*Review*

# Potentiometric Biosensing Applications of Graphene Electrode with Stabilized Polymer Lipid Membranes

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**Abstract:** This review provides informations and details for the fabrication of biosensors that are composed from lipid membranes and have been utilized and applied to rapidly detect food toxic compounds, environmental pollutants and analytes of clinical interest. Biosensors based on polymeric lipid membranes have been used to rapidly detect a wide range of these analytes and offer several advantages such as fast response, high sensitivity and selectivity, can be portable for in the field applications, and small size. A description of the construction of these devices and their applications for the rapid detection of food toxic substance, environmental pollutants and analytes of clinical interest is provided in this review.

**Keywords:** Biosensors; lipid membranes; potentiometry; graphene electrodes

## 1. Introduction

A biosensor is an instrument that analyses a sample and provides the chemical concentration and composition of the sample. A chemical sensor consists by two parts: A biological element that chemically recognizes the unknown compound and provides this chemical information in a physical unit that transforms the signal into a measurable electrical, optical, or piezoelectric signal.(eg. voltage, current, absorbance, etc).

The advantages of biosensors as compared to the classical analytical devices (eg. liquid and gas chromatography, etc) areas follows: faster response times, much higher throughput of samples, smaller size and can be used for in the field measurements. It is also cheaper and does not require training of personnel.

Chemosensors are similar to biosensors but are composed from a synthetically prepared molecule instead of a biological element. They recognize small molecules or metal ions by binding to them. The synthesis, design, and applications of chemosensors are of special interest for the detection of these analytes. The present review provides applications of chemosensors that have not been well covered elsewhere, for example the design and synthesis of calixarenes that have been used to rapidly determine carbamates [1] and naphthalene acetic acid (NAA) [2].

Nanobiosensors are based on the merging of nanotechnology with biosensors. Such materials include graphene, carbon nanotubes and nanowires, etc. Metal based nanoparticles are also excellent hosts for the contruction of electrical and optical devices that can be applied to detect nucleic acid sequences. Various nanomaterials have been explored and their properties were analyzed for possible applications in biosensors. The research in nanobiosensor technology has prompted the construction of novel devices and their number increases in an exponential rate.

The present review paper deals with the fabrication of biosensing devices that are composed from lipid membranes on graphene electrodes and have been utilized and applied to rapidly detect food toxic substances, environmental pollutants and analytes of clinical interest.. These devices are composed from a polymerized lipid films on graphene electrodes. The construction of these devices and their applications in the rapid detection of the above analytes are described in the present paper. Figure 1 provides a schematic of a lipid based biosensor on a grphene electrode that has been used for the potentiometric detection of urea

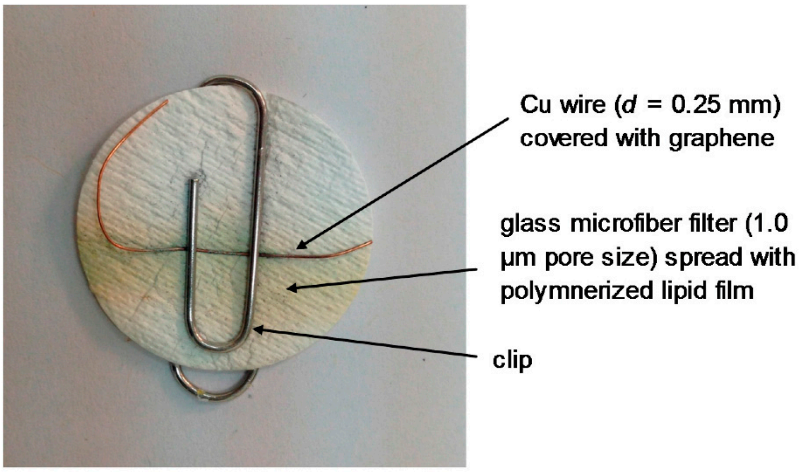


**Figure 1.** Schematic of a lipid membrane based biosensor on graphene electrode. This device was used for the potentiometric determination of urea (reprinted from reference 3 with permission).

## 2. Polymer lipid membranes

Stabilized in electrolyte solution lipid film based biosensors were first constructed by Nikolelis group [4] by using glass fiber filters to support and stabilize the lipid membrane. However, these membranes were not stable outside an electrolyte solution in the air. Later on, lipid polymer membranes supported on glass fiber were shown to be stable in air [5]. Recently, the deposition of these polymer lipid films on graphene electrode [3] has shown to offer many advantages such as increased sensitivity and selectivity, rapid response times and provided biosensors for urea for remote sensing.

Biosensors have been developed during the last 40 years and a pioneer in this field of science was Antony Turner, Prof. Emeritus at Linköping University. Lipid membrane biosensors appeared in 1990 with the pioneer work of Ulrich J. Krull and Dimitrios P. Nikolelis in University of Toronto and Athens; Prof. Tibor Hianik also pioneer the work of lipid membrane based biosensors focused mainly on metal supported lipid films. Recently nanotechnology has offered a route to prepare nanosensors based on lipid membranes by using graphene electrodes.

Biosensors that are based on lipid membranes offer an alternative to the standard analytical methods (i.e., chromatography or mass spectroscopy) such as rapid response times, small size, are not time consuming, they have a low cost, they can be portable for uses in the field and most importantly they are biocompatible. Recent advances in the construction of stabilized in the air lipid films has allowed their applications for the detection of a wide range of compounds that are toxic in foods or are of environmental or clinical interest.

The design, construction and storage of polymer lipid membranes on microporous filtering media such as glass fibers has been described extensively in one of our previous papers [5]. This paper has examined the simplicity for membrane construction and how stable are these lipid membranes after they are stored in the air so that they can be used a large number of times specially in a repetitive way. The preparation technique of the polymer lipid membranes has a standard route and is as follows, 5 mg of phosphatidyl choline (PC) is stirred in a small vial with 0.070 ml of methacrylic acid, 0.8 ml of ethylene glycol dimethacrylate, 8 mg of 2,2′-azobis-(2-methylpropionitrile)

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and 1.0 ml of acetonitrile. The roles of methacrylic acid, ethylene glycol dimethacrylate and 2,2′-azobis-(2-methylpropionitrile) (AIBN) were explained in one of our previous paper [5]. Nitrogen passed through this mixture by sparging for ca. 1 min, then was sonicated for ½ hour and stored in the refrigerator. In order to prepare stabilized polymer lipid membranes, 0.15 ml of this mixture was injected on the surface of a microfiber filter and was then irradiated with a UV deuterium lamp for 4 hours. This polymer lipid film can directly be used to construct devices based on lipid membranes.

These stabilized polymeric lipid membranes based biosensors can be used as excellent host matrices for to maintain the activity of a biological compound and used for the transduction of the activity of a wide number of biological active compounds such as enzymes, antibodies and artificial or natural receptors [5]. These devices are stable in air for more than one month and provide a response to a wide range of compound/ analytes [6-8]. The advantages of polymerization by irradiation by using a UV deuterium lampI instead of heating at 60 $^0$C for 4 hours has been described in one of our previous papers [9] .

In this paper [9], the UV irradiation was used instead of heating the mixture at 60 $^0$C because this process retains the activity of the enzyme, antibody or receptor, heating may deactivate these biological "receptors". This method to prepare stable biosensors based on lipid membranes by heating at 60 $^0$C was empirical; on the other hand, the mechanism of polymerization was never investigated. In this paper [9], the preparation technique of stable lipid films was explored by using physicochemical methods i.e. Raman and IR spectrophotometry, differential scanning calorimetry (DSC) and scanning electron microscopy (SEM) experiments The results of Raman and IR have shown that the polymerization kinetics finishes after 4 h. The polymerization mechanism and generation of the signal was further on explored by Raman and IR spectroscopy, micro-Raman spectroscopy, DSC and SEM experiments [2,3,9,10]. These membranes were stable for storage in air for a period of longer than two months.

Through this route the practical use of biosensors based on lipid membranes was possible, because the technique has allowed incorporation of natural ion-channels in these devices and eventually will permit these devices to be commercialized.

This procedure by irridation with a UV light has shown to retain the activity of acetylcholinesterase for the detection of carbamates [9]; heating at 60 $^0$Cmay would deactivated the activity. A very important criteria to for practical applications of lipid membrane based biosensors is the ability to store these membranes at temperatures of 25 $^0$C for long period of time. A number of practical characteristics of these lipid membrane based biosensors such as stability at ambient temperatures, reproducibility and reusability have been shown in their analytical applications in our recent papers [9-12].

## 3. Polymer Lipid membranes on Graphene electrode

We recently have reported a method on how to construct devices based on polymeric lipid films incorporated in a graphene electrode [3} This technique is as follows: a homogeneous dispersion (ca. 0.4 mg/mL) of graphene in a solvent N-methyl-pyrrolidone (NMP) was sonicated for ca. 180 h and then was centrifugated at 700 rpm for 2 h [21]. the prolong sonication time is requisite in order to reduce the size of the flakes which is required in many applications.

At this stage, this suspension was deposited on a copper wire (with d=0.25 mm) which was placed on a glass fiber filter and the organic solvent was evaporated by a fan heater. The use of the wire was to help the connection in order to provide the voltage as the output of the signal.

The "receptor" molecules were incorporated in the lipid films at the stage of polymerization by spreading 10 mL of a "receptor" suspension onto the polymerization mixture (for instance, in order to prepare devices for the detection of cholera toxin, 10 mL of the ganglioside (which acts as a receptor in this case) suspension was mixed with 0.15 mL of the polymerization mixture using a microsyringe at the surface of the microfiber filter. The graphene electrodes with incorporated lipid membranes are stored at 4 $^0$C when not in use for periods of more than 3 months..

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The construction of the potentiometric biosensor concluded after the encapsulation of the filter-supported polymer lipid membrane onto the copper wire which contained the graphene nanosheets (Figure 2).

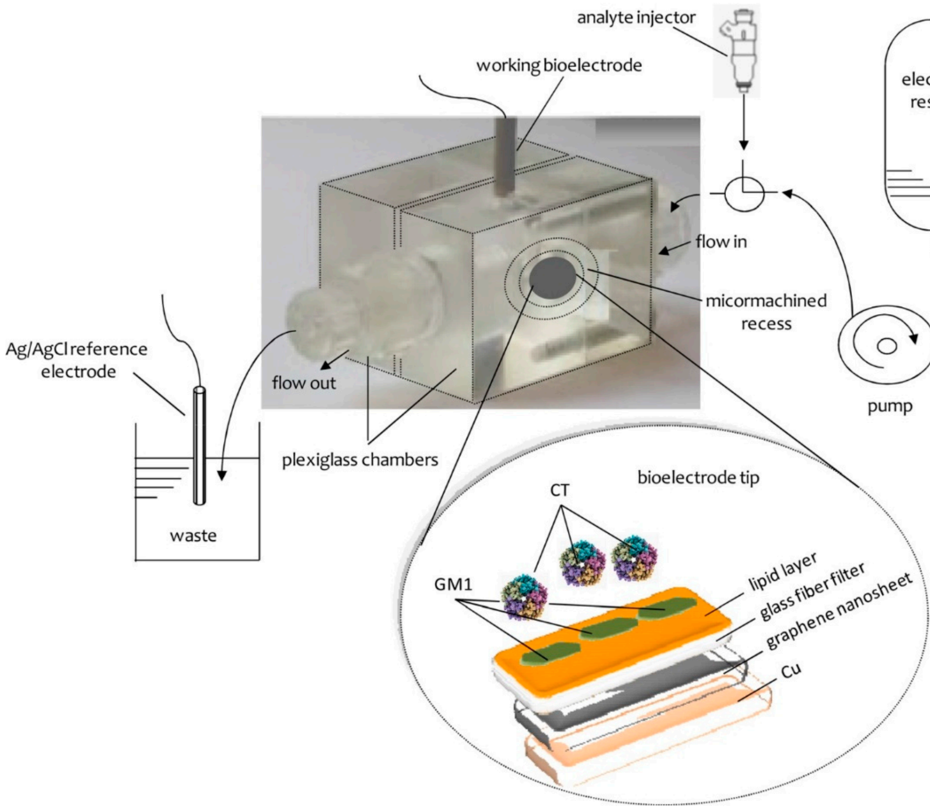


**Figure 2.** A simplified scheme of the experimental device and the bioelectrode edge surface .(Reprinted from ref. 11).

**4. Potentiometric applications on biosensors based on lipid membranes on graphene electrodes**

An article was written in the literature which describes a potentiometric urea lipid membrane based nanosensor incorporated on graphene [3]. The investigation of the structure of graphene nanosheets was made using atomic force microscopy (AFM) and transmission electron microscopy (TEM) measurements. UV-Vis and Fourrier transform IR (FTIR) spectrophotometry were used to examine the pre- and postconjugated surfaces of graphene nanosheets. This potentiometric urea nanosensor had a good reproducibility, reusability, selectivity, fast response times (~4 s), long shelf life and high sensitivity having a slope of ca. 70 mV/decade in the concentration range of urea between $1\times10^{-6}$ M to $1\times10^{-3}$ M.

A cholesterol potentiometric nanosensor has also appeared in the literature by fabrication using the immobilization techniques of stable polymer lipid film which was deposited on graphene [12]. This nanosensor used the enzyme cholesterol oxidase to retain a response towards cholesterol. This device has shown a good reproducibility, high selectivity and excellent sensing capability having a linear slope curve of ~64 mV per decade. Given that there is a biocompatibility between lipid films and human biofluids (eg., serum and urine) this gives the possibility to use this device for human blood samples and other biological fluids.

An article that provides the construction of a potentiometric D-dimer nanosensor on graphene using polymer lipid membranes has appeared in the literature [13]. This graphene biosensor was applied to construct a selective and sensitive immunosensor for the determination of D-dimer; this was accomplished by using as "receptor" the mouse anti human D-dimer antibody which was immobilized on stabilized polymer lipid membranes on graphene bioelectrode. The range of response towards D-dimer concentrations was between $10^{-6}$ μg/L to $10^{-3}$ μg/L and the response times were ca. 15 s. This potentiometric D-dimer device can be constructed very easily and has a good

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reproducibility, reusability, selectivity, rapid response times, long shelf life and high sensitivity with a linear logarithmic slope of ca. 59 mV/decade in the D-dimer concentration range from $10^{-6}$ µg/L to $10^{-3}$ µg/L.

A carbofuran chemosensor based on graphene electrode using lipid membranes has appeared in the literature for the determination of carbofuran in real samples of fruits and vegetables [1]. The graphene electrode was used as a basis to developing a chemosensor to determine carbofuran very sensitive and selectively using the immobilitation technique of an artificial receptor deposited onto stable lipid membranes. The method of preparation of this receptor was described in this paper [1] and the technique included altering the hydroxyl groups of resorcin[4]arene in phosphoryl groups. The chemosensor has a response the mM concentration range of carbofuran and has rapid response times (ca. 20 s). This potentiometric carbofuran chemosensor is constructed easily and is very reproducible and sensitive; its response times are very short (20 s), exhibits, a long period of stability having logarithmic concentration response with a slope of. 59 mV/decade in the range between $10^{-6}$ to $10^{-3}$ M.

A potentiometric miniature cholera toxin device which was based on a graphene electrode with deposited lipid membranes has appeared in the literature [14]. Ganglioside GM1 (which is the natural receptor for cholera toxin) was mixed with the lipid mixture prior to polymerization. This device had a good selectivity and sensitivity with nanomolar detection limits and rapid response times (ca. 5 min). The nanosensor was easily constructed and shown excellent characteristics (i.e., reproducibility, reusability, selectivity, long shelf life). The slope was ca. 60 mV/decade over a logarithmic cholera toxin concentration. The method applied for the determination of this toxin in water samples of lakes.

A naphthalene acetic acid (NAA) potentiometric device using the described graphene electrode in which the lipid membrane transducer was deposited has been described in the literature [2]. The receptor (auxin-binding protein 1) was placed in the lipid mixture prior to polymerization. The selectivity and sensitivity of response were very good, the detection limits were in the mM range and the response times were ca. 5 minutes. The device can be constructed very easily and shows excellent reproducible results and can be reused many times. The shelf life was very long and the slope of the potentiometric nanosensor was 56 mV/decade of NAA concentration. An evaluation/ validation of this device was made by using spiked fruits and vegetables.

A saxitoxin nanosensor based on graphene electrodes with deposited lipid membrane and in mixture with anti-STX (the natural saxitoxin receptor) was described in the literature [15]. Saxitoxin was selectively determined in concentrations between of $1 \times 10^{-9}$ M to $1 \times 10^{-6}$ M, the response times were between. 5–20 min, and the detection limit was 1 nM. This nanosensor can be construct,ed very easily and shows a good reproducibility and selectivity. This device can be used many times, it shows a stability at ambient temperatures and the shelf life is long. The slope of the electrode is. 60 mV/decade of saxitoxin. The method was applied in real samples (i.e., validated in lake waters and shellfish samples). The present technology can be adapted to detecting other toxins and can be a weapon against bioterrorism.

## 5. Conclusions

This review article deals with construction of biosensors based on polymer lipid membranes on graphene electrode and their potentiometric applications. These applications include the rapid detection of a wide range of toxic substances in foods, environmental pollutants and compounds of biomedical attention. These biosensors have shown adequate selectivity, sensitivity, reproducibility, rapid response times, they are easy to construct. The person who uses these devices can be non-skilled and these nanosensors can be used for in the field measurements. Research is now directed to the use of nanotechnological advances to construct nanosensors with even more improved characteristics that could be portable and used for the rapid determination of these analytes.

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