Advanced lipid based biosensors for food analysis

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Abstract

The investigation of lipid films for the construction of nanosensors has recently given the opportunity to manufacture devices to selectively determine a wide range of food toxicants. Biosensor miniaturization using recent advances in nanotechnology has given the opportunity to investigate novel techniques to immobilize a wide range of enzymes, antibodies and receptors within the lipid film. This chapter reviews novel revent platforms in nanobiosensors based on lipid membranes that are used in food chemistry to determine various food toxicants. Examples of applications are described with an emphasis on novel systems, sensing techniques and nanotechnology-based transduction schemes. The compounds that can be monitored are insecticides, pesticides, herbicides, metals, toxins, hormones, etc. Finally, limitations and future prospects are presented herein on the evaluation/validation and eventually commercialization of the proposed sensors.

1. Introduction

The reconstitution of lipid bilayers 50 years ago in vitro gradually established a lipid film technology and a set of instrumentation, yet highly

Advances in Food and Nutrition Research
ISSN 1043-4526
https://doi.org/10.1016/bs.afnr.2019.07.002

demanding in expertise when the extremely fragile lipid bilayer membrane was produced (freely suspended between two electrolyte interfaces) should remain intact for the duration of an experiment (Siontorou, Nikoleli, Nikolelis, & Karapetis, 2017). Today, lipid membrane technology provides much architecture in lipid membranes (bilayers, multilayers, mixed layers, branched, doped, nanodiscs, tethered, functionalized, etc.) that serves, a dual purpose: analytical detection (Mazur, Bally, Städler, & Chandrawati, 2017) and simulation studies (Su, Leitch, & Lipkowski, 2018). Both research fields benefit from the capability to reproduce some properties of biological membranes and package them into sensing platforms for biosensor devices or membrane interaction studies, respectively. Interestingly, the former field uses the term *artificial lipid membranes* (Mazur et al., 2017) while the latter exploits similar principles and methods to yield *biomembranes* or *membrane mimics* (Su et al., 2018).

The number of devices based on lipid film technology that were used to monitor food toxicants, environmental pollutants and compounds of clinical interest has increased during the last decade. During this period of time, a number of efforts to construct stable lipid film membranes were successful and this offered the opportunity to prepare devices to monitor food toxicants and environmental pollutants in real samples and in the field. The advantages of lipid membrane devices are summarized as follows: the membranes have an appropriate biocompatible structure with rapid response times, high sensitivity and selectivity, small size, portability, and provide advantages as compared to the bulky liquid chromatographic (LC) instrumentation; the size of the lipid film devices is much smaller and faster than those of the LC instruments. The new generation of stabilized lipid membrane nanosensors has the potentiality to construct site-specific analytical devices with respect to analytical performance, operational stability and response.

Lipid membrane technology minimizes the complex membrane processes to well-controlled and defined interactions between biological moieties, lipids and transducers (Fig. 1). Although the very nature of biological membrane still remains elusive (Gould, 2018), it is easy to isolate specific membrane properties for drug permeability (Ebert, Hannesschlaeger, Goss, & Pohl, 2018) or protein-lipid interaction studies (Vacek, Zatloukalova, & Novak, 2018) or even to control nano-processes on the lipid bilayer by changing macro-parameters (pH, temperature, ionic strength, lipid composition, etc.) (Nikoleli, Nikolelis, Evtugyn, & Hianik, 2016). Biological enzymes, antibodies, receptors, ligands, DNA, etc. can now be easily immobilized or embedded in the surface of lipid film

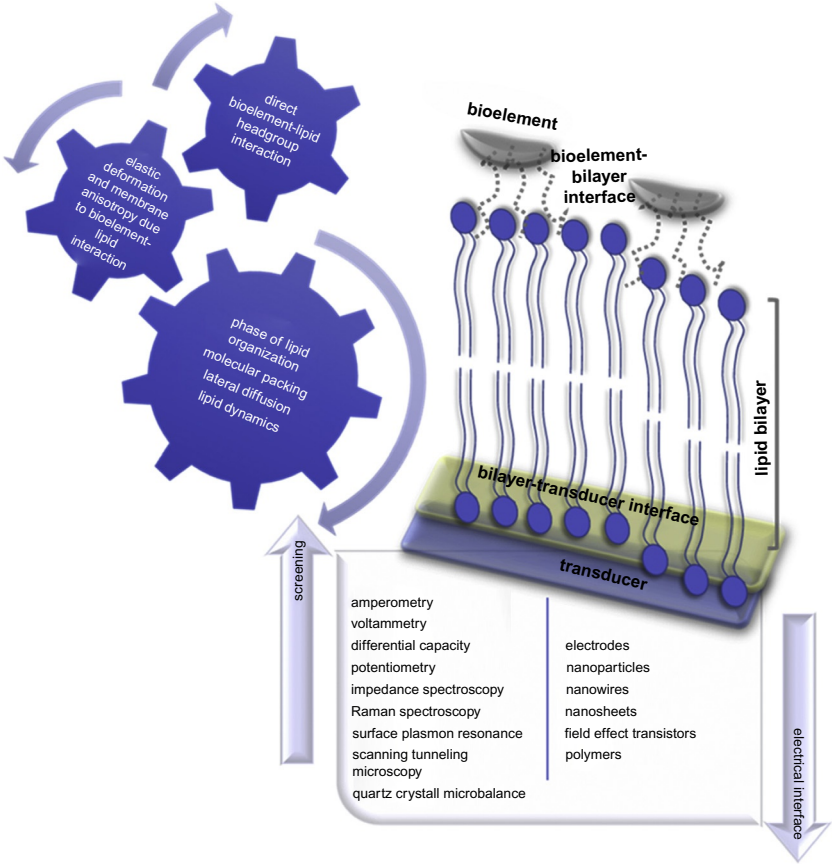

Fig. 1 Simplified scheme of the lipid membrane platform for biosensor development, illustrating, also, the critical physical chemistry that gears the biochemical information produced on the membrane surface for electrochemical transduction and the most common screening strategies employed. *From ref. Nikoleli, G.-P., Siontorou, C. G., Nikolelis M.-T., Bratakou, S., & Bendos, D. K. (2019). Recent lipid membrane-based biosensing platforms.* Applied Sciences, *9(9), 1745–1764.*

(Mazur et al., 2017; Vacek et al., 2018) by using novel recently developed techniques such as patterning (Raghunathan & Kenworthy, 2018) or surface printing (Kalyankar et al., 2006). The lipid membrane represents a biocompatible structure and offers an intrinsic signal transduction that is suitable for electrochemical experimentation; when the analyte is attached on or embedded in the lipid membrane, this affects the flux of ions through the membrane and can be readily detected as current alterations.

A quick look at the history of the field of lipid membrane technology reveals that the 1990s had witnessed an intense research towards stabilized

lipid membrane devices (Siontorou et al., 2017). These efforts have included a variety of construction techniques (Siontorou, 2015). The recent advances in nanotechnology have allowed to perform device engineering in order to simulate membrane energetics and provide platforms that address certain requirements for practical implementation:

- Adequate robustness for long-term storage and operation.
- Simple and non-skilled demanding reproducible fabrication.
- Stable membrane physical structure.
- Stable and reproducible membrane morphology, preferably defect-free to facilitate control for ion transport.
- Adequate hydration in the hydrophilic region in order to support protein-based interactions.
- Compatible coupling to the physical transducers.
- Fit-for-purpose detection strategy that will ensure optimal detection without catastrophic perturbations of the lipid organization.

Lipid membrane technology adsorbed rapidly the scientific and technological output of many fields in order to evolve into the diverse cluster of today (Fig. 2). This paper presents the electroanalytical aspects of artificial film based devices, the processes and tools that are widely used to construct membrane platforms, the current challenges and the future trends. What is expected in the future of the market availability of these lipid-based sensing devices is also within the scope of this chapter.

2. Methods for preparation of stabilized biosensors based on lipid films

During the last decade, the preparation of stabilized lipid membrane based devices that are not prone to electrical or mechanical shock and are stable outside an electrolyte solution has been the outcome of a large number of reports; these investigations provide lipid membrane based devices that can be commercialized. Recent nanotechnological advances have given a route to the construction of devices that their size is less than 100 nm size and therefore belong to the class of nanosensors. Further on, we provide the description of the methods for the preparation of this class of biosensors that are based on lipid membranes and provide advantages that are the following: ease of construction, fast response times, nanosize very high selectivity and sensitivity and are stable outside an electrolyte solution which allows them to eventually be commercialized.

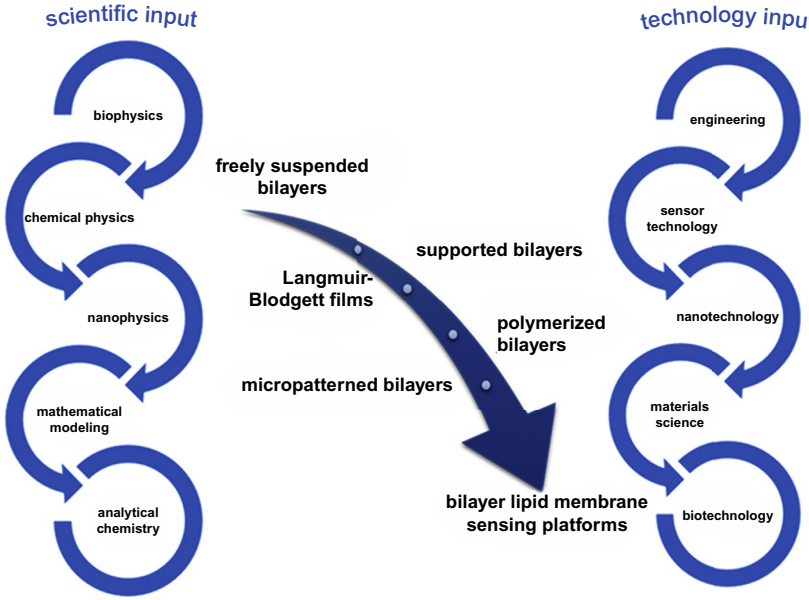

Fig. 2 The science and technology input that enabled lipid membrane technology to progress from freely suspended fragile bilayers (black lipid membranes) to rugged nanopatterned sensing platforms. *From ref. Nikoleli, G.-P., Siontorou, C. G., Nikolelis M.-T., Bratakou, S., & Bendos, D. K. (2019). Recent lipid membrane-based biosensing platforms.* Applied Sciences, *9(9), 1745–1764.*

2.1 Metal supported lipid membranes

Tien and Salamon (1989) reported a simple method for the construction of stabilized bilayer lipid membrane (sBLM) by using the freshly cut tip of a Teflon coated metal wire and providing the advantage of the interactions between the amphiphatic lipids 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 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 simplified scheme of this procedure is shown in Fig. 3.

sBLMs have been fully characterized (Nikolelis, Siontorou, Krull, & Katrivanos, 1996; Siontorou, Nikolelis, Krull, & Chiang, 1997; Tien & Salamon, 1989). The time of stabilization of the device depends upon the diameter of the wires and whether the organic solvent used was chloroform, decane or hexane (Nikolelis et al., 1996; Siontorou et al., 1997). Metallic

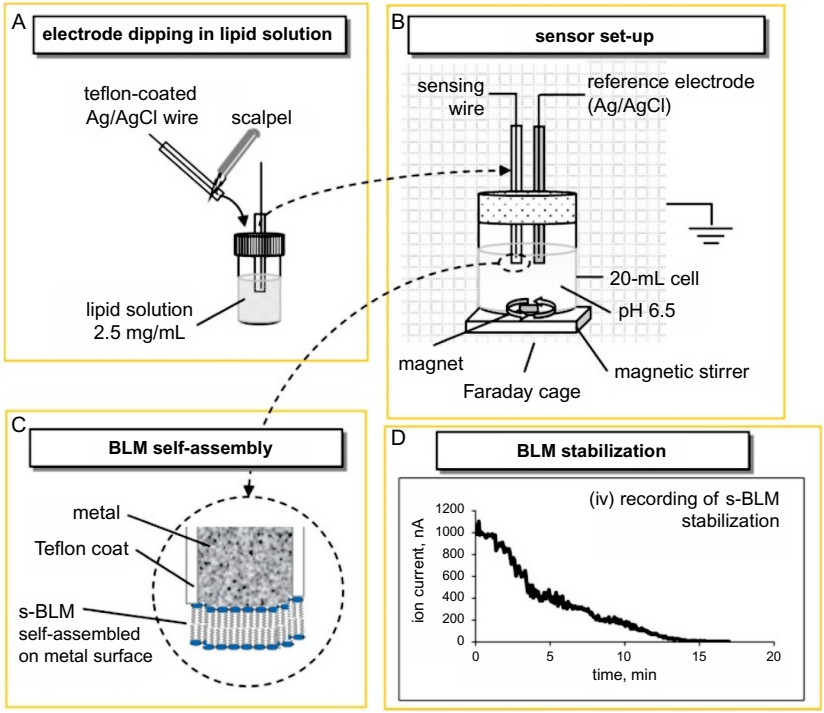

Fig. 3 A simplified scheme of the device setup, and the lipid self-assembly process for the construction of metal supported sBLMs (Mazur et al., 2017): (A) the tip of the sensing electrode is cut with a scalpel and immediately dipped in lipid solution before transferred in the electrolyte solution. (B) The electrochemical setup uses a two-electrode configuration (i.e., the sensing electrode and a Ag/AgCl reference electrode). The setup 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 solution. (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. Nikoleli, G.-P.; Nikolelis, D.; Siontorou, C. G.; & Karapetis, S. (2018). Lipid membrane nanosensors for environmental monitoring: The art, the opportunities, and the challenges.* Sensors, 18*(1), 284.*

wires that had a diameter of 0.25 mm should be avoided because the biosensor had increased electrical noise; chloroform or decane should not also be used as solvents because the lipid films belong to "black" lipid membranes and 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 period of times over 48 h.

Some efforts have investigated to model the potential profile across metal supported lipid membranes and the structure of the lipid bilayer that exists in the lipid/metal interface. A possible theory involves the interactions of oxygen atoms of the phosphate headgroups of the lipids with the Ag^+ ions in the metal lattice (Hianik, Dlugopolsky, & Gyepessova, 1993; Hianik, Passechnik, Sargent, Dlugopolsky, & Sokolikova, 1995). The ion mobility through membranes can be attributed to the presence of Cl^- ions at the space between the metal ions and the inner lipid layer. The sources for chloride ions: could be two: through the lipid film during the initial BLM stabilization process and through the partial wire insulation (Nikolelis et al., 1996; Siontorou et al., 1997). Chloride ions would also react with the Ag^+ ions to form silver chloride (Nikolelis et al., 1996; Siontorou et al., 1997). Electrochemical potentiometric experiments (against a Ag/AgCl reference electrode) (Siontorou et al., 1997) has exhibited small voltages when the bilayer lipid membrane was 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 (Passechnik, Hianik, Ivanov, & Sivak, 1998).

2.2 Stabilized lipid films formed on a glass fiber filter

The construction of stabilized lipid membranes on ultrafiltration glass fiber disks has been provided in the literature (Nikolelis, Siontorou, Andreou, & Krull, 1995) and allowed a number of practical applications in real samples, such as the determination of aflatoxin M_1 in milk and milk preparations (Andreou & Nikolelis, 1998). 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, UK) (Andreou & Nikolelis, 1998; Nikolelis et al., 1995).

The experimental set up which was used for the construction of these stabilized lipid membrane devices consisted of two plexiglas cells which were separated by a thin plastic partition (10 μm thick Saran-Wrap film). This partition was folded in half and a 0.32 mm hole was punched through the double layer of the plastic film. The microfiber filter was placed between the two plastic layers, centered on the 0.32 mm hole. The partition which contained the filter disk was then clamped between the two plexiglas chambers. One of the plastic cells had a circular shape (diameter 1.0 cm and depth 0.5 cm); this cell was connected with a carrier electrolyte KCl flow injection system. An Ag/AgCl reference electrode was positioned in the waste of the carrier solution. The second chamber was cylindrical and had its longitudinal

axis perpendicular to the flow of the electrolyte solution. The upper hole of this chamber 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. 4. Details for the procedure followed for the formation of the stabilized BLMs can be found in (Andreou & Nikolelis, 1998; Nikolelis et al., 1995).

2.3 Polymer lipid membranes

The methods of polymer stabilized in air lipid film devices were given in previous reports (Nikolelis, Raftopoulou, Chatzigeorgiou, Nikoleli, & Viras, 2008; Nikolelis, Raftopoulou, Nikoleli, & Simantiraki, 2006). These techniques include polymerization using UV irradiation and

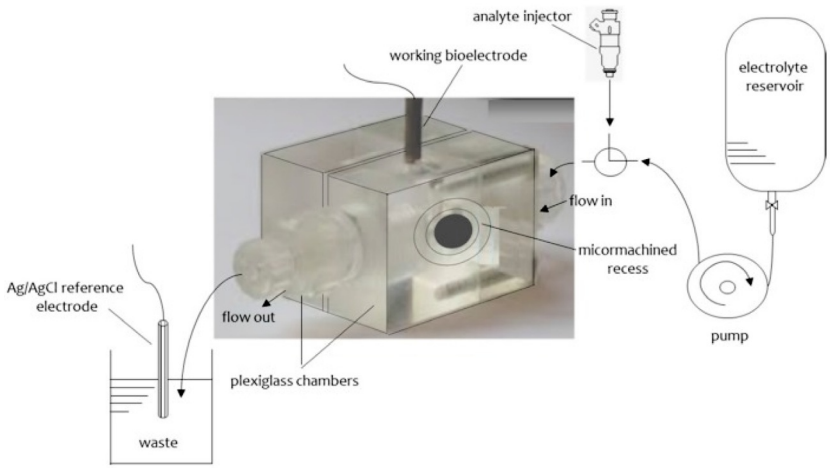

Fig. 4 The experimental set-up used for the formation of stabilized lipid films on glass fiber filters; the micromachined chambers are separated by a thin (12.5 μm thick) polyvinylidene chloride wrap and enclose the microfiber disk. For more details, see text. *From ref. Nikoleli, G.-P.; Nikolelis, D.; Siontorou, C. G.; & Karapetis, S. (2018). Lipid membrane nanosensors for environmental monitoring: The art, the opportunities, and the challenges.* Sensors, 18*(1), 284.*

not heating at 60 °C because the latter deactivates the enzyme or antibody molecules. Differential scanning calorimetry and Raman spectrophotometry experiments have shown that 4 h are required to finishing the polymerization stage.

2.3.1 Polymeric lipid membranes supported on graphene microelectrodes

Graphene nanomaterials have been used in biosensors because of their advantages such as enhanced physicochemical properties (mechanical, electrical and thermal), large surface-to-volume ratio, high biocompatibility and low toxicity. The large surface-to-volume ratio minimizes the size of the device and fast response times and lower sensitivity without biofouling are achieved. Our group has constructed a device that was composed from a stabilized lipid membrane on a copper wire that contained graphene nanosheets (Bratakou, Nikoleli, Nikolelis, & Psaroudakis, 2015; Nikoleli et al., 2012). These nanodevices have been used for the rapid determination of food toxicants, environmental pollutants and compounds of clinical interest (Bratakou et al., 2017; Bratakou, Nikoleli, Siontorou, Nikolelis, & Tzamtzis, 2016; Karapetis et al., 2016).

The preparation of graphene microelectrodes has been extensively described in the literature (Bratakou et al., 2016, 2017; Karapetis et al., 2016). *N*-methyl-pyrrolidone (NMP) was mildly sonicated for 180 h and centrifuged at 700 rpm for 2 h which provides a homogeneous dispersion (~0.4 mg/mL). This dispersion was poured onto a copper wire (0.25 mm in diameter) which was placed on a glass microfiber filter and the solvent was evaporated. The copper acted as the connection for the electrochemical experiments.

The method of preparation of the lipid membrane biosensors was previously described in detail (Bratakou et al., 2016, 2017; Karapetis et al., 2016): The stabilized lipid films were prepared by polymerization, as described above.

The "receptor" molecules were incorporated in these lipid film devices prior to polymerization by injecting 15 μL of the "receptor" suspension on the polymerization mixture. These filter-supported polymer lipid membranes were finally mounted onto the copper wire that contained the graphene nanosheets.

2.3.2 Polymer lipid films supported on ZnO microelectrodes

Nanostructured zinc oxide (ZnO) is a very promising material to preparing nanoelectrodes to construct devices for electrochemical experiments to

monitor food toxicants, environmental pollutants or analytes of clinical interest due to the large number of advantages of these electrodes which include their low cost, ease of preparation, biocompatibility and catalytic surface activity, high isoelectric point (IEP). The IEP of ZnO is 9.5 which is higher than the IEP of a large number of analytes of biomolecular interest and therefore these nanoelectrodes can be used as a matrix to immobilize these biomolecules through electrostatic bonding. Nanostructured ZnO nanoelectrodes have also high sensitivity and small size, high surface area and high electrical transport. Note that the electrical transport ZnO properties depend on its crystal structure, surface polarity, etc. and these properties can be modeled. ZnO nanoelectrodes has widely been used for the construction of nanodevices to monitor food toxicants such as hormones, insecticides, pesticides, toxins. Glucose, urea, cholesterol, L-lactic acid, metal ions, etc.

2.3.2.1 Potentiometric biosensors based on ZnO nanowalls and stabilized polymerized lipid film

The unmodified ZnO nanowalls electrodes on an aluminum (Al) foil can be constructed by the well known sonochemical technique of Nayak et al. (Naval, Katzenmeyer, Gosho, Tekin, & Islam, 2012) which briefly is as follows: Unimolar solutions of zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$, 99.9% purity) and hexamethylenetetramine ($C_6H_{12}N_4$, 99.9% purity) are stirred with a magnetic stir bar at 350 r.p.m. for 5 min. An Al coated substrate was then immersed in this solution and sonicated at 50% of the maximum amplitude for 10 min. The electrode was then rinsed with distilled water and dried with a N_2. The foil was covered by a scotch tape by 1/3 during sonication; this part of the foil was uncovered during measurements and used as a contact for the potential measurements.

The preparation of the polymerized stabilized lipid films for the determination of cholesterol was previously described (Psychoyios, Nikoleli, Tzamtzis, et al., 2013). Cholesterol oxidase was incorporated in these lipid membranes prior polymerization by spreading on the microfilter 15 μL of the enzyme suspension with the polymerization mixture using a microliter syringe.

The final stage to construct the device was to encapsulate the filter-supported polymerized lipid film onto the wire containing the ZnO-nanowalls electrode. Fig. 5 shows a simplified diagram of the device.

2.3.2.2 Potentiometric biosensors based on lipid stabilized membranes ZnO nanowires

The sensor electrodes were constructed following the method as previously described (Usman Ali et al., 2011). The plastic substrate was affixed on a

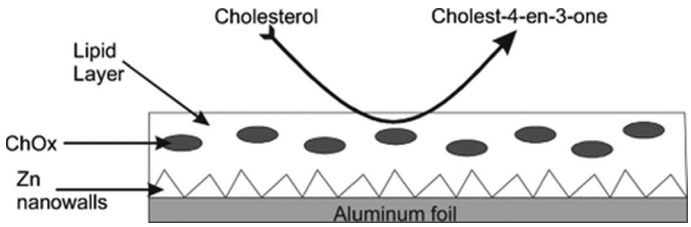

Fig. 5 A simple diagram of the device design. *From ref. Psychoyios, V. N., Nikoleli, G.-P., Tzamtzis, N., Nikolelis, D. P., Psaroudakis, N., Danielsson, B., et al. (2013). Potentiometric cholesterol biosensor based on ZnO nanowalls and stabilized polymerized lipid film.* Electroanalysis, 25*(2), 367–372 with permission.*

support in the vacuum chamber of an evaporator. Firstly, the titanium film was deposited (thickness ca. 10 nm) and then 50 nm of gold was deposited on the surface of the plastic substrate. The gold coated electrode (4 cm long and 1 mm width) was rinsed with acetone and then with de-ionized water and dried at room temperature. A chemical approach was used to prepare the ZnO nanowires on the electrode (Vaface & Youzbashizade, 2007). The electrode was inserted twice into a solution that contained zinc acetate (2 min) and then was dried in the air. The electrodes then were inserted in a 025 M Zn $(NO_3)_2$ $6H_2O$ solution that contained 0.025 M hexamethylenetetramine and placed an oven (2–4 h, 90 °C). The ZnO nanowires were finally rinsed with de-ionized water and dried at room temperature. The diameters were 80–150 nm and were uniform.

The enzyme (uricase) was incorporated in the lipid membranes prior to polymerization as previously described (Tzamtzis, Psychoyios, Nikoleli, Willander, & Qadir Israr, 2012). Construction of the uricase device was finalized by encapsulation of the filter-supported polymerized lipid film onto copper wire containing the ZnO nanowires.

3. Applications on biosensors based on lipid membranes in food analysis

3.1 Applications of lipid film devices based on glass fiber disks

An atrazine lipid membrane nanosensor was described in the literature (Nikolelis & Andreou, 1996). When atrazine interacted with bilayer lipid membranes (BLMs) free of solvent, a transient ion current signal appeared (i.e., transient) with duration of seconds; this transient reproducibly

appeared within ca. 1 min after atrazine came in contact with the lipid. The sensitivity of the biosensor increased by using 35% (w/w) DPPA in the lipid mixture and Ca^{2+} in the electrolyte solution; calcium ions promoted changes in the phase structure of the acidic lipid (i.e., DPPA) and increased the peak height of the detection. The detection limits were on the order of μM concentration range. Similar results were obtained by using platelet-activating factor (PAF; an ether analog of PC) in membranes.

The flow injection analysis of mixtures of simazine, atrazine and propazine using mixtures of phosphatidyl choline and dipalmitoyl phosphatidic acid on filter-stabilized lipid films was described in the literature (Nikolelis & Siontorou, 1996). An ion current transient appeared after exposure of membranes to a mixture of these herbicides in less than 2 min after injection. The peak heights were linearly related to the herbicides concentration and these compounds were determined at μM range. Repetitive cycles of injections have shown that the signal did not decrease (i.e., 10 cycles of injection). The time of appearance of these signals changed for each compound and increased on the order of simazine, atrazine and propazine; this has permitted the simultaneous detection and monitoring of mixtures of these herbicides.

Lipid membranes biosensors were used for the monitoring of carbofuran using flow injection analysis (FIA) techniques (Nikolelis, Simantiraki, Siontorou, & Toth, 2005). The principle was based on the inhibition degree and reactivation of enzyme when the substrate was injected. Carbofuran could be determined at the nM concentration range. Proteins and lipids were investigated as interferents and no interferences were noticed. The sensor has been applied in a wide range of food samples, such as fruits, vegetables and dairy products. A recovery of 96% to 106% was observed which shows that there were no interferences from the matrix effects.

A synthetic "receptor" incorporated on stabilized lipid membranes using glass fiber disks was reported in the literature (Nikolelis, Raftopoulou, Simantiraki, et al., 2008). The stabilized lipid film devices were modified with calixarenes. The method had a high sensitivity and selectivity so that it can be applied for the fast determination of insecticides in fruits and vegetables (Nikolelis, Raftopoulou, Simantiraki, et al., 2008). Other nanosensors that were described include a chemical sensor for the selective and rapid determination of hormones in foods which included naphthalene acetic acid (Nikolelis, Ntanos, Nikoleli, & Tampouris, 2008) and a device for the rapid detection of zinc in waters (Nikolelis, Raftopoulou, Psaroudakis, & Nikoleli, 2009).

3.2 Applications of lipid film devices based on polymeric lipid membranes

3.2.1 Applications of graphene based devices

A potentiometric urea lipid membrane based minisensor on graphene has been appeared recently (Nikoleli et al., 2012). A potentiometric urea device based on lipid film technology on graphene nanosheets has been constructed; a simplified set up of this biosensor is shown in Fig. 6. The main characteristics of this biosensor are excellent reproducibility, sensitivity, selectivity, reusability, and rapid response times; the slope of the electrode is ca. 70 mV/decade over the urea logarithmic concentration range which can be determined from 1×10^{-6} M to 1×10^{-3} M.

A nanosensor for naphthalene acetic acid (NAA) was described in the literature and was based on polymerized stable in air lipid membrane devices in which auxin-binding protein 1 receptor was incorporated with the lipid membranes (Bratakou et al., 2016). The sensor was evaluated in real samples of fruits and vegetables using a flow through injection system, NAA was injected into the flowing 0.1 M KCl electrolyte solution and the flow stopped. An ion current transient was obtained; the peak height was correlated to the concentration of NAA with mM detection limits and analysis times of ca. 5 min. Interference studies have shown no interferences from compounds usually found in foods and vegetables (e.g., lipids, proteins, ascorbic acid). The sensor has been used to determine NAA in fruits and vegetables with satisfactory results.

A potentiometric carbofuran minisensor on graphene in which lipid films were polymerized was provided in the literature (Bratakou et al., 2015).

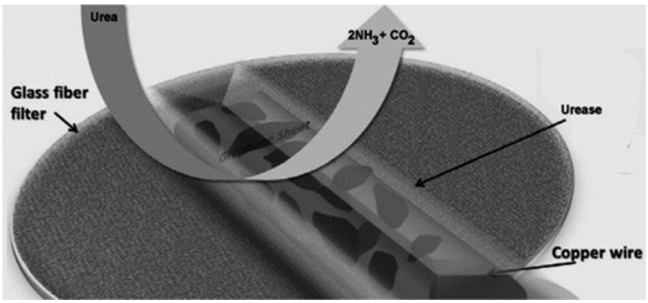

Fig. 6 Picture of the lipid film device on graphene minielectrode which was used for the potentiometric detection of urea. *Reprinted from reference Nikoleli, G.-P., Israr, M. Q., Tzamtzis, N., Nikolelis, D. P., Willander, M., & Psaroudakis, N. (2012). Structural characterization of graphene nanosheets for miniaturization of potentiometric urea lipid film based biosensors.* Electroanalysis, 24, *1285–1295 with permission.*

This device was applied to construct a carbofuran chemical sensor by codepositing resorcin[4]arene selective receptor. The detection limits were at the nM levels with times of response of 20 s. The device was constructed easily and showed excellent characteristics such as good reproducibility, reusability, selectivity, and long shelf life; the electrode slope was 59 mV/decade and carbofuran was logarithmically related in the range 10^{-6} to 10^{-3} M.

The electrochemical interactions of cholera toxin with polymerized lipid films with incorporated ganglioside GM1 was reported in the literature (Nikoleli, Nikolelis, & Tzamtzis, 2011). An injection of cholera toxin in the flowing streams of a KCl 0.1 M carrier electrolyte provided a current signal. The peak height of the ion current signal was related with the concentration of cholera toxin with detection limits of 0.06 μM.

The response times and detection limits were improved using polymerized lipid membranes on graphene nanosheets (i.e., response times of 5 min, and detection limits of 1 nM) (Karapetis et al., 2016). The construction of this sensor was easy and the sensor has showed good reproducibility, reusability, selectivity, long shelf life and sensitivity of 60 mV/decade of toxin concentration. The method was applied in lake water samples.

An electrochemical nanosensor for the detection of saxitoxin based on graphene nanosheets with stabilized in air lipid membranes and incorporated anti-STX was reported in the literature (Bratakou et al., 2017). An excellent selectivity, sensitivity and detection limits (1 nM) for the determination of saxitoxin with fast response times (i.e., 5–20 min) were observed. The sensor was easily constructed and had good storage stability and a slope of 60 mV/decade over saxitoxin concentration. The method was applied for the determination of STX in lake water and shellfish samples.

3.2.2 Applications of the ZnO nanoelectrode based devices

A potentiometric cholesterol device was constructed by incorporating cholesterol oxidase into polymerized lipid films on ZnO nanowalls (Psychoyios et al., 2013). The enzyme was codeposited into the lipid film prior to polymerization on the ZnO nanowalls surface and provided a sensitive, selective, stable and reproducible cholesterol device. The electrode slope was 57 mV/decade of cholesterol. No interferences were noticed by ascorbic acid, glucose, urea, proteins and lipids. The present biosensor has shown biocompatibility and could be implanted in the human body.

An uric acid electrochemical device was reported in the literature by immobilizing the enzyme uricase into polymerized lipid membranes on

Zn nanowires (Usman Ali et al., 2011). The enzyme was codeposited with the lipid membrane prior to polymerization on the surface of the electrode. The biosensor was sensitive, selective, stable and reproducible. The presence of a cationic lipid in membranes has increased the electrode slope by two-fold. No interferences were observed by the presence of ascorbic acid, glucose, urea, proteins and lipids.

ZnO nanowires (NW) were tailored for the immobilization of glucose oxidase in order to construct a glucose biosensor (Zang et al., 2007). The high specific surface area and isoelectric point provide the electrode efficient immobilization of high concentration of acidic enzymes. The apparent Michaelis constants were adjusted by tailoring the thickness of the GOD/ZnO nanowire layer and the enzyme loading in the nanowires. Through this route, a linear region of sensitivity and reaction rates could be obtained. The long-term stability of this biosensor was high due to the inorganic ZnO nanowires.

Well-aligned ZnO nanowires were constructed on gold-coated plastic substrates using a low-temperature aqueous chemical growth method (Ali et al., 2011). These arrays had 50–130 nm diameters and were applied to construct a urea biosensor using urease within the concentration range 0.1 mM to 100 mM with logarithmic response. The electrode slope was 52.8 mV/decade for 0.1–40 mM of urea and response times were less than 4 s; this urea biosensor had excellent selectivity, reproducibility and did not showed any response to interferents such as ascorbic acid and uric acid, glucose. K(+) and Na(+) ions.

Well-aligned ZnO nanowires decorated with Pt nanoparticles (NPs) were recently used to construct a non-enzymatic glucose biosensor (Hsu et al., 2017). The use of Pt NPs decoration increased the sensitivity by 10-fold. A similar glucose biosensor on silicon NWs (ZnO/Si NWs) was also reported in the literature (Miao, Lu, Tao, Li, & Chu, 2016). These nanowire nanocomposites have shown an excellent amperometric sensitivity to glucose ($129\,\mu A\,mM^{-1}$), low detection limits (12 μM), good stability and reproducibility and selectivity in the presence of common interferents. Recent works applied a roll-to-roll flexographic printing technique that was used to construct a three electrode electrochemical unit that consisted of ZnO NWs (Fung, Lloyd, Samavat, Deganello, & Teng, 2017). This unit exhibited a sensitivity of $1.2 \pm 0.2\,\mu A\,mM^{-1}\,cm^{-2}$ and the calibration graph was linear over the glucose concentration between 0.1 and 3.6 mM (Table 1).

Table 1 Summary of biosensor devices based on lipid membranes that were used in food analysis, their characteristics, main applications in food analysis and detection limits towards target analytes in particular food matrices.

Target analyte	Sensor device	Enzyme or receptor	Food analysis	Detection limit	Reference
Atrazine	Lipid films on fiber glass			μM	Nikolelis and Andreou (1996)
Herbicides (simazine, atrazine, propoazine)	Lipid films on fiber glass			μM	Nikolelis and Siontorou (1996)
Carbofuran	Polymer lipid films on fiber glass disks	Acetylcholinesterase (AchE)	Fruits, vegetables, dairy products	nM	Nikolelis et al. (2005)
Carbofuran	Polymer lipid films on fiber glass disks	Phosphoryl derivative of a resorcin[4]arene receptor	Fruits and vegetables	nM	Nikolelis, Raftopoulou, Simantiraki, et al. (2008)
Naphthalenic acid	Polymer lipid films on fiber glass disks	Auxin binding protein-1 receptor	Fruits	nM	Nikolelis, Ntanos, Nikoleli, et al. (2008)
Zinc	Polymer lipid films on fiber glass disks	Calix4arene phosphoryl receptor	Real samples of waters	5.00×10^{-8} M	Nikolelis et al. (2009)
Urea	Polymer lipid films on fiber glass disks on graphene microelectrodes	Urease		1×10^{-6} M	Nikoleli et al. (2012)
Naphthalenic acid	Polymer lipid films on fiber glass disks on graphene microelectrodes	Auxin binding protein-1 receptor	Fruits and vegetables	nM	Bratakou et al. (2016)

Carbofuran	Polymer lipid films on fiber glass disks on graphene microelectrodes	AchE	Fruits and vegetables	nM	Bratakou et al. (2015)
Cholera toxin	Polymer lipid films on fiber glass disks	Ganglioside GM1		0.1 μMi	Nikoleli et al. (2011)
Cholera toxin	Polymer lipid films on fiber glass disks on graphene microelectrodes	Ganglioside GM1		0.1 nM	Karapetis et al. (2016)
Saxitoxin	Polymer lipid films on fiber glass disks on graphene microelectrodes	Anti-STX	Real lake and shellfish samples	1.00×10^{-9} M	Bratakou et al. (2017)
Cholesterol	ZnO nanowalls and stabilized polymerized lipid film	Cholesterol oxidase		1×10^{-6} M	Psychoyios et al. (2013)
Uric acid	Zinc oxide (ZnO) nanowires	Uricase		1 μM	Usman Ali et al. (2011)
Glucose	Zinc oxide (ZnO) nanowires	Glucose oxidase		1 μM	Zang et al. (2007)
Urea	Zinc oxide (ZnO) nanowires	Urease		0.1 mM	Ali et al. (2011)
Glucose	Zinc oxide (ZnO) nanowires via decorated Pt nanoparticles	Glucose oxidase		1.00 mM	Hsu et al. (2017)
Glucose	ZnO nanowires/ferrocenyl alkanethiol array	Glucose oxidase	Food analysis	0.2 mM	Miao et al. (2016)
Glucose	ZnO nanowires	Glucose oxidase		1 μM	Fung et al. (2017)

4. Conclusions

The recent novel nanotechnological advances have brought many changes, opportunities and prospects for advancing science and technology in many fields and especially those in the biosensor field. This paper presents the current directions in the field of artificial lipid membranes nanosensor technology and their applications in the determination of food toxicants, more in an effort to attract new researchers in the field than to present an exhaustive description of recent accounts.

The paper describes the recent advances which are based on lipid membranes and used for food analysis. These technologies include the construction of stable in solution and in air and are supported on microfiber glass filters or polymerized on graphene and ZnO microelectrodes. The polymer lipid membrane nanobiosensors can be portable and used in the field. These nanosensors have detection limits in the nM concentrations. It is expected soon to commercially prepare units for market production.

The results have exhibited that these lipid membrane based detectors can be stored and used after remaining in the air for periods of 1 month and can be easily constructed at low cost. The response times of these nanosensors are on the order of seconds, are not bulky and much cheaper than chromatographic units; these detectors can be complimentary to LC and gas chromatographic instruments for in-field applications for the rapid detection of food toxicants. These toxicants include toxins, carbamates, hormones, glucose, cholesterol, urea, etc. with high sensitivity and selectivity, rapid response times, portability, etc.

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