surfactant modification, polymer wrapping, and polymer absorption via various adsorption forces, such as van der Waals and π -stacking interactions. The advantage of non-covalent modification is that the structures and mechanical properties of CNTs remain intact.

However, the force between the CNTs and the wrapping molecules is very weak, which means that the load may not be transferred efficiently from the polymer matrix to the CNT filler (Islam et al., 2003). The covalent functionalization of CNT surfaces can improve the efficiency of load transfer from matrix to nanotubes. However, it must be noted that the process to obtain functional groups may introduce defects on the walls of nanotubes. These defects will lower the strength of the reinforcing component. Therefore, there will be a trade-off between the strength of the interface and the strength of the CNT filler (Eitan et al., 2003). The covalent functionalization of CNTs is most frequently initiated by introducing carboxylic acid groups using nitric acid oxidation (Men et al., 2008; J. Shen et al., 2007; Kitano et al., 2007). Thereafter, small molecules (Kooi et al., 2002; Liu, 2005; Tasis et al., 2003) or polymer chains (Kang & Taton., 2003; O. K. Kim et al., 2003) can be chemically attached to the CNTs by esterification and amidation reactions via the carboxylic acid moieties. The chemical modification is an especially attractive target, as it can improve solubility (Bahr et al., 2001) and processing ability and allows the unique properties of CNTs to be coupled to other types of materials.

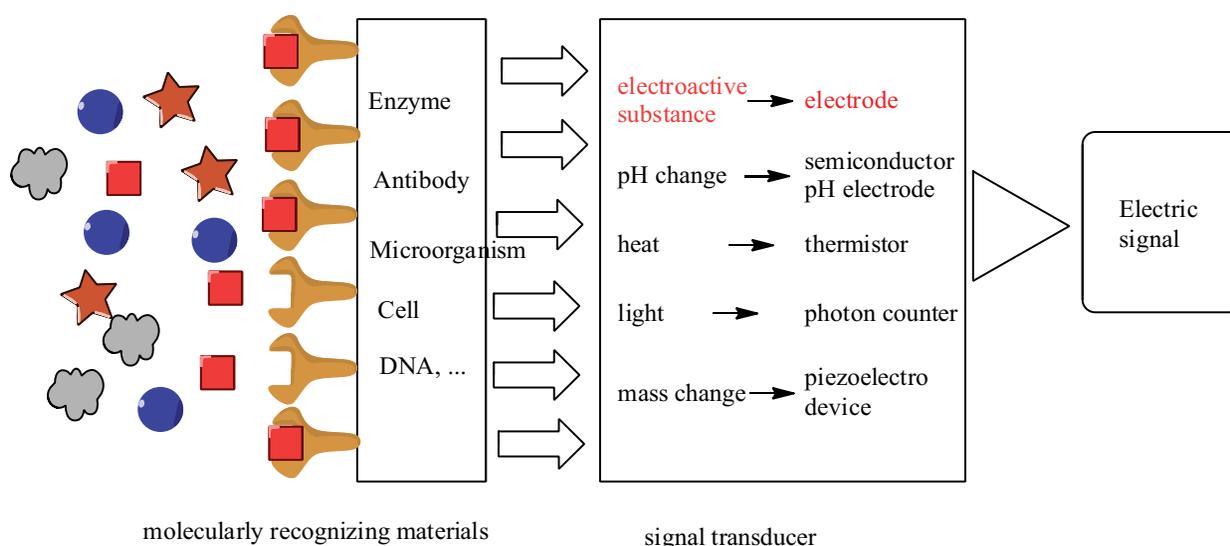


Fig. 1. Schematic diagram for the principal of the biosensors.

Radiation-induced graft polymerization (RIGP) is a useful method for the introduction of functional groups into polymer *matrices* using specially selected vinyl monomers. There have been several reports on RIGP of polar monomers onto the surface of polymer substrates with hydrophobic properties to obtain hydrophilic properties for versatile applications (Choi & Nho, 1999a, 1999b; Choi et al., 1999, 2000a, 2000b, 2001; S. K. Kim et al., 2010). RIGP can be easily modified for the surface of CNTs to induce free radicals on the surface of nanotubes in aqueous solution and organic solvents at room temperature. Figure 2 shows the introduction of functional groups, such as hydroxyl, carboxyl, and sulfonic acid onto the CNT surface (Oh et al., 2006a) and fullerene (Chung et al., 2011) using free radicals generated during γ -ray irradiation.

In this chapter, we describe the fabrication of biosensors using vinyl polymer-grafted carbon nanotubes prepared by RIGP. Various vinyl monomers used for functionalization of CNTs will be introduced. The obtained vinyl polymer-grafted CNTs are used as biosensor supporting materials to increase sensitivity and affinity for biomolecules. The characterization and application of the four biosensor types are: (1) Enzyme-free biosensors

based on chemical reaction, (2) Enzymatic biosensors based on functional group-MWNTs, (3) Bacterial biosensors based on polymer grafted MWNTs, and (4) E-DNA biosensors based on polymer grafted MWNTs, as summarized in Fig. 3.

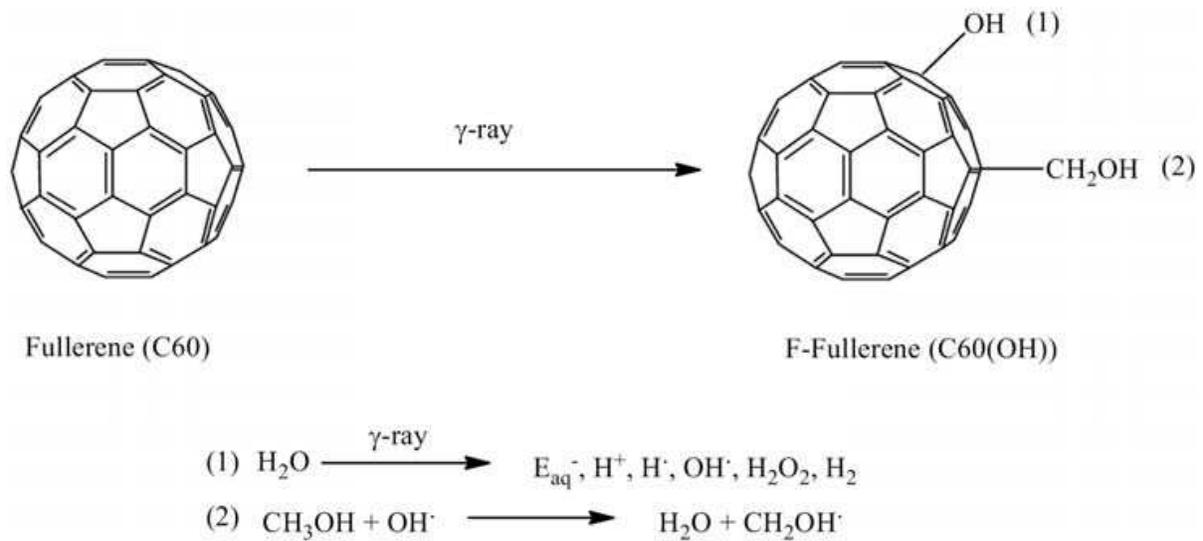


Fig. 2. Radiolytic functionalization possible mechanism of Fullerene (C60) in H₂O/MeOH mixture solution.

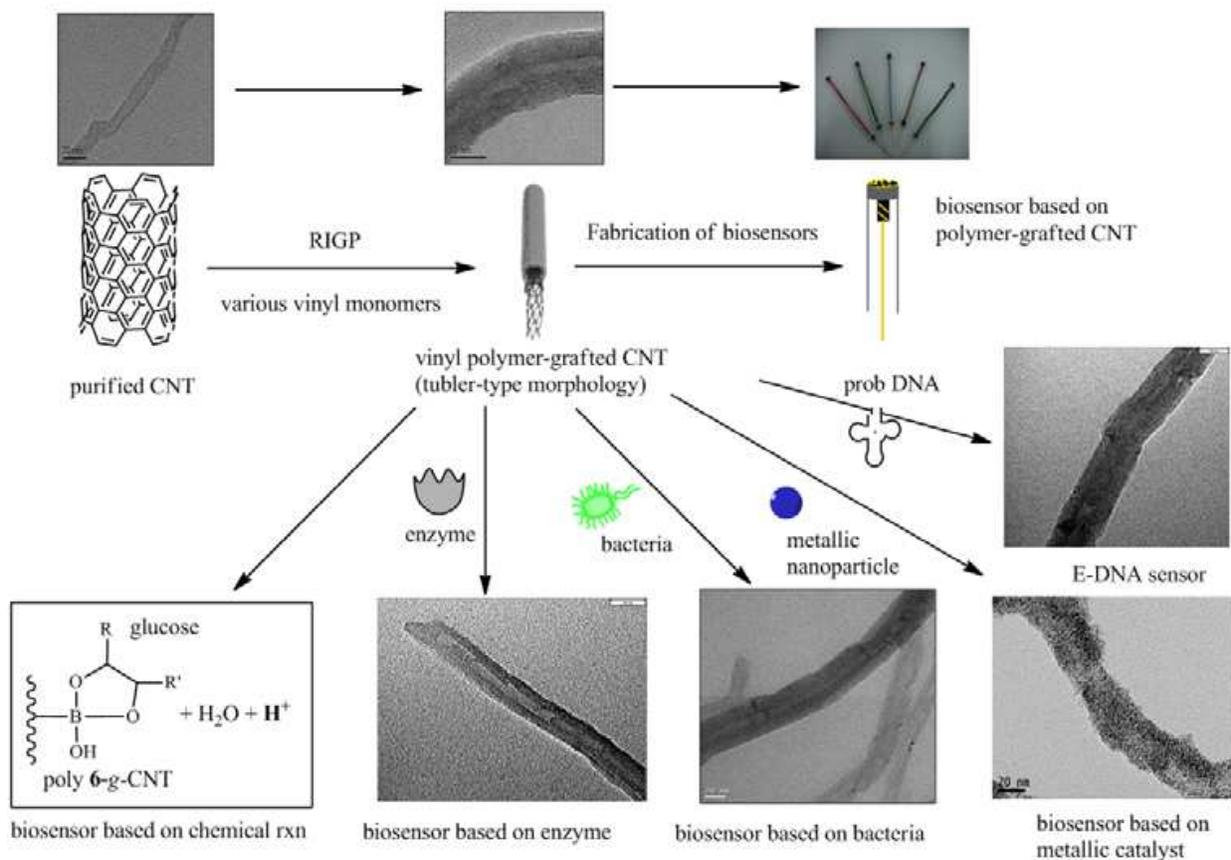


Fig. 3. Schemetic fabrication of biosensor based on vinyl polymer-grafted MWNT.

2. Enzyme-free biosensor based on chemical reaction

Numerous methods based on enzyme immobilization for human cholesterol assays have been developed, including colorimetric, spectrometric and electrochemical methods (Crumbly et al., 1993; Shumyantseva et al., 2004). The use of enzymes in the fabrication of sensors has advantages due to their rapid, selective, and sensitive nature. However, there exist some practical problems related to the use of enzymes in analytical devices due to their short lifetime and low reusability since they are easily affected by temperature, humidity, and pH (Gavalas & Chaniotakis, 2000, 2001). To address these issues, non-enzymatic sensors based on the direct electrocatalytic oxidation of glucose are being investigated for their stability, simple fabrication, reproducibility, low cost, and freedom from oxygen limitation, unlike enzyme-based sensors (Lee et al., 2009). In this section, we discuss the preparation and characterization of enzyme-free biosensors based on boronic acid-modified and metallic nanoparticles-immobilized CNTs.

2.1 Chemical reaction between boronic acid containing group modified carbon surface and target molecules

Boronic acids ($-B(OH)_2$) can interact with cyanides (Badugu et al., 2004a) and fluorides (Cesare & Lakowicz, 2002a), which have been explored for sensor development. Boronic acids binding with diols (J. Wang et al., 2005) have been mostly studied in developing fluorescent carbohydrate sensors. Boronic acids act as an electron withdrawing group in its neutral form, $-B(OH)_2$, and as an electron donation group in its anionic form, $-B(OH)_3^-$.

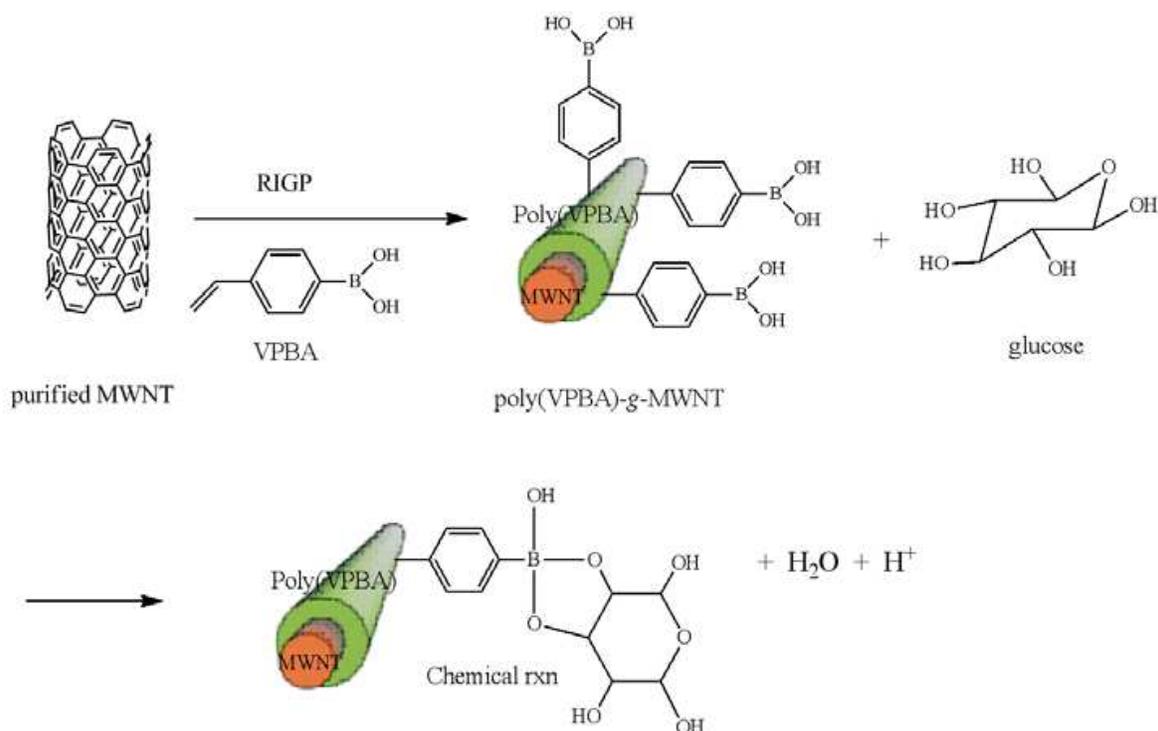


Fig. 4. Preparation of non-enzymatic biosensor based on chemical reaction by RIGP.

The feasibility of tear glucose sensing was tested using a daily disposable contact lens embedded with boronic acid-containing fluorophores which act as a potential alternative to current invasive glucose monitoring techniques (Badugu et al., 2004b). The boronic acid

probes in the contact lens could continuously monitor the tear glucose levels in the range of 50-500 μM . The boronic acid group showed higher affinity for D-fructose and smaller affinity for D-glucose (Cesare & Lakowicz, 2002a). This means that the use of the boronic acid group for sensing sugars is strongly dependent on the molecular geometry and the aromatic species where boronic acid is present.

Poly(VBAC)-grafted MWNTs are of great interest for the preparation of enzyme-free sensors because of the boronic acid group they contain. 4-Vinylphenyl boronic acid (VPBA) was used to functionalize the surface of multi-walled carbon nanotubes (MWNTs) since it possesses both hydrophobic and hydrophilic properties (D. S. Yang et al., 2010). The vinyl group of the monomer attaches to the surface of MWNTs because of a hydrophobic-hydrophobic interaction, while the functional group of the monomer comes to the surface in an aqueous solution because of a hydrophilic-hydrophilic interaction. When irradiated, the radical polymerization of the monomer on the surface of MWNTs occurs to form grafted vinyl polymer PVBAc-g-MWNTs. Boronic acid in PVBAc-g-MWNTs couples with diols of glucose to form a boronic acid diester group, as shown in Figure 4 (D. S. Yang et al., 2010). The boronic acid content of PVBAc-g-MWNTs was 296 mg/g, as determined by titration. The diols are linked covalently and the reaction is fast and completely reversible (Cesare & Lakowicz, 2002b). The cyclic voltammograms of PVBAc-g-MWNTs in 0.1 M phosphoric buffer solution displayed an excellent linear response to glucose concentration in the range 1.0-10 mM.

2.2 Catalytic reaction of the target molecules on the surface of metallic nanoparticle-modified MWNT

Recently, an enzyme-free hydrogen peroxide (H_2O_2) biosensor was developed based on nano-conducting polymer composites (MWNT-PEDOT nanoparticles) (K. C. Lin et al., 2010). The use of enzyme-free H_2O_2 biosensors is important in chemical, food and environmental applications. More enzyme-free H_2O_2 biosensors have been developed based on modified carbon fiber microelectrodes (Y. Wang et al., 1998), vanadium-doped zirconias (Domenecha & Alarcon, 2002) and Fe_3O_4 (M. S. Lin & Leu, 2005).

Nanoscale materials of metal (Ag, Au, Pd, Pt, etc.), alloy (Pt-Ru), carbon and polymers are very attractive for a variety of applications including optical and electronic nanodevices, and chemical and biological nanosensors. Nanoparticles offer higher catalytic efficiency than bulk materials due to their large surface-to-volume ratio (Yu et al., 1999; S. J. Kim et al., 2008).

Initial research developing non-enzymatic sensors focused on the use of nanocrystalline metals, such as Pt and Au, especially Pt-based amperometric electrodes (S. J. Park et al., 2003; Song et al., 2005). However, such Pt-based glucose sensors lacked sufficient selectivity and sensitivity due to chemisorbed intermediates and electroactive species. The desire for better and cheaper electrocatalysts has resulted in bimetallic systems being developed. Pt-Au (Habrioux et al., 2007; H. Liu et al., 1992), Pt-Pb (Cui et al., 2007; Bai et al., 2008; J. Wang et al., 2008; Sun et al., 2001), and Pt-Ru (Xiao et al., 2009) have all displayed high electrocatalytic activity for glucose oxidation.

Effective fabrication of electrocatalysts also depends on the support material (Hsu et al., 2008). Catalyst dispersion and utilization have been shown to improve supporting Pt-Ru nanoparticles on high-surface area carbon materials, such as CNTs, carbon nanofibers, carbon nanocoils and carbon nanohorns (Steigerwalt et al., 2002; Hyeon et al., 2003; K. Park

et al., 2004; R. Yang et al., 2005). A systematic study has shown that MWNTs are the best of the carbon based electrocatalyst supports (Reddy & Ramaprabhu, 2007). In principle, MWNTs are seamless cylinders. However, they often have defects where the attachment of Pt-based alloy nanoparticles most likely occurs.

In a preliminary report, Pt-Ru nanoparticles were deposited on the surfaces of various carbon supports, including Vulcan XC-71, Ketjen-300, Ketjen-600, single-walled carbon nanotubes (SWNTs), and MWNTs for use as fuel cell catalysts using γ -ray irradiation without anchoring agents (Choi et al., 2003a, 2003b; Oh et al., 2006; Hwang et al., 2008). The metal (Ag or Pd) and alloy (Pt-Ru) nanoparticles were also deposited on the surfaces of SWNTs (Oh et al., 2005, 2006a, 2006b, 2008) and porous carbon supports using γ -irradiation without anchoring agents (Seo et al., 2008). However, metallic alloy nanoparticles aggregated on the surfaces of the carbon supports due to their hydrophobic nature. This aggregation was overcome by modifying the surface of the carbon support to give it hydrophilic properties. This was done by *in-situ* polymerization of β -caprolactone, methacrylate and pyrrole using oxidizing agents as initiators (Bae et al., 2010). The polymer-stabilized bimetallic (Pd-Ag) nanoparticles were prepared by γ -irradiation in organic solvents and used as catalysts for hydrogenation of *cis,cis*-1,3-cyclooctadiene (Choi et al., 2005). Pt-Ru nanoparticles were then deposited on the polymer-wrapped MWNT supports to produce a direct methanol fuel cell (DMFC) anode catalyst (Choi et al., 2010).

Pt-Ru nanoparticles have also been deposited on functional polymer (FP)-grafted MWNTs by RIGP, to produce an anode catalyst for DMFCs (D. S. Yang et al., 2011). This method involved two steps: grafting the functional polymer onto the MWNTs by RIGP; and then depositing the Pt-Ru nanoparticles onto the MWNTs by radiation-induced reduction. Pt-M nanoparticles on FP-MWNT supports have also been prepared via a one-step process initiated by free radicals and hydrated electrons generated during γ -irradiation in an aqueous solution.

The catalytic efficiencies of the Pd/C and Pd-M/C particles in various Suzuki-type and Heck-type reactions were examined (S. J. Kim et al., 2008; M. R. Kim & Choi, 2009). In the Suzuki-type reactions, the catalytic efficiency (measured by the yield of the product) decreases in the order of Pd-Cu/C > Pd/C > Pd-Ag/C > Pd-Ni/C. The reaction yield with Pd-Ni/C was much lower than those with other particles. Generally the carbon-supported Pd and Pd-M nanoparticles showed excellent capabilities as a catalyst for carbon-carbon coupling reaction such as Suzuki- and Heck-type reactions.

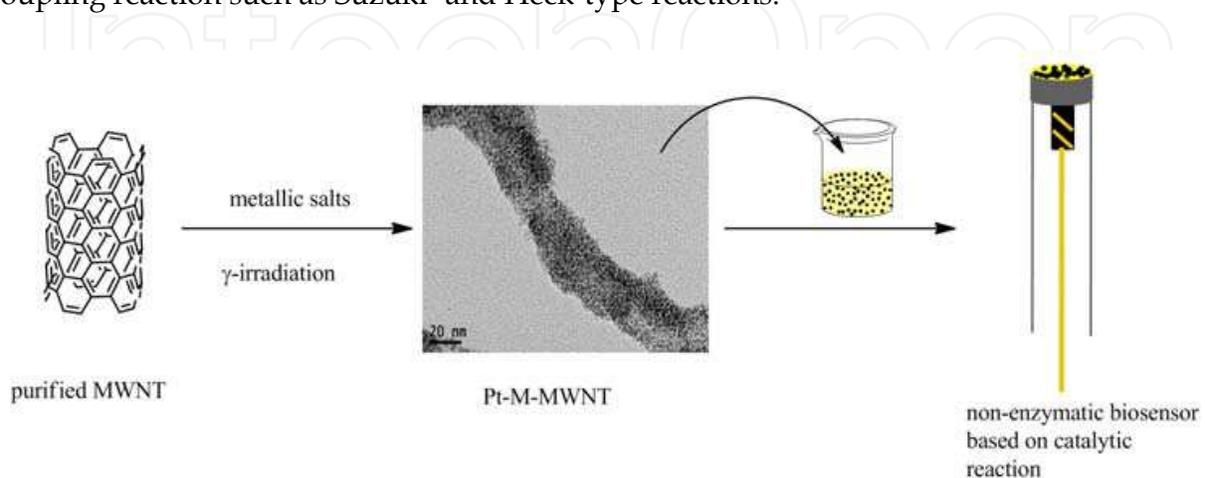


Fig. 5. Radiolytic preparation of non-enzymatic biosensor based on catalytic oxidation.

Non-enzymatic glucose sensors employing polyvinylpyrrolidone (PVP) modified-MWNTs with highly dispersed Pt-M (M = Ru and Sn) nanoparticles (Pt-M/PVP@MWNTs) were fabricated by radiolytic deposition (Kwon et al., 2011). The Pt-M nanoparticles were found to be well-dispersed and exhibit alloy properties on the MWNT supports. Electrochemical testing showed that these non-enzymatic sensors had larger currents (mA) than that of a bare glassy carbon (GC) electrode and PVP modified-MWNTs. The prepared biosensor with Pt-Ru nanoparticles for glucose has good sensitivity, linear range, and a lower detection limit in NaOH electrolyte. These non-enzymatic sensors can effectively avoid interference from ascorbic acid and uric acid in NaOH electrolyte.

3. Enzymatic biosensors based on vinyl polymer grafted-MWNTs prepared by RIGP

3.1 Enzymatic biosensors

The first enzyme electrode was an amperometric type of biosensor developed by Clark and Lyons (Clark & Lyons, 1962). A soluble biomaterial glucose oxidase was held between membranes and the oxygen uptake was measured with an oxygen electrode. Since then, enzyme-based electrochemical biosensors have been widely used in medical and pharmaceutical applications, food safety and environmental monitoring, defense and security. Health care is the main area using biosensor applications today for monitoring blood glucose levels and diabetes. Also, potential applications exist for the reliable detection of urea in renal disease patients either at home or in the hospital. Industrial applications are used to improve manufacturing processes leading to better yield and product quality, such as monitoring the production of alcohol during the fermentation process. Furthermore, biosensors help to meet environmental legislation through monitoring of phenolic compounds contained in industrial waste water, much of which is toxic to the environment. Electrochemical biosensors incorporating enzymes with nanomaterials, which combine the recognition and catalytic properties of enzymes and the electronic properties of various nanomaterials, are the desired materials with synergistic properties originating from the components of the hybrid composites. Many enzymes have been employed to prepare various kinds of biosensors using carbon nanotubes (CNTs) (Chakraborty & Raj, 2007; Male et al., 2007; Arvinte et al., 2008). Usually, enzymes are immobilized onto CNTs by physical adsorption (Guan et al., 2005) and covalent bonding (Patolsky et al., 2004; Y. J. Zhang et al., 2005). Various vinyl monomers, such as acrylic acid (AAc), methacrylic acid (MAc), glycidyl methacrylate (GMA), maleic anhydride (MAN), and 4-vinylphenylboronic acid (VPBac), are used to functionalize CNTs prior to immobilizing enzymes onto CNTs. Figure 6 shows the vinyl monomers which possess both hydrophobic and hydrophilic properties. Polymer-CNT nanocomposites have been obtained by γ -irradiation polymerization of various vinyl monomers. The obtained vinyl polymer-grafted CNTs are used as biosensor support materials to increase sensitivity and affinity for biomolecules.

Tyrosinase-immobilized biosensors were fabricated based on Poly(AAc)-g-MWNT and Poly(Man)-g-MWNT by RIGP of AAc and MAN on the surface of MWNTs, (#1 and 4 in Fig. 6). The biosensor was then prepared on an indium tin oxide (ITO) glass electrode via a hand-casting of chitosan solution with tyrosinase-immobilized Poly(AAc)-g-MWNT (#1 in Fig. 6) and Poly(Man)-g-MWNT (#4 in Fig. 6) respectively. The sensing ranges of biosensors were 0.2–0.9 mM and 0.1–0.5 mM concentrations for phenol in phosphate buffer solution. Various parameters influencing biosensor performance have been optimized for pH,

temperature, and the response to various phenolic compounds. The biosensor was then tested on phenolic compounds contained in commercial red wines (K. I. Kim et al., 2010).

A tyrosinase-immobilized biosensor with hydroxyl group-functionalized MWNTs was also developed for phenol detection (J. H. Yang et al., 2009). The hydroxyl group-modified MWNTs include poly(GVPB)-g-MWNT and poly(HEMA) prepared by RIGP (#5 and 6 in Fig. 6). The biosensor response was in the range of 0.6–7.0 mM and 0.05–0.35 mM for phenol in a phosphate buffer solution, respectively. The biosensor was then optimized for pH, temperature, and other phenolic compounds in commercial red wines. As a result, the amount of phenolic compounds in commercial red wines are in the range of 68.5–655.0 mg/L, which was calculated from a calibration curve of phenol on a biosensor based on poly(GVPB)-g-MWNTs.

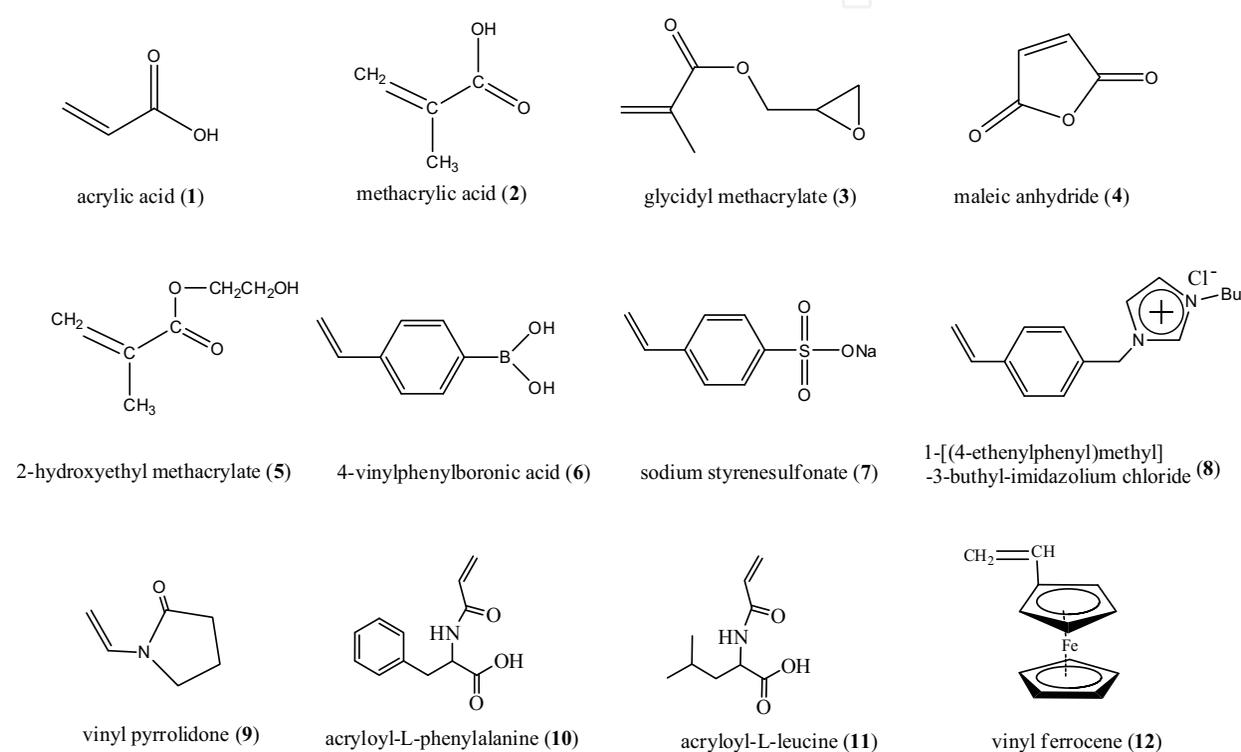


Fig. 6. The used vinyl monomers in Choi lab. for radiation-induced grafting onto carbon nanotubes.

Direct electrochemistry of biological enzymes has been studied with ion liquids (ILs) in both theoretical and practical applications since ILs are considered to be suitable media for supporting a biocatalytic process with high polarity, non-coordination power, high selectivity, fast rates, and great enzyme stability (Compton & Laszlo, 2002; Sweeny & Peters, 2001). The cationic property of modified MWNT-based sensors was developed by RIGP of vinyl monomers with ionic properties, such as 1-butylimidazole bromide (#8 in Fig. 6), 3-(butyl imidazol)-2-(hydroxyl)propyl methyl methacrylate and 1-[(4-ethenylphenyl)methyl]-3-butyl-imidazolium chloride, in aqueous solution at room temperature (K. I. Kim et al., 2009; Ryu & Choi, 2010a). Subsequently, the *tyrosinase*-immobilized biosensor was fabricated by hand-casting the ionic property-modified MWNTs, *tyrosinase*, and chitosan solution as a binder onto the ITO glass surface. The biosensors were used to determine phenolic compounds in red wines or caffeine in commercial coffee. As a result, the amount of

phenolics in commercial red wines has been determined to be in the range of 383.5–3,087 mg/L in a phosphate buffer solution (K. I. Kim et al., 2009), and the amount of caffeine in commercial coffee was in the range of 144.8 ~ 1765 mg/L in a phosphate buffer solution (Ryu & Choi, 2010a).

In addition, colloidal gold nanoparticles (Au-NPs) have been widely used as a model system because of their ease of synthesis and surface modification (Feng et al., 2007), good biocompatibility (Qu et al., 2006), as well as their ability to act as tiny conduction centers which facilitate electron transfer (Jia et al., 2002). Fabrication of a glucose oxidase (GODox) immobilized biosensor has been attempted by two methods (Piao et al., 2010). In one of the methods, gold nanoparticles (Au-NPs) prepared by γ -irradiation were loaded into the poly(MAn)-g-MWNT electrode via physical entrapment. In the other method, the Au-NPs were prepared by electrochemical reduction of Au ions on the surface of the poly(MAn)-g-MWNT electrode and then GODox was immobilized into the Au-NPs. The GODox immobilized biosensors were tested for electrocatalytic activity to sense glucose. The sensing range was from 30 μ M to 100 μ M for the glucose concentration, and the detection limit was 15 μ M. Interference of ascorbic acid and uric acid were below 7.6%. The physically Au deposited poly(MAn)-g-MWNT paste electrodes appear to be good sensors in detecting glucose.

Hydroxyl group was introduced onto the surface of fullerene by RIGP of VPBA in a methanol/1,2-dichlorobenzene mixture solution. The obtained functionalized-fullerene, F-fullerene, was then used as a sensor support material. The enzyme electrode was prepared on the ITO electrode via hand-casting of the chitosan solution based on F-fullerene and tyrosinase. The sensing range of the biosensor was 0.1 ~ 0.6 mM for phenol in a phosphate buffer solution. Furthermore, the prepared biosensor was used to determine concentration of phenolics in commercial red wines (Chung et al, 2011).

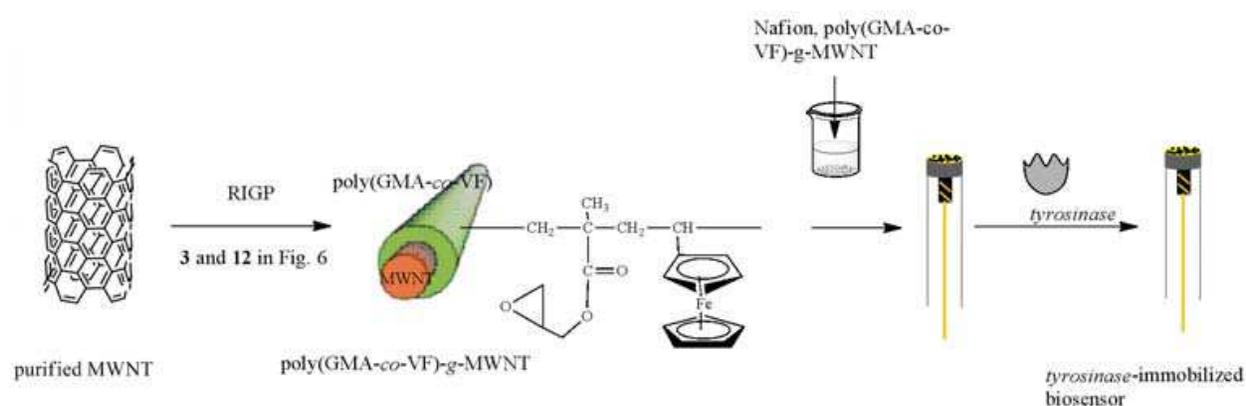


Fig. 7. Preparation of enzymatic biosensor based on poly(GMA-co-VF)-g-MWNT prepared by RIGP.

Ferrocene derivatives are widely used as electron transfer mediators for amperometric glucose biosensors since ferrocene meets the criteria of a good mediator, such as inert behavior with oxygen, stable in both redox forms, independent of pH, shows reversible electron transfer kinetics, and reacts rapidly with enzymes (Eggins, 1996). A polymeric mediator is necessary since polymers allow the incorporation of reagents to achieve reagentless sensing devices. Some examples of redox copolymers trialing the covalent attachment of ferrocene include poly(vinylferrocene-co-hydroxyethyl methacrylate),

poly(N-acryloylpyrrolidine-co-vinylferrocene) and ferrocene-containing polythiophene derivative (T. Saito & Watanabe, 1998; Koide & Yokoyama, 1999).

The electrochemical phenol biosensor was fabricated by immobilizing tyrosinase on poly(glycidyl methacrylate-co-vinylferrocene)/MWNT [poly(GMA-co-VFc)/MWNT] film, as shown in Fig. 7. A polymeric electron transfer mediator, containing copolymers of glycidyl methacrylate (GMA) and vinylferrocene (VFc) with different molar ratios, was prepared by RIGP. The prepared poly(GMA-co-VFc)/MWNT was confirmed via Fourier transform infrared spectrometer (FT-IR), thermogravimetric analysis (TGA), transmission electron microscopy (TEM) and X-ray photoelectron spectra (XPS). Also, the sensing efficiency of the fabricated electrochemical phenol biosensor was evaluated by cyclic voltametric (CV) (Lee & Choi, 2010).

3.2 ECL biosensor

Electrogenerated chemiluminescence (ECL) is a light emission produced from a high energy electron transfer (redox) reaction between electrogenerated species, which is usually accompanied by the regeneration of emitting species. In ECL, light emission is controlled by turning on/off the electrode potential. ECL has been receiving great attention as an important and valuable detection method in analytical chemistry. Application of ECL is widely found in chemical sensing (Knight, 1999), imaging (Wightman et al., 1998), and optical studies (Fan et al., 1998). Moreover, it is also used in chromatography (Noffsinger & Danielson, 1987) and capillary electrophoresis (Arora et al., 2001). Among the various ECL systems, Ru(bpy)₃²⁺ is the most widely used complex due to its excellent intrinsic characteristics and capability to produce ECL with a wide range of analysis, such as oxalate, amines, and amino acid.

Chemiluminescence Sensor was fabricated based on conducting polymer@SiO₂/Nafion composite film (Jung et al., 2010). The Tris(2,2'-bipyridyl)ruthenium (II) (Ru(bpy)₃²⁺) ECL sensor was fabricated by immobilization of Ru(bpy)₃²⁺ complex on the functionalized MWNT-Nafion composite film coated glass carbon (GC) electrode. The functionalized MWNT was prepared by coating polythiophene (PTh), polyaniline (PANI), and poly(3-thiopheneacetic acid) [P(3-TAA)] on the surface of the carboxylic acid-modified MWNTs. The sensitivity and reproducibility of the prepared ECL sensor to tripropylamine (TPA) was evaluated. As a result, the carboxylic acid-modified MWNT composite electrode showed higher sensitivity and better reproducibility than that of other functionalized MWNT composite electrodes (S. H. Kim et al., 2008).

A SO₃H-F-MWNT-Nafion-Ru(bpy)₃²⁺-ADH ECL electrode for ethanol sensing is shown in Fig. 8. The ECL sensor was fabricated by immobilization of Ru(bpy)₃²⁺ on the SO₃H-functionalized MWNT (SO₃H-F-MWNT)-Nafion composite film coated on a GC electrode. Finally, ADH was immobilized on the electrode in a phosphate buffer solution at 4 °C. The SO₃H-F-MWNT ECL biosensor showed higher sensing efficiency for ethanol than that of the ECL biosensor prepared by purified MWNT. Experimental parameters affecting ethanol detection were also examined in terms of pH, and the content of SO₃H-F-MWNT in Nafion. Little interference of other compounds for assay of the ethanol was observed. Results suggest that the ECL biosensor could be applied for ethanol detection in real samples (Ryu & Choi, 2010b).

A COOH-F-MWNT-Nafion-Ru(bpy)₃²⁺-Au-ADH ECL electrode using COOH-functionalized MWNTs (COOH-F-MWNT) and Au nanoparticles synthesized by γ -irradiation was fabricated for ethanol sensing (Piao et al., 2009). Here, Au atoms were produced in solution

by radiation-induced reduction of Au ion-precursors without chemical reducing agents. The species arising from the radiolysis of water, solvated electrons, e_{aq}^- , and $H\cdot$ atoms are the strongest reducing agents. They easily reduce Au ions to produce Au nanoparticles. A higher sensing efficiency for ethanol for the ECL biosensor prepared by Poly(AAc)-g-MWNT (#1 in Fig. 6) was measured compared to that of the ECL biosensor prepared by Poly(Mac)-g-MWNT (#2 in Fig. 6), and purified MWNT. Experimental parameters affecting ethanol detection were also examined in terms of pH and the content of Poly(AAc)-g-MWNT in Nafion. The sensors can effectively avoid interference from the oxidation of citric acid, ascorbic acid, acetic acid and oxalic acid. The experimental results show that the ECL biosensor could be applied for ethanol detection in real samples (Piao et al., 2009).

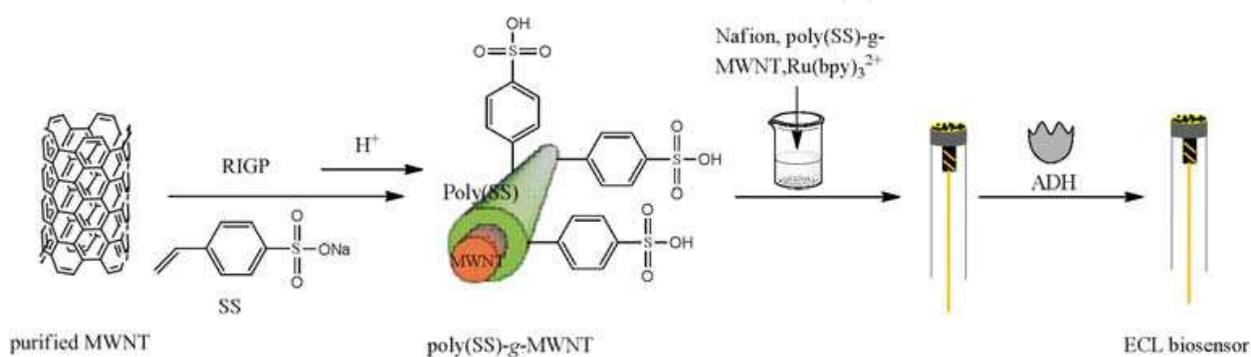


Fig. 8. Preparation of ECL biosensor based on poly(SS)-g-MWNT prepared by RIGP.

4. Bacteria biosensors based on polymer grafted MWNTs prepared by RIGP

Various bio-elements, including enzymes, antibodies, microorganism, organelles, tissues, and cells have been used in the fabrication of biosensors (Lei et al., 2006). Enzymes are the most widely used bio-element due to their unique specificity and sensitivity (D'Souza, 2001). However, the use of enzymes is often hampered by costly and time-consuming procedures in purification. Also, the enzyme activity could be decreased in *in vitro* operating environments (Byfield & Abuknesha, 1994). Microbes, such as algae, bacteria, and yeast can be alternatively used in the fabrication of biosensors since they can be massively produced by cell culturing. In addition, microbial cells are relatively easier to be manipulated and have better viability and stability *in vitro* compared to the cells from higher organisms such as plants, animals, and human beings (Byfield & Abuknesha, 1994).

Bacterial sensors using *Pseudomonas fluorescens* (*Pseudomonas putida* DSM 6521) and *P. putida* DSM 50026 cells entrapped with chitosan matrix onto the surface of graphite electrodes have been prepared (Odaci et al., 2008). The measurements were based on the respiratory activity of the cells. The sensor showed good linearity and repeatability with high operational stability. Also prepared were CNT-modified chitosan membranes to test the effect of nanoparticles on the biosensor performance. The results showed that combining the properties of carbon nanotubes and the versatility and biocompatibility of chitosan created chitosan surface coated carbon nanotubes.

As discussed earlier, nanomaterials have been used to improve the efficiency of electron transfer between the redox center of enzymes and electrodes (Li et al., 2006; Zhang & Fang, 2010; Guo et al., 2008). Owing to their unique properties, quantum dots (QDs) have

generated considerable interest as electron transport nanomaterials for biodetection. The direct electron transfer of glucose oxidase (GOD) adsorbed on a CdS QDs modified pyrolytic graphite electrode has been reported, where the enzyme demonstrated significantly enhanced electron-transfer reactivity (Huang et al., 2007). The CdS QDs-coated electrode was displayed as a pair of well-defined redox peaks of GOD.

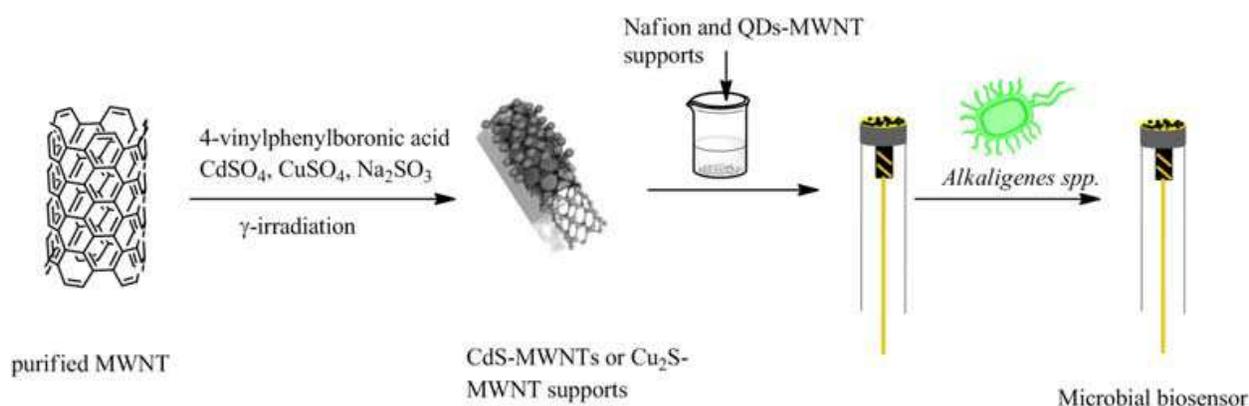


Fig. 9. Preparation of microbial biosensor based on QD-MWNT prepared by radiation reaction.

The *Acaligense sp.*-immobilized biosensor was fabricated based on QD-MWNT composites as an electron transfer mediate and a microbe immobilization support for sensing phenolic compounds in commercial red wines, as shown in Fig. 9 (S. K. Kim et al., 2011). First, the quantum dot-modified MWNT composite (QD-MWNTs) was prepared in an aqueous solution at room temperature. The successful preparation of the QD-MWNT composite was confirmed through XPS, TEM, and elemental analysis. Second, the microbial biosensor was fabricated by immobilization of *Acaligense sp.* on the surface of the GC electrode, which was prepared by hand-casting with the mixture of obtained composite and Nafion solution. The sensing ranges of the microbial biosensor based on CdS-MWNT and Cu₂S-MWNT supports were 0.5-5.0 mM and 0.7-10 mM for phenol in phosphate buffer solution, respectively. Total concentration of phenolic compounds contained in commercial red wines was also determined using the prepared microbial immobilized biosensor (S. K. Kim et al., 2011).

RIGP of the poly(GMA) onto MWNT surfaces in an aqueous solution at room temperature was performed to introduce an epoxy group (Shin et al., 2011). The epoxy group can be easily converted to alcohols, amines, phosphonic acid, and sulfonic acid. Subsequently, triethylene diamine (TEDA) was introduced onto the epoxy group of the grafted poly(GMA) to prepare MWNT supports with an amine group. Finally, the electrochemical microbial biosensor (EMB) was fabricated after immobilization of a microbe (*Alkaligenes spp.*) on the modified electrode. The EMB was evaluated for its sensing efficiency for phenol in a phosphate buffer solution. The total concentration of phenolic compounds in commercial red wines was determined using the EMB.

5. E-DNA biosensor based on polymer grafted MWNT prepared by RIGP

Currently, direct detection of nucleic acids is an area of tremendous interest, as it plays a major role in forensics (Jelly et al., 2008), pharmaceutical applications (Kranaster & Marx, 2007), medical diagnosis (Marras et al., 2006), food and agricultural analysis (Timko et al.,

2008), and environmental control (X. C. Shen et al., 2008). Electrochemical transducers offer many potential advantages that include sensitivity, accurate specificity, simplicity and low-cost, in converting nucleic acid hybridization events into useful analytical signals (Millan et al., 1994; Hashimoto et al., 1994; S. F. Wang et al., 2005). Electrochemical DNA (E-DNA) biosensors have been popularly developed for DNA sequence analysis with immobilization of single-stranded DNA (ssDNA) probes on various electrode surfaces (Niu et al., 2009).

Fabrication of E-DNA biosensors has been reported by the physical immobilization of probe DNA, 5'-NH₂-GGA GCT GCT GGC ATT ATT GAA-3', on ionic-liquid (IL) modified MWNTs with ITO electrodes to detect *Salmonella typhi* (*S. typhi*), as shown in Fig. 10 (Chung et al., 2009). IL-MWNTs were prepared by the introduction of 1-butylimidazole bromide onto an epoxy group on poly(GMA)-grafted MWNTs, which were synthesized by RIGP of GMA onto MWNTs in aqueous solution. Subsequently, IL-MWNTs were coated onto the ITO electrode surface, and then the physical immobilization of the probe DNA performed in solution at room temperature for 1 h. The IL-MWNTs were characterized by elemental analysis, XPS, and TGA. The electron transfer resistance (R_{et}) of the E-DNA biosensor was evaluated after hybridization of the probe DNA and target DNA using electrochemical impedance spectroscopy (EIS). The R_{et} increased after the hybridization of probe DNA and target DNA. The target DNAs used were: (1) Complementary DNA, 5'-TTC AAT AAT GCC AGC AGC TCC-3', (2) Single-base mismatch DNA, 5'-TTC AAT AAT GGC AGC AGC TCC-3' and (3) Three-base mismatch DNA, 5'-TTC ATT AAT GGC AGC ACG TCC-3' (Chung et al. 2009).

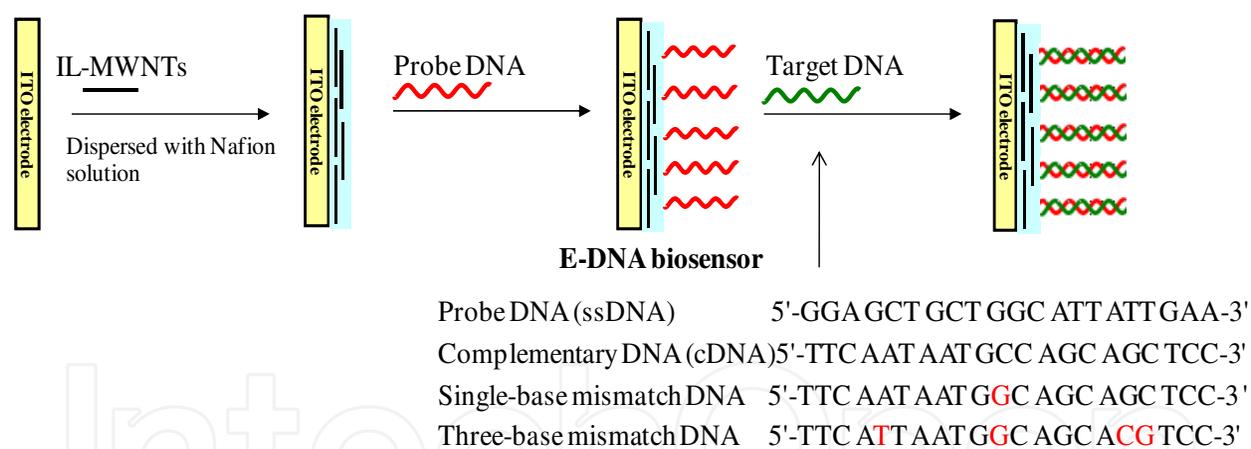


Fig. 10. Preparation of E-DNA biosensor based on IL-MWNTs prepared by RIGP.

6. Conclusion

This chapter has discussed fabrication of biosensors using vinyl polymer-grafted carbon nanotubes prepared by RIGP. To fabricate a biosensor, biomolecules should be immobilized on transducers or support matrices either by chemical methods or by physical methods. Nanostructured materials have been applied in biosensing due to their good biocompatibility, enhanced electron transfer property, and high surface area. Immobilization of bio-elements on nanostructured electrodes could potentially improve the sensitivity of biosensors

The small size and electronic properties of CNTs make them ideal for use in electrochemical biosensors and nanoscale electronic devices. However, the insolubility of CNTs in most

solvents is a major barrier for developing such CNT-based biosensing devices. Therefore, surface modification is necessary for CNT materials to be biocompatible and to improve solubility in common solvents and selective binding capability to biotargets. Various vinyl monomers have been used to functionalize CNTs since they possess both hydrophobic and hydrophilic properties. The vinyl group of the monomer comes to the surface of CNTs because of a hydrophobic-hydrophobic interaction, while the functional group of monomer comes to the surface in an aqueous solution because of a hydrophilic-hydrophilic interaction.

When irradiated by γ -rays, the radical polymerization of the monomer on the surface of CNT occurs. RIGP is a useful method for the introduction of functional groups into polymer matrices using specially selected vinyl monomers. Two active species such as a free radical and a hydrated electron generated during γ -irradiation are used in preparing polymer nanocomposites. Various polymer-CNT nanocomposites using RIGP of the desired vinyl monomers can be prepared in a one-step process at room temperature and ambient pressure. The obtained vinyl polymer-grafted CNTs are used as biosensor supporting materials to increase sensitivity and affinity for biomolecules.

Catalyst dispersion and utilization have been shown to improve supporting Pt-Ru nanoparticles on high-surface area carbon materials. The metal (Ag or Pd) and alloy (Pt-Ru) nanoparticles have been deposited on the polymer-wrapped MWNT supports using γ -irradiation without anchoring agents. Non-enzymatic glucose sensors employing PVP modified-MWNTs with highly dispersed Pt-M (M = Ru and Sn) nanoparticles were fabricated by radiolytic deposition. The biosensor with Pt-Ru nanoparticles for glucose showed good sensitivity, linear range, and a lower detection limit in NaOH electrolyte.

The obtained vinyl polymer-grafted CNTs have been successfully used as biosensor supporting materials for various types biosensors, including enzyme-free biosensors, enzymatic biosensors, bacterial biosensors, and E-DNA biosensors. Poly(VPBAC)-grafted MWNTs were prepared and used in enzyme-free glucose sensors for the detection of glucose without enzymes. The electrode displayed an excellent linear response to glucose concentration in the range 1.0–10 mM. The *tyrosinase*-immobilized biosensor was fabricated by a hand-casting of the modified MWNTs, *tyrosinase*, and chitosan solution as a binder onto ITO glass surface. The sensing ranges of the *tyrosinase*-biosensor for phenol in phosphate buffer solution was in the range of 0.005 ~ 0.2 mM. The biosensors were used to determine phenolic compounds in red wines or caffeine in commercial coffee. The *Acaligense sp.*-immobilized biosensor was also fabricated based on QD-MWNTs composites as an electron transfer mediator and a microbe immobilization support for sensing phenolic compounds in commercial red wines. E-DNA biosensor has been fabricated by physical immobilization of probe DNA on IL-MWNTs modified glassy carbon (GC) electrode in order to detect *Salmonella typhi* (*S. typhi*).

7. References

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