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Lipid Membrane Based Biosensors

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Abstract: The exploitation of lipid membranes in biosensors has provided the ability to reconstitute a considerable part of their functionality to detect trace of food toxicants and environmental pollutants. Nanotechnology enabled sensor miniaturization and extended the range of biological moieties that could be immobilized within a lipid bilayer device. This chapter reviews recent progress in biosensor technologies based on lipid membranes suitable for environmental applications and food quality monitoring. Numerous biosensing applications are presented, putting emphasis on novel systems, new sensing techniques and nanotechnology-based transduction schemes. The range of analytes that can be currently detected include, insecticides, pesticides, herbicides, metals, toxins, antibiotics, microorganisms, hormones, dioxins, etc. Technology limitations and future prospects are discussed, focused on the evaluation/ validation and eventually commercialization of the proposed sensors.

Keywords: biosensors, enzyme-based systems, receptor-based systems, toxins, food analysis, environmental monitoring, nanotechnology

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1. Introduction

Biosensors, in general, translate a chemical or biochemical interaction into a signal, eg., voltage, current, absorbance, etc. The sector is very dynamic, continuously evolving and well-established, almost in all continents, with remarkable infrastructure and human potential. To no surprise, most research teams were fast to adopt nano-tools, processes and concepts in order to solve their technology problems and optimize their product. Yet, size reduction to nano-dimensions is neither straightforward nor problem-free. The fabrication of sensor sub-systems or materials with nanometers sizes may be adequately addressed with nano-manufacturing strategies (e.g., self-assembly or 3D printing), but the efficient communication between these sub-systems might prove to be tricky. Biosensors have a large number of applications in food analysis and environmental monitoring and provide distinct advantages as compared to liquid and gas chromatographic techniques such as fast response times, portability, high sensitivity and selectivity, very small preparation of sample, etc. There is a clear difference between the multiple-use and single-shot because the latter characterizes the devices that are used only for one test.

Nanosensing currently involves many research areas, of which the most important are the field of nano-material-based bio- and chemical sensors. The use of nanomaterials has harmonized the scale between biological species and transduction platforms, thus resulting in the development of devices with higher rates of information flow. The range of strategies, architectures and materials for biomedical sensing, for example increased drastically to include non-enzymatic catalysis schemes and enzyme

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wiring. Therefore, the number of affordable devices has increased tremendously and have been integrated into systems for market applications. These applications include a large number of food toxicants and environmental pollutants, such as cholera toxin, aflatoxin M1 and B1, saxitoxin, carbamates, arochlor 1242, hydrazines, naphthalene acetic acid (NAA), doping materials (such as dopamine, adrenaline and ephedrine), urea, uric acid, etc.

Since Mueller's et al. work on bilayer lipid membranes (BLMs) [1], the number of biosensor devices based on lipid films for applications in food toxicants detection or environmental pollutants monitoring has tremendously increased. However, the so called "black" lipid films produced were very fragile and were prone to electrical and mechanical breakage and were not stable outside an electrolyte solution. This has prohibited their practical applications. Recent advances in the preparation of stabilized lipid bilayer have resulted in lipid membrane based devices for the detection of a large diversion of toxicants and pollutants in real samples. Lipid membranes based biosensors represent an appropriate biocompatible structure with rapid response times, high sensitivity and selectivity, small size, portability, and offer many advantages compared with the bulky analytical instrumentation such as liquid chromatographic units. The new generation of stabilized lipid membrane nanosensors has the potential to develop site-specific monitors with respect to analytical performance, operational stability and response .

This work reviews the devices based on lipid films that were explored for applications in various fields of science such as for biomedical applications, food analysis and environmental monitoring. The chapter

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provides novel reports on the design and microfabrication of prototype lipid membrane nanosensing devices for the rapid in the field detection of food toxicants, environmental pollutants, for biomedical applications and the challenges that lie ahead.

2. **Methods for preparation biosensors based on lipid films**

Over the last two decades, a variety of techniques have been proposed for the construction of stabilized lipid membranes that are not susceptible to electrical or mechanical failure. Most of these techniques provide lipid membranes that are stable enough for practical applications, whereas their less than 1 μm size can describe the resultant devices as nanosensors. These biosensors have been used for electrochemical experimentation and belong therefore in electrochemical biosensors. An exception is the development of stabilized polymerized lipid films on a filter paper that switch on and off their fluorescence and therefore belong to optical biosensors. Below we provide an overview of the most common techniques for the preparation of mini- or nano-biosensors based on lipid membranes.

2.1. Metal supported lipid membranes

Tien and Salamon [2] proposed a simple and reliable technique for the preparation of stabilized bilayer lipid membrane (sBLM) using the freshly cut tip of a Teflon coated metallic wire and taking advantage of the interaction between the amphiphatic lipid molecule with the nascent metallic surface. The procedure required the cutting of a Teflon-coated stainless steel metal wire (0.1-0.5 mm in diameter) while it was immersed in lipid solution in chloroform

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using a miniature guillotine. The tip of the wire is coated with lipid solution that turns into a lipid film; when transferred in electrolyte (0.1 M KCl), the lipid film spontaneously thins into a self-assembled lipid bilayer membrane (sBLM). A more recent and easier version of this approach is shown in Figure 1.

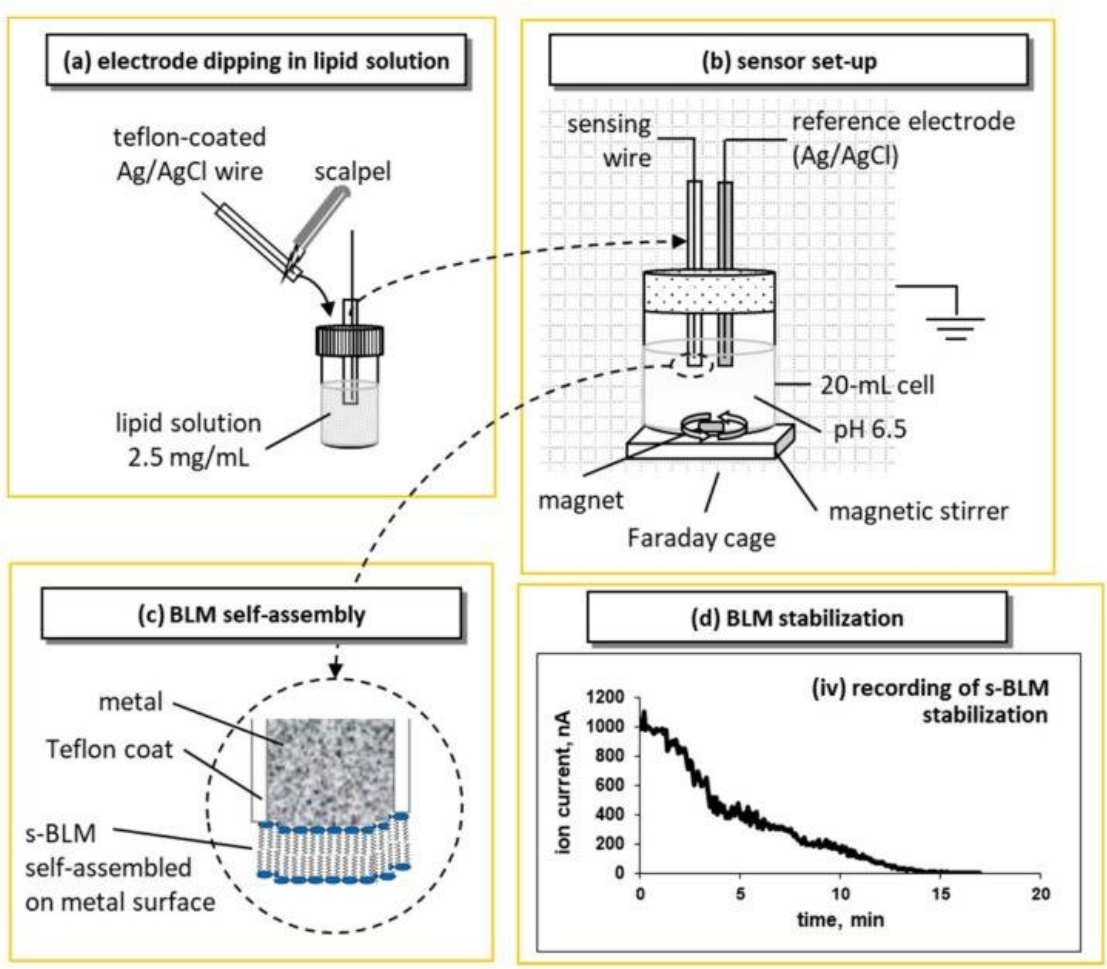

Figure 1

Representation of the device setup, and the lipid self-assembly process for the preparation of metal supported sBLMs (not drawn to scale) based on the original idea of [2]: (**a**) the tip of the sensing electrode is cut with a scalpel and immediately dipped in lipid solution before transferred in the electrolyte solution. (**b**) The electrochemical setup uses a two-electrode configuration

(i.e., the sensing electrode and a Ag/AgCl reference electrode) in a magnetically stirred 20-mL cell. The set-up is placed in a grounded Faraday cage; 25 mV external DC potential is applied between the electrodes; the ionic current through the BLM is measured with a digital electrometer. (**c**) Upon immersion, the lipid drop attached to the tip of the wire is self-assembled into a bilayer; one layer is adsorbed on the metal surface and the other faces the electrolyte. (**d**) Recording of the ion current decrease during the self-assembly process. The recording starts with the immersion of the sensing electrode in the electrolyte solution [reprinted from ref. 3].

sBLMs have been fully characterized [2,4,5]. Device stabilization depends upon the diameter of the wires and the organic solvent used [4,5]. Wires of 0.25 mm diameter should be avoided due to increased sensor noise; the use of decane as a solvent should be also avoided as it enhances the tendency for “black” lipid membranes that do not provide reproducible results. Hexane solvent and silver wires with diameters of 0.5 and 1.0 mm provide BLMs that are mechanically and electrically stable for over 48 hours.

Some attempts have been made to model the potential profile across sBLMs and the structure of the lipid layer that faces the metal surface. A plausible theory involves the interactions of oxygen atoms of the phosphate groups of the lipid headgroups with the silver ions in the metal lattice [6,7]. Transmembrane ion mobility can be attributed to the presence of chloride ions at the space between the metal and the inner lipid layer. There could be two sources for chloride ions: through the lipid film during the initial BLM stabilization process and through the partial wire insulation [4,5]. Chloride would react with the silver metal to form silver chloride [4,5]. Potentiometric

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experiments (against a Ag/AgCl reference electrode) [5] showed only small voltages (relative to a silver wire against a Ag/AgCl reference electrode) when the BLM had been removed using an organic solvent rinse. These results suggest that (a) the metal surface is possibly coated with a thin layer of silver chloride and (b) the lipid membrane actually consists of a network of nm-sized BLMs [8].

2.2. Stabilized lipid films formed on a glass fiber filter

The preparation of stabilized lipid membranes supported on ultrafiltration glass fiber filters has been reported in the literature [9] and allowed several practical applications in real samples, such as the determination of aflatoxin M_1 in milk and milk preparations [10]. The lipid membrane is formed on a microporous filter glass fiber disk (namely GF/F glass microfiber, 0.9 cm in diameter and 0.7 μm nominal pore size; Whatman Scientific Ltd., Kent, U.K.) [9,10].

The experimental set up which was used for the formation of these stabilized BLMs consisted of two plexiglas chambers separated by a thin plastic partition (10 μm thick Saran-Wrap film). The plastic partition was folded in half and a 0.32 mm hole was punched through the double layer of the plastic film. A microporous glass GF/F microfiber disk was placed between the two plastic layers, centered on the 0.32 mm hole. The partition containing the filter was then clamped between the two plexiglas chambers. One of the chambers had a circular shape (diameter 1.0 cm and depth 0.5 cm); this chamber was connected with a carrier electrolyte flow system. An Ag/AgCl reference electrode was immersed in the waste of the carrier electrolyte solution. The second chamber was cylindrical and had its longitudinal axis

perpendicular to the flow of the carrier solution. The upper hole of this cell was circular (surface area of about 0.2 cm^2) and the lower was elliptical (with diameters 0.5 and 1.4 cm parallel and vertical to the flow of the carrier electrolyte solution, respectively). The lower hole faced the opposing cell. An Ag/AgCl reference electrode was positioned at the center of the cylindrical cell. An external voltage of 25 or 50 mV d.c. was applied between the two reference electrodes. A Keithley digital electrometer was used as a current-to-voltage converter. A peristaltic pump was used for the flow of the carrier electrolyte. Sample injections were made with a Hamilton repeating dispenser. The electrochemical cell and electronic equipment were isolated in a grounded Faraday cage. A simple scheme of the apparatus used is presented in Fig. 2. Details for the procedure followed for the formation of the stabilized BLMs can be found in [9,10], Briefly, a drop of the lipid solution (ca. 10 μL) was added to the electrolyte surface in the cylindrical cell near the plastic partition. The level of the electrolyte solution was dropped below the 0.32 mm hole and then raised again within a few seconds. The formation of the BLMs could be immediately verified by the ion current magnitude or/and by the electrochemical characterization using gramicidin D.

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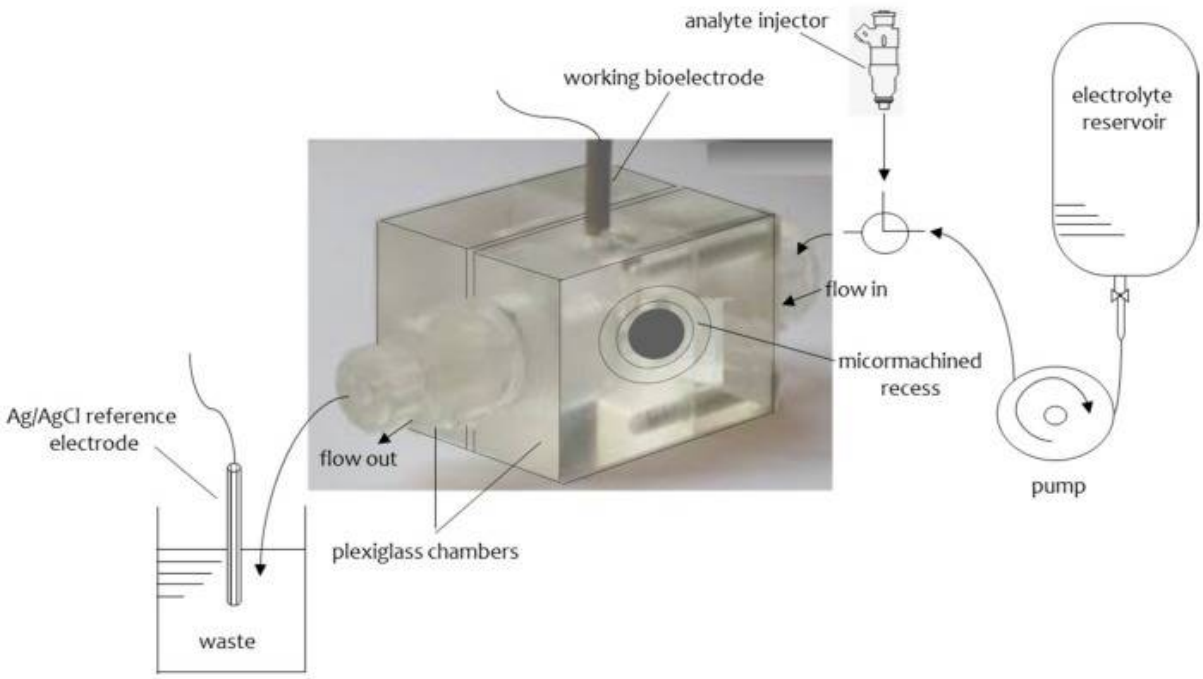

Figure 2

The experimental set-up used for the formation of stabilized lipid films on glass fiber filters; the micromachined chambers are separated by a thin (12.5 μm thick) polyvinylidene chloride wrap and enclose the microfiber disk. For more details, see text.[from ref. 3]

2.3. Polymer-supported bilayer lipid membranes

The preparation of polymer stabilized has been recently described in literature. UV irradiation is much preferred [11,12], mainly because enzymes retain their activity whereas heating the lipid mixture to 60 °C deactivates them. Physicochemical methods, such as DSC, IR or Raman spectrophotometry, indicated that the polymerization process requires 4 hours. This method facilitates a reliable and reproducible incorporation of even heat-sensitive or extremely fragile “receptor” molecules in the lipid films

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while the devices developed are stable outside the solution, i.e., in the air, for more than 48 h.

The preparation of these stabilized lipid films was as follows [11,12]: 0.8 mL of a mixture containing 4% w/v egg phosphatidylcholine (PC) in n-hexane were mixed with 0.07 mL of methacrylic acid, 0.8 mL of ethylene glycol dimethacrylate, 8 mg of 2,2'-azobis-(2-methylpropionitrile) and 1.0 mL of acetonitrile; n-hexane was used because it evaporates quickly leaving and the films solvent-free. The mixture was sparged with nitrogen (1 min) and then sonicated for 30 min. An aliquot of 0.15 mL of this mixture was spread on a microfilter (microporous glass GF/F microfiber disk with a diameter of ca. 0.9 cm and nominal pore size of 0.7 μm) and irradiated using a UV deuterium lamp. Raman spectrometry and differential scanning calorimetry (DSC) were used to monitor the kinetics of the polymerization process. The measuring set up was similar to that presented in Fig. 2. These membranes were stable in storage in air for repetitive uses.

2.4. Polymer lipid films supported on graphene microelectrodes

Graphene nanomaterials have been extensively experimented upon in an effort to seize their unique physicochemical properties: good sensing ability, excellent mechanical and electrical properties, enhanced thermal stability, large surface-to-volume ratio, improved biocompatibility, high electron-transfer rates, limited toxicity and bio-safety. Their implementation in electrochemical biosensing is quite beneficial as the large surface-area-to-volume ratio enables device size reduction and increases speed of

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response; the former might be proven critical for commercialization whereas the latter allows for lower detectabilities while adequately handling biofouling problems. Several nanobiosensors have been described using enzymes and antibodies. A reliable system presented involves stabilized lipid films wrapped around a copper wire containing graphene nanosheets [13,14]. These nanosensors have been implemented in the rapid detection of food toxicants, environmental pollutants and toxins in real samples, such insecticides [14], naphthalene acetic acid [15], cholera toxin [16], and saxitoxin [17].

The preparation of graphene microelectrodes was as follows [13-17]: using *N*-methyl-pyrrolidone (NMP) and mild sonication for 180 hours followed by centrifugation at 700 rpm for 2 h yielded a homogeneous graphene dispersion (~0.4 mg/mL). The graphene suspension has been poured onto a copper wire (0.25 mm in diameter) mounted on a glass fiber filter; the organic solvent evaporated under a fan heater. The copper wire established the connection for the extraction of voltage signals for the calibration curve. Following a simple protocol, the drop wise dispersion of graphene suspended in NMP solution has been utilized to scatter the graphene nanosheets on the copper wire. The extended sonication time results in a good fraction of monolayer sheets but with smaller lateral sizes.

The procedure of construction of these devices is in brief as follows [13-17]: The stabilized lipid films were prepared by polymerization, as described in [11,12]: 0.15 mL of a mixture containing 5 mg of a lipid powder composed of 35 (w/w) dipalmitoyl phosphatidic acid (DPPA) (and 1.75 mg) 65

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(w/w) of dipalmitoyl phosphatidyl choline (DPPC) (3.25 mg) were mixed with 0.070 mL of methacrylic acid, 0.8 mL of ethylene glycol dimethacrylate, 8 mg of 2,2′-azobis-(2-methylpropionitrile) and 1.0 mL of acetonitrile. Phosphatidyl choline (PC) is a more common lipid but because it can be oxidized by air and does not provide reproducible results it has been replaced by DPPC. The mixture was spumed with nitrogen for about 1 min and sonicated for 30 min. This mixture could be stored in the refrigerator. For the preparation of the stabilized lipid films, 0.15 mL of this mixture was spread on the glass filer microfilter and irradiated. Raman spectrometry was used to monitor the kinetics of the polymerization process [11,12].

The enzyme, antibody or receptor ("receptor") was incorporated in these BLMs prior to polymerization by spreading 15 µL of the "receptor" suspension over the polymerization mixture. The filter-supported polymerized lipid film was then mounted onto the copper wire containing graphene nanosheets to produce the nano-device.

3. Applications of lipid film based biosensors in food analysis and environmental monitoring

The stabilized supported lipid membranes biosensors were used for the detection of pesticides through flow injection analysis (FIA) [18]. The typical pesticide studied was carbofuran. The analysis method was based on the degree of inhibition and reactivation of enzyme upon substrate injections.

Carbofuran could be determined within the concentration range 10^{-7} - 10^{-9} M. Interference studies used proteins and lipids that can be typically found in foods. The results have shown no interferences from these compounds. The sensor has been implemented in various real food samples, such as fruits, vegetables and dairy products. The recovery ranged between 96% and 106%, indicating no interferences from the sample matrix.

A paper was reported in the literature using a synthetic “receptor” immobilized on supported lipid films on glass fiber filters. The supported lipid films were modified by calixarenes and proved adequate for the sensitive and rapid determination of various insecticides in fruit and vegetable samples [19]. Other devices similarly developed include a disposable chemosensor for the selective and fast detection of food hormones such as naphthalene acetic acid in fruits and vegetables [20] and a sensor for the detection of zinc in water [21].

A potentiometric urea lipid film based minisensor on graphene nanosheets has been recently reported in the literature [22]. The structural characteristics of graphene nanosheets have been extensively studied using atomic force microscopy (AFM) and transmission electron microscopy (TEM). The pre- and post-conjugated surfaces of graphene nanosheets has been studied with UV-Vis and Fourrier transform IR (FTIR) spectroscopy. A potentiometric urea biosensor has been developed (Figure 3) exhibiting good reproducibility and reusability, high selectivity and fast response times (on the order of 4 s), long shelf life under storage and a high sensitivity of ca. 70 mV/decade over the urea logarithmic concentration range from 1×10^{-6} M to 1×10^{-3} M.

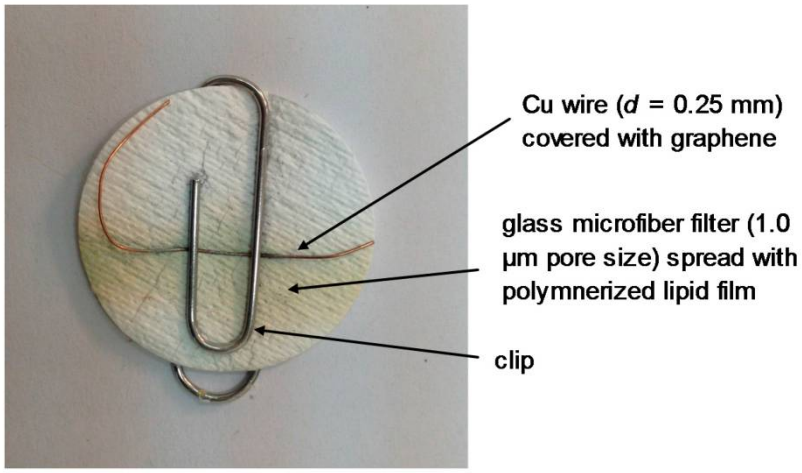

Figure 3. Photo of the lipid membrane based biosensor on graphene electrode developed for the potentiometric determination of urea (reprinted from reference 23).

A nanosensor for naphthalene acetic acid (NAA) was based on stabilized lipid films supported on a methacrylate polymer on a glass fiber filter with incorporated auxin-binding protein 1 receptor [24]; the sensor has been tested in reals samples of fuits and vegetables. Using a FIA system, NAA was injected into the flowing carrier electrolyte solution and the flow stopped until an ion current transient (peak) was obtained; the height of the peak could be correlated to the concentration of the hormone in the sample. A micromolar detection limit could be obtained. The analysis time was about 5 min. The effect of interferences was studied using a wide range of compounds. The study indicated no interferences from these compounds at concentration levels usually found in real food samples. The sensor has been implemented for the detection of NAA in fruits and vegetables and the reproducibility obtained was quite satisfactory.

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A potentiometric carbofuran minisensor on graphene nanosheets with incorporated lipid membranes has been reported [25]. The graphene electrode was used to develop a carbofuran sensor using an artificial selective receptor (resorcin[4]arene receptor) on stable lipid films. The detection range was at the nanomolar levels, response times were ca. 20 s. The sensor was easy to construct and exhibited good reproducibility, reusability, selectivity, long shelf life and high electrode slope of ca. 59 mV/decade over the carbofuran logarithmic concentration range from 10^{-6} to 10^{-3} M.

An atrazine lipid membrane sensor has been also described with micromolar detection limit [26]. The interactions of atrazine with solventless bilayer lipid membranes (BLMs) were found to be electrochemically transduced by these films in the form of a transient current signal with duration of seconds and reproducibly appearing within 1 min after the membranes had been exposed to atrazine. The sensor could be optimized by the introduction pf 35% (w./w.) DPPA in the lipid mixture and calcium ions in the electrolyte solution; calcium induced alteration of the phase distribution of DPPA doped membranes, thus increasing signals manifold. Alternatively, doping PC membranes with platelet-activating factor (PAF; an ether analog of PC) provided similar results.

The flow injection analysis of mixtures of the triazine herbicides simazine, atrazine and propazine on PC/DPPA filter-supported BLMs has been described in the literature [27]. When a sample containing a mixture of these herbicides injected into the flowing electrolyte, a transient current signal with a duration of seconds reproducibly appeared in less than two min after injection. The magnitude of the peak heights was linearly related to the

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concentration of the herbicides, which could be determined at μM range. Repetitive cycles of injection of herbicides have shown no signal degradation during each cycle. The time of appearance of the transient signal was different for each triazine and increased to the order of simazine, atrazine and propazine, thus permitting the simultaneous detection and analysis of these triazines in mixtures.

A strategy was described in the literature that was based on monitoring of changes of ion current through a lipid film with immobilized DNA probes caused by interaction of these lipid membranes with hydrazine compounds [28]. A s-BLM that was consisted of egg PC was deposited on a silver metal electrode. The single stranded deoxyribonucleic acids used were thymidylic acid icosanucleotide terminated with a C-16 alkyl chain to assist incorporation into s-BLMs (dT_{20}-C_{16}), and deoxyadenylic acid icosanucleotide (dA_{20}). These s-BLMs with incorporated DNA interact with hydrazines, and it is possible to monitor ppb levels of hydrazine, methylhydrazine, dimethylhydrazine and phenylhydrazine. This BLM/DNA biosensor showed a highly sensitive, selective, fast, and portable biosensor for monitoring these environmentally and toxicologically significant compounds.

A paper appeared in the literature that describes the electrochemical interactions of cholera toxin with polymerized lipid films incorporated with ganglioside GM1 [29]. The analyte was injected into the flowing streams of a carrier electrolyte solution, the flow of the solution stopped for 5 min and an ion current transient was obtained. The magnitude of the signal could be correlated with the concentration of cholera toxin in the sample. The detection

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limits was 0.06 μM. Further work is directed to investigate the rapid detection of other toxins used in bioterrorism and uses this novel ultrathin film technology.

Switching to polymerized lipid membranes on graphene nanosheets, Ganglioside GM1 provided better results, i.e., response time of ca. 5 min, and detection limits of 1 nM [30]. The proposed sensor is easy to construct and exhibits good reproducibility, reusability, selectivity, long shelf life and sensitivity of 60 mV/decade of toxin concentration. The method was evaluated, implemented and validated in lake water samples. The sensor is currently adapted for the detection of other toxins.

A novel electrochemical biosensor based on a supported polymeric lipid membranes with immobilized Sheep anti-PCB antibody for the rapid determination of arochlor 1242 in flowing solution streams (FIA systems) has been described [31]. The antibody was immobilized in the lipid membrane during polymerization of the film; the injections of antigen were made into flowing streams of a carrier electrolyte solution. The experimentation was made in a stopped-flow mode; the lipid mixtures were composed of 15 % (w/w) PA and 85% of DPPC to provide only one and only single transient current signal with a peak height related to the concentration of the antigen. Lipid films that were composed of 35 % DPPA were used to investigate the regeneration of the active sites of antibody after complex formation; the results showed adequate regeneration with intensive washing with the carrier electrolyte solution. Repetitive cycles of injection of antigen have exhibited that the maximum number of cycles is ca. 5.

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A potentiometric saxitoxin minisensor based on graphene nanosheets with incorporated lipid films and immobilized anti-STX (which is the natural saxitoxin receptor) on stabilized lipid films was recently reported in the literature [32]. A good selectivity and sensitivity for the detection of saxitoxin, fast response times of ca. 5–20 min, and detection limits of 1 nM were observed. The sensor is easy to construct, it has good reproducibility, reusability, and selectivity, adequate storage stability and sensitivity (ca. 60 mV/decade over saxitoxin concentration). The method was evaluated and validated in lake water and shellfish samples. This sensor can be easily adapted for other toxins, as well.

An electrochemical biosensor that is suitable for the rapid and sensitive screening of the sweetener sucralose based on surface-stabilized bilayer lipid membranes (s-BLMs) composed of PC was recently described in the literature [33]. The interactions of sucralose with s-BLMs provided an ion current increase, that appeared within a few seconds after exposure of the membranes to the sweetener. Differential scanning calorimetry was used to investigate the mechanism of signal generation. The mechanism was found to be related to changes of the electrostatic fields of the lipid membrane. These studies have shown that there is an increase of the molecular area of the lipids at the membranes and a stabilization of a gel phase structure; this was due to adsorption of the sweetener in the membrane surface. The current signal increases were correlated to the concentration of sucralose in bulk solution in the μM concentration range. The present lipid film based biosensor has provided a rapid response (order of seconds) to alterations of sucralose concentration (5-50 μM) in the bulk solution. The electrochemical transduction

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of interactions of this sweetener with s-BLMs was evaluated by its determination in granulated sugar substitute products.

A method that reports the FIA of mixtures of the artificial sweeteners acesulfame-K, cyclamate, and saccharin using stabilized systems of filter-supported BLMs has been proposed [34]. A transient current with duration of seconds appeared in less than 1 min after exposure of the lipid membranes to the artificial sweeteners. The peak height of the signal could be linearly related to the concentration of artificial sweeteners, within a μM concentration range; 30 analyses could be performed before the sensor showed signs of signal degradation. The time of appearance of the signals was different for each artificial sweetener and increased in the order of cyclamic acid, acesulfame-K, and saccharin. This has allowed the simultaneous detection of these artificial sweeteners in mixtures. Interference studies indicated no interference from a large range of compounds commonly found in food samples. The method has been implemented in real food samples (i.e., artificial sweetener tablets, diet soft drinks, wines, and yogurts) that contain mixtures of artificial sweeteners. A comparison of results using this method and that of an Official Method of Analysis showed good agreement between the two methods.

Investigation of the transport phenomena through channels/pores it is very important for various biological, medical, and technical applications. The scope of a paper appeared in the literature is the development of nanofluidics for the creation of biosensors capable of detecting single molecules and manipulating them [35]. The detection of molecules was based on the measurement of the current through a channel when a molecule enters the

channel, which has a diameter comparable with the molecule size, the current reduces. In order to improve transport properties of such channels, their walls are often coated with a lipid bilayer, which behaves as two-dimensional liquid and thus is capable of supporting transport phenomena. Presently, this property of lipid membranes was utilized for the development of a technique for detecting and controlling transport of single-stranded DNA through channels formed by membrane cylinders with the luminal radii of 5–7 nm. It was demonstrated that in the conditions of small ion strength, the appearance of a DNA molecule inside such channel is accompanied by an increase of its ion conductivity and can be controlled by the polarity of the applied voltage. The peak height of the current increase permits to evaluate the number of DNA molecules inside the channels. It was also demonstrated that upon adsorption of DNA molecules on the lipid bilayer surface, the membrane cylinder behaves as a voltage-sensitive selective ion channel.

Biological membranes have been also studied. Eggshell, for example, was used for the immobilization of urease in a potentiometric urea device [36]. Eggshell was treated with polyethyleneimine (PEI) to gain polycation properties. Urease was adsorbed on the PEI treated eggshell membrane. A SEM study was conducted in order to investigate the changes in surface morphology and an FTIR study was carried out to observe the changes in IR spectra after immobilization of the enzyme. The biosensor exhibited a sigmoidal response for a urea concentration range from 0.5 to 10 mM. The response time was 120 s. A single membrane could be used for 270 reactions without loss of activity. The urease–eggshell membranes were stable for 2 months when stored in buffer even at room temperature.

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Bilayer lipid membranes (BLMs) can be also produced by polymers electrodeposited on a solid metallic support. Avidin–biotin interactions were employed for enzyme immobilization on the BLM surface. A BLM glucose biosensor based on glucose oxidase immobilized on a platinum support modified with several polymers [37] showed improved storage stability and better selectivity towards certain interfering electroactive species. Especially good results were obtained for a mediated system in which the BLM was formed on a Pt support covered with a layer of evaporated Nafion with incorporated ferrocene. The stable and sensitive response with minimized interference appears very promising for practical applications.

A chemiluminescence biosensor formed on a supported lipid layer incorporated with ganglioside GM1 was reported for cholera toxin. The planar supported lipid membrane was prepared as a biosensing interface via spontaneous spread of ganglioside-incorporated phospholipid vesicles on the octadecanethiol-coated gold surface [38]. The specific interaction of multivalent toxin by ganglioside GM1 molecules enabled the implementation of the sensor in a sandwiched format using a GM1 and horseradish peroxidase (HRP) functionalized liposome probe, where the presence of the toxin could be determined via the HRP-catalyzed enhanced chemiluminescence reaction. This approach offers several advantages over conventional strategies, especially as regards easy construction and renewal of the sensing interface, small background noise (due to limited non-specific adsorption of serum matrix constituents on the membrane), and effective immobilization of many biocatalytic amplifiers and recognition species via

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shared phospholipid reagents. The sensor could detect cholera toxin within the range of 1 pg mL^{-1} to 1 ng mL^{-1} and a detection limit of 0.8 pg mL^{-1}.

A work that reports a BLM-based nucleic acid biosensor supported by modified patch-clamp pipette electrode was developed for staphylococcus enterotoxins B (SEB) gene [39]. Hydrophobic dodecane tail (C_{12}) modified 18 bp single-stranded DNA (ssDNA) probe was immobilized on the membrane to yield linear correlations. The sensor was constructed by selecting the ssDNA probe as the signal sensing element with the concentration of 273.65 ng/mL. The electrochemical performance of the biosensor for SEB detection was studied, showing a linear relationship between the current and ln(concentration) from 20 to 5000 ng/mL, with a detection limit of 20 ng/mL. In addition, the biosensor has shown a specific response to SEB gene and no significant current alteration in the absence of the SEB gene. AFM images were used to evaluate the microstructure of BLMs, ssDNA immobilized on BLMs and BLMs after hybridization. The sensor could be developed in a reliable tool for the detection of *Staphylococcus aureus,* which produce SEB.

A nanostructured electrochemical biosensor was developed for screening estrogenic substances using only the estrogen receptor (ER) [40]. ERs were immobilized in s-BLM modified with Au nanoparticles, and the properties of the modified electrodes were characterized by cyclic voltammetry and impedance spectroscopy. The results have shown that the biosensor was able to detect 17β-estradiol (the natural estrogen) with a linear correlation for the concentration range 5 - 150 ng/L and a detection limit of 1 ng/L. The biosensor could also detect other known xenoestrogens such as

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bisphenol A and 4-nonylphenol with adequate sensitivity. The reliability of the biosensor was good and the Au nanoparticles greatly enhanced the sensitivity and stability of the sensor. The sensor was implemented for screening the estrogenic activity of water samples and the results have been found to be in good agreement with those determined by MCF-7 cell proliferation assay.

4. Conclusions and future prospects

The present paper describes a variety of approaches and strategies to construct nanosensors based on lipid film technology and implement them for food and environmental analyses. The recent technological advances include the engineering of stabilized supported lipid film on graphene nanoelectrodes with an incorporated “receptor” of any kind, natural or artificial. These films remain stable in air and are suitable for the development of portable devices for in the field applications. The sensors exhibit detection limits in the nM concentration range. In effect, a portable unit that can be used for in-field and market applications might be developed in the near future.

The results have shown that a variety of lipid film based detectors can be reused after storage in air, even after few months, and can be reproducibly fabricated with simplicity and low cost. These nanosensors have fast response times and are easy to construct at quite lesser cost than chromatography-based instrumentation; they can be also used as rapid hand-held detectors complimentary to these methods for in-field and market measurements in foods and for environmental monitoring.

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The present review describes biosensors based on lipid film technology that can be used for the rapid detection of food toxicants and environmental pollutants such as toxins, carbamates, hormones, polycyclic aromatic hydrocarbons, etc and highlights their advantages which are high sensitivity and selectivity, rapid response times, portability, etc. It is of common sense that the use of nanotechnology to construct lipid membrane based biosensors will provide devices with even improved characteristics.

Author Contributions

All authors contributed equally to this work.

Conflicts of Interest

The authors declare no conflict of interest.

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