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Detection of odorant molecules via surface acoustic wave biosensor array based on odorant-binding proteins

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ABSTRACT

In this paper, we present an array of biosensors for vapour phase detection of odorant molecules based on surface acoustic wave (SAW) resonators coated with odorant-binding proteins (OBPs). For the first time, the sensing capabilities of three different OBPs, as sensitive layers for SAW devices, are studied and compared. The SAW biosensor array is composed of three SAW devices coated by the droplet method with the wild-type OBP from cow (wtbOBP), a double mutant of the OBP from cow (dmbOBP) and the wild-type OBP from pig (wtpOBP). An uncoated device is used to compensate the variations of the environmental parameters. The SAW devices consist of two-port resonators fabricated on quartz (ST-cut, x propagation) with electrodes made of aluminium covered with a thin gold film (2 nm thick). The obtained surface densities of OBP layers are between 1.18×10^{-6} kg/m^2 and 2.31×10^{-6} kg/m^2 and were calculated measuring the resonant frequency shift of the SAW devices after the coating. The SAW biosensor array was tested in nitrogen upon exposure to vapours of R-(−)-1-octen-3-ol (octenol), in the range of concentration between 13 and 61 ppm, and R-(−)-carvone (carvone), in the range between 9 and 80 ppm. The highest sensitivity for detection of octenol (25.9 Hz/ppm) was obtained using the wtpOBP-based SAW biosensor, while the highest sensitivity for detection of carvone (9.2 Hz/ppm) was obtained using the dmbOBP-based SAW biosensor.

1. Introduction

The demand for versatile technologies that can be used for developing chemical and biological sensors for the detection of volatile species has rapidly increased in the last decade (Fanget et al., 2011). Multiple areas of application include homeland security (Demirev et al., 2005), environmental pollution control, health and wellness (Varriale et al., 2012), to mention some examples. Specifically, the mandatory need for detection of volatile organic compounds (VOCs), which have a potential impact on climate and long-term health effect (Cheol Gil et al., 2000), and of chemical warfare agents for counter-terrorism actions (Joo et al., 2007), has stimulated an extensive research activity for the development of several types of sensitive devices. These sensors have to provide precision, high sensitivity, reversibility, selectivity, low cost, fast response time and compactness (Fernández et al., 2007; Pellejero et al., 2012). Finally, the methodologies devoted to the sensory assessment of foods and based on the global evaluation of the odour intensity require sensor arrays to develop artificial olfactory systems (Garcia-Gonzalez and Aparicio, 2002).

The capabilities of SAW devices to measure physical parameters, such as force, acceleration, pressure, electric and magnetic fields, potentials, etc., or chemical and biochemical values, such as gas, vapours or ion concentrations, are widely exploited since many years (Ballantine et al., 1997; Benetti et al., 2004). In particular, in the last two decades, SAW devices have attracted the attention of the biochemical scientific community for bio/sensing applications. Some examples of SAW based biosensor systems suitable to detect bacteria, proteins, DNA, sugars, viruses and cells in liquids are reported in the literature (Berkenpas et al., 2006; Branch and Edwards 2007; Länge et al., 2007; Rupp et al., 2008).

For vapour phase applications, SAW biosensors seem to be a powerful tool to measure small concentrations of volatile compounds (Sang-Hun et al., 2005). In fact, they can overcome the intrinsic low-selectivity of polymer coated SAW chemical sensors (Alizadeh and Zeynali 2008), and, at the same time, ensure the high sensitivity and fast response time typical of these sensors. However, the development of SAW biosensors for in-air applications has been delayed because of the knowledge that biomolecules maintain their three-dimensional structure and, hence, their prescribed functionality, only in an aqueous environment (Stubbs et al., 2002). On the contrary, some works on acoustic wave biosensors for vapour phase detection based on commercially available quartz crystal microbalance (QCM), which operate on thickness shear mode resonation, were reported in the literature several years ago (Guilbault 1983; Ngeh-Ngwainbi et al., 1986). QCM sensors for mass detection of formaldehyde and organophosphorous pesticides based on films of enzymes and antibodies are

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described previously (Guilbault 1983; Ngeh-Ngwainbi et al., 1986).

To date, only a limited number of works on SAW devices utilizing biological molecules as sensing material and able to detect small molecules in air have been proposed (Hunt et al., 2003). A SAW resonator immunosensor array is described in (Sang-Hun et al., 2005) demonstrating the detection of low vapour pressure plastic explosives containing nitro groups such as RDX and TNT. The monoclonal anti-RDX and anti-TNT have been immobilized on the metal electrodes of the SAW resonator via protein-A cross linker. Stubbs et al., (2005) detected cocaine vapours by anti-benzoylecgonine antibodies using a SAW platform.

Vertebrate OBPs are small extracellular proteins belonging to the lipocalin superfamily (Briand et al., 2002; Dal Monte et al., 1993; Lobel et al., 2002; Spinelli et al., 1998). It has been hypothesized that OBPs play a role in receptorial events of odour detection by carrying, deactivating, and/or selecting odorant molecules (Blanchet et al., 1996; Herent et al., 1995; Tegoni et al., 1996). The OBPs share a conserved folding pattern, an 8-stranded β-barrel flanked by an α-helix at the C-terminal end of the polypeptide chain. The β-barrel creates a central apolar cavity whose role is to bind and transport hydrophobic odorant molecules. These proteins reversibly bind odorants with dissociation constants in the micromolar range. Although their functions are still not fully understood, OBPs are also believed to participate in the deactivation of odorants (Briand et al., 2002; Dal Monte et al., 1993). Since the discovery of the first vertebrate OBP in the bovine nasal mucus, OBPs have been identified in a variety of species, and different OBP subtypes have been reported to simultaneously occur in the same animal species (Briand et al., 2002). The binding properties investigated in the three OBPs from rat demonstrated that they were specially tuned towards distinct chemical classes of odorants (Garibotti et al., 1997; Pes et al., 1992; Vincent et al., 2000).

In this work, we present a comparison of the sensing capabilities of three different OBPs as probes for odorant molecules by using a SAW biosensor system.

The proposed solution exploits the flexibility and the well-known features of SAW based sensors in conjunction with the adaptable selectivity of the OBPs. In particular, the use of this class of proteins was suggested by the fact that they can be easily modified by genetic engineering techniques to modulate their binding specificity (Ramoni et al., 2007) and preserve their full functionality when exposed to air environment. Moreover, the possibility to use wtbOBPs to implement SAW biosensors, able to detect odorant molecules, has been demonstrated (Di Pietrantonio et al., 2009).

The proposed sensor system is based on an array configuration composed of three SAW resonators coated with three different OBPs, characterized by different binding specificity, plus an uncoated SAW device used as reference. The chosen proteins are the wtbOBP, a dmbOBP and the wtpOBP. Tests were performed exposing the SAW biosensor array to concentrations of octenol and carvone vapours in nitrogen atmosphere. These odorants are largely used in food industry and their detection is a first approach towards the recognition of food flavours.

Experimental results showed different sensitivities of the three OBP-based SAW biosensors for detection of the investigated compounds.

2. Material and methods

2.1. OBPs purification and functionality test with 1-amino-antharacene

A 6xHis affinity tag was placed at the N-terminal of the wtbOBP and mutant the double protein dmbOBP by Polymerase Chain Reaction (PCR) with specific primers (Ramoni et al., 2007). The fused cDNAs were sub-cloned in the expression vector pT7-7 and the expressions of the two proteins were realized in BL21-DE 3 *Escherichia coli*. The purification of the two proteins was obtained by affinity chromatography with a Ni-NTA Agarose (Quiagen, Germany) according to the manufacturer's instructions, followed by a second chromatography step on the anion exchange column Resource Q (Amersham Biosciences, Italy), on an Fast Performance Liquid Chromatography (FPLC) chromatographic system. The purity of the two proteins was determined by SDS-PAGE (Poly-Acrylamide Gel Electrophoresis) and the concentration of the two purified proteins was calculated by the absorbance value at 280 nm (48,000 M^{-1} cm^{-1}) (Ramoni et al., 2007).

Pig (*Sus scrofa*, breed Large White) nasal mucosa was obtained from the local slaughterhouse. The tissue was collected within 20 min after death and quickly utilized for the extraction of pOBP. Protein was purified from fresh porcine nasal tissue according to the procedure described by (Dal Monte et al., 1991). This procedure yielded on average 15 mg of purified pOBP from a single animal.

The functionalities of the recombinant wtbOBP, dmbOBP and pOBP were determined by direct titrations using the fluorescent ligand 1-amino-anthracene (AMA). Briefly, 1.0 ml of 1.0 μM OBPs, in 20 mM Tris–HCl buffer pH 7.8, was incubated overnight at 4 °C in the presence of increasing concentrations of AMA (0.156–10 μM).

Fluorescence emission spectra were recorded between 450 nm and 550 nm by fluorometer (ISS K2 model) (excitation and emission slits were set at 2.0 nm) at a fixed excitation wavelength of 380 nm. The formation of the AMA-OBPs complex was followed as the increase of the fluorescence emission intensity at 480 nm. In particular, the functional assay is based on the resonance energy transfer phenomenon between the indolic residues of the protein and the AMA localized in protein binding site. In fact, the indolic residues of OBP are excited upon irradiation at 295 nm. Since in the very proximity of them are located AMA molecules (Forster distance), there is no emission from the indolic residues of OBP, but there is energy transfer to AMA molecules that emit at about 480 nm. The presence of the odorant molecules competes with AMA molecules for the protein active site, and since odorant molecules have a higher affinity than AMA for the protein active centre, they displace AMA molecules and interrupt the energy transfer phenomenon. This means that in the presence of the odorant molecules we cannot observe emission from AMA molecules.

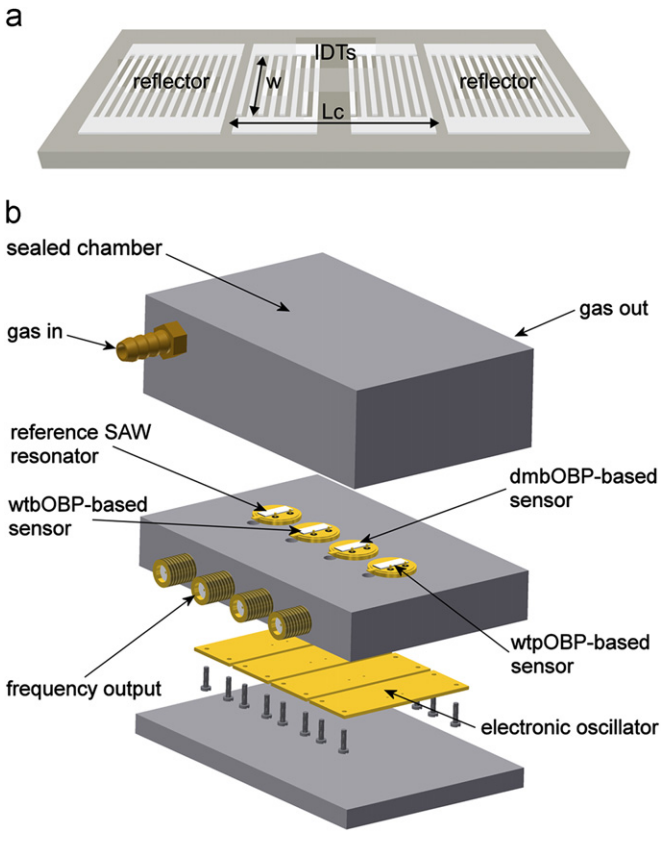
2.2. SAW biosensor system

SAW biosensors consist of two-port SAW resonators obtained by two inter-digital transducers (IDTs) synchronously arranged between reflecting gratings and operating at approximately 392 MHz. The devices were implemented on α-quartz substrates (ST-cut, x propagation) and the metallic electrodes are made of Al film (100 nm thick), which is conventionally used in SAW devices for the low density, good conductivity (Zhu et al., 1996). A thin Au film (2 nm thick) is deposited on the Al electrodes to increase the adhesion of protein. In fact, Au forms strong bonds with the thiol groups of the proteins (Lee et al., 2003; Wilson et al., 2001). Both Au and Al films were grown by radio frequency (RF) magnetron sputtering technique, using a commercial MRC 8620 system, from a 99.9% and 99.999% pure Au and Al target (6.5 in.), respectively. The metals were deposited in an Ar atmosphere at a pressure of 3 mTorr and a flow rate of 90 sccm. The deposition rate was 0.167 nm/s for Al and 0.087 nm/s for Au films. The thickness of the films was estimated measuring the deposition time. The patterns were transferred by a photolithography process, using poly(methyl methacrylate) (PMMA) resist exposed by deep UV radiation, and lift-off procedure. Each IDT

consists of 76 couples with a finger width of 2 μm and a metallization ratio of 0.5, resulting in a wavelength (λ) of 8 μm, and is effective for excitation and detection of Rayleigh waves. The maximum finger overlap is of 450 μm (w) and IDTs are shaped with a Gaussian apodization profile in order to minimize any spurious transverse mode. The number of fingers for each reflecting grating is 300 and the cavity length is 1278 μm (L_c). The schematic drawing of the SAW resonator used in this work is shown in Scheme 1(a).

The SAW resonators were designed to allow the use of microwave probes (Picoprobe 40 A-GSG-200-T by GGB industries) to measure the frequency response of the sensors with the Network Analyzer (HP8753A) for the characterization of the OBP depositions. This design permits also the bonding of the SAW devices with the packages and the conditioning electronics. In fact, the SAW device is used as a frequency control element in the feedback path of a RF oscillator. This configuration provides a simple, effective and accurate method for monitoring small variations in SAW velocity typical of the SAW biological- and chemical-based sensors (Yadava et al., 2009).

The SAW biosensor system is composed of four electronic oscillators: three oscillators for the SAW devices coated with the three different types of OBPs and one oscillator for a reference resonator. The SAW devices were mounted on TO39 packages, placed inside a stainless steel sealed chamber and connected to the conditioning electronic circuits by feed through pin-connectors. The schematic drawing of the system is shown in Scheme 1(b). Each feedback oscillator use an integrated amplifier (Mini-Circuits\MAR-8) and was fabricated on a high dielectric constant substrate ("Arlon 1000"; thickness: 0.25 inches; $\varepsilon_r = 10$).



Scheme 1. (a) Diagram of the 2-port SAW resonator obtained on a quartz substrate by two IDTs synchronously arranged between reflecting gratings: *w* is the finger overlap and L_c the cavity length. (b) Schematic drawing of the system composed of the SAW biosensor array and the electronic oscillators embedded in the measuring chamber.

All traces carrying RF signals were designed to have a characteristic impedance of 50 Ω and, to reduce the effects of interference and noise, multiple vias providing low-impedance connections to the ground plane were used.

De-coupling capacitors were added to the power supply voltage to prevent RF signals from coupling to the supply. A phase shift filter was used to fulfil the *Barkhausen* phase condition on the oscillator loop. A tuneable capacitor allowed us to fine adjust the oscillation frequency to the serial resonance frequency of the SAW device. An output buffer was implemented by a BJT transistor (BFR92P) in emitter follower configuration.

2.3. Odorant molecules

SAW biosensor array performances were tested by using two odorant compounds: octenol and carvone. Octenol was purchased from Fontarome Chemical inc. and carvone from Sigma Aldrich, both in liquid phase at room temperature with purity higher than 98%.

R-(−)-1-Octen-3-ol (octenol or mushroom alcohol), is an enantiomer produced by several plants and fungi, including edible mushrooms and Lemon balm. Octenol is also contained in human breath and sweat, and it is an odorant molecule that attracts mosquitoes and biting flies. Octenol may be used in conjunction with carbon dioxide together with electronic devices that in turn kill the trapped insects (Environmental-Protection-Agency 2007). When released into air, it is not harmful to humans, other non-target organisms, or to environment. There is the potential for toxicity if it is ingested (LD 50 of 340 mg/kg (Environmental Protection Agency 2007)), but it is approved by the U.S. Food and Drug Administration as a food additive for human consumption (US FDAs Center for Food Safety and Applied Nutrition 2008), and actually it is also used in food industry.

Carvone is a member of a family of chemicals named "terpenoids" (Simonsen, 1953), and it forms two enantiomers. The R-(−)-carvone is the enantiomer used in this work and its main characteristic is to smell like spearmint. R-(−)-carvone is the most abundant compound in the essential oil from several species of mint, particularly spearmint oil (*Mentha spicata*), which is composed of 50–80% R-(−)-carvone (Chauhan et al., 2009). This odorant molecule is employed in different application areas, such as the food and flavour industries (De Carvalho and Da Fonseca 2006). In particular, it is used for air freshening products, in aromatherapy and in alternative medicine. R-(−)-carvone has been proposed for use as a mosquito repellent, and the U.S. Environmental Protection Agency is reviewing the request to register it as a pesticide (Environmental Protection Agency 2009).

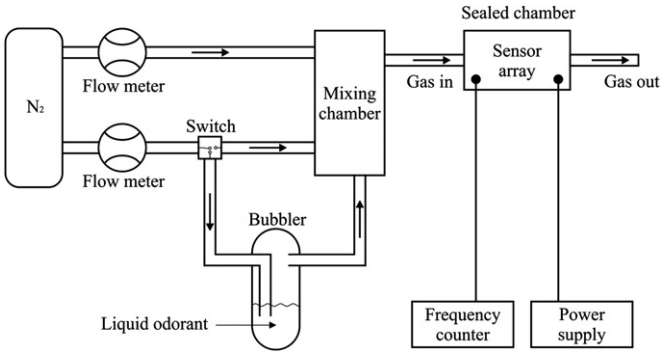
2.4. Sensor measurement setup and test procedure

The SAW biosensor array was tested in N_2 atmosphere upon exposure to concentrations of octenol and carvone vapours (each analyte was tested separately). In order to obtain different concentrations of odorants in N_2, the sensor system, composed of three biosensors and the reference, was exposed to a total flux of 100 sccm, controlled by two flow meters: the main for the gas carrier (N_2) and the second for the odorant. Different concentrations of vapour were obtained fluxing N_2 in a bubbler containing pure and liquid odorant at room temperature. The concentrations of the odorants at room temperature (20 °C) were calculated considering the vapour pressure reported on the safety data sheets: 0.667 hPa for octenol (Fontarome Chemical, inc.) and 0.5 hPa for carvone (Sigma Aldrich). A flow switch positioned before the bubbler allowed the flux to remain constant both in the absence and in the presence of the odorant compounds. In addition, a mixing chamber allowed us to obtain the desired gas

mixture (odorant/N_2). The schematic drawing of the measuring setup is shown in Scheme 2.

The SAW biosensor array responses, given by frequency shifts of the oscillators, were measured with a frequency counter (HP 53131 A) and a multiplexer module (Agilent 34980 A and 34941 A). All data were acquired using a custom LabVIEW™ routine. The frequency of the reference device was measured and subtracted from the measurements of the coated sensors. This differential configuration allowed the system to compensate the influence of humidity, temperature and pressure on the sensor responses.

Before measurements, the frequency baseline was obtained exposing the SAW biosensor array to a flux of pure N_2. Then, the odorant concentration was added to the system until saturation of the frequency responses was reached. The performances of system were evaluated considering the sensitivity, repeatability, and detection limit of the SAW biosensors. For each odorant, measurements at different concentrations, in the range between 13 and 61 ppm for octenol and between 9 and 80 ppm for carvone, were performed and the frequency shifts at saturation were recorded to evaluate the sensitivity. The repeatability was tested executing three repeated experiments at the same concentration. Each experiment was started when the initial conditions (baseline) were restored. Finally, the detection limits were calculated considering a noise level of 10 Hz.



Scheme 2. Schematic drawing of the setup used to test the SAW biosensor array. The first flow meter is used for the gas carrier (N2). The output of the second flow meter is switched to a bubbler containing pure and liquid odorant to obtain different concentrations of the analyte.

2.5. Immobilization and surface density measurements of the three OBPs

The three OBPs were deposited on the SAW resonators by means of the droplet method. The deposition based on this method is a simple and fast technique that did not require an elaborated optimization but, at same time, allowed us to obtain SAW sensors with comparable surface densities.

In particular, the wtbOBP and the dmbOBP were dissolved in Tris/HCl (10 mM, pH=8.0), whereas the wtpOBP was dissolved in PBS (10 mM, pH=7.4). An amount of 5 μl of the protein solutions with a concentration of 2 mg/ml was dropped on the surface of the SAW device. After deposition, the SAW biosensors were placed under a N_2 flux to promote the evaporation of the solvent. Then, they were washed by a gentle deionized water flow to remove the unbounded biomolecules. An additional N_2 flux was applied to dry the surface of the device. The SAW biosensors were tested before and after OBP depositions by microwave probes. The S_{21} scatter parameter was measured in both magnitude and phase formats using the Network Analyzer.

The surface densities (m/A) of the three OBP coatings were calculated considering the mass sensitivity given by the following equation (Grate and Klusty, 1991):

$$\Delta f = (k_1 + k_2) f_0^2 \frac{m}{A} \quad (1)$$

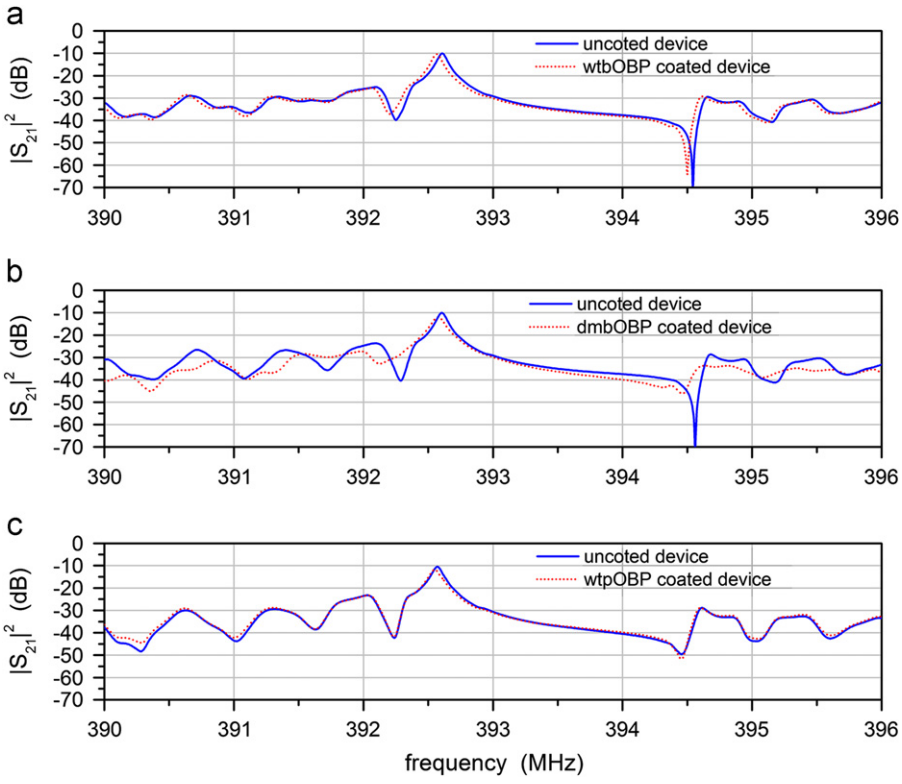


Fig. 1. Frequency response of the SAW resonator (amplitude of S_{21}) before (continuous line) and after (dotted line) deposition of OBPs: (a) wtbOBP coated device, (b) dmbOBP coated device, and (c) wtpOBP coated device. The frequency shift is proportional to surface density of the protein on the SAW device.

where f_0 is the fundamental resonant frequency, $k_1$and k_2 are material constants of the piezoelectric substrate and Δf is the frequency shift caused by the deposition of a mass m on the active area (A) of the SAW biosensor. For α-quartz (ST-cut, x propagation), $k_1 = -8.7 \times 10^{-8}$ and $k_2 = -3.9 \times 10^{-8}$ (Grate and Klusty, 1991).

3. Results and discussion

3.1. Surface densities of OBP depositions

The surface densities of the proteins on the SAW devices were measured with the method described in Section 2.5. For the SAW biosensor coated with the wtbOBP, considering a molecular weight (MW) of 37,000 Da and a frequency shift of 45 kHz (Fig. 1(a)), the obtained surface density is 2.31×10^{-6} kg/m^2, corresponding to 3.7×10^4 molecules/μm^2. The surface density obtained for the SAW biosensor coated with the dmbOBP (MW=18,500 Da) and characterized by a frequency shift of 24 kHz (Fig. 1(b)) is 1.23×10^{-6} kg/m^2 (4.0×10^4 molecules/μm^2). The SAW biosensor coated with the wtpOBP (MW=22,000 Da) shows a frequency shift of 23 kHz (Fig. 1(c)) and a surface density of 1.18×10^{-6} kg/m^2 (3.2×10^4 molecules/μm^2).

3.2. SAW biosensor measurements

The SAW biosensor system was exposed to different concentrations of the odorants, in the range between 13 and 61 ppm for octenol and between 9 and 80 ppm for carvone, in N_2 atmosphere and the frequency shifts of the difference between the resonance frequency of the uncoated reference device and the resonance frequency of the SAW sensors were measured. The SAW biosensor system showed a fast, remarkable and reversible response to the selected odorants, indeed the main response was registered within 30 s from exposure. As an example, the real-time response of the wtbOBP coated SAW sensor for different concentrations of octenol in the range from 13 ppm to 61 ppm is reported in Fig. 2(a).

Moreover, the SAW biosensor system showed a good repeatability as observed by the multiple responses obtained with the same concentration of the odorant compound. The time response of the wtbOBP coated sensor to 61 ppm of octenol repeated three times, showed a mean frequency shift of about 205 Hz (Fig. 2(b)).

The response curves related to the three different proteins used (wtbOBP, dmbOBP and pOBP) to octenol and carvone showed a linear behaviour in the tested concentration ranges. In Fig. 3(a) the response curves for the three proteins upon exposure to different concentrations of octenol, i.e. from 13 ppm to 61 ppm, are shown. Each point in Fig. 3(a) is an average of three independent measurements shown with standard deviation as an error bar. The linear fit of these points is reported as a straight line.

For wtbOBP-based SAW biosensor, a sensitivity of 3.5 Hz/ppm was obtained with a calculated detection limit of 2.8 ppm. In the case of the dmbOBP-based SAW biosensor, the sensitivity and detection limit were 6 Hz/ppm and 1.6 ppm, respectively. Finally, for the pOBP-based SAW biosensor a sensitivity of 25.9 Hz/ppm was obtained and a detection limit of 0.39 ppm was calculated.

The higher sensitivity to the octenol was obtained for the pOBP-based SAW biosensor. This result suggested that octenol molecule fits in the hydrophobic funnel of the pOBP better than the two bovine OBPs used in the other two SAW biosensors. This finding points out that the three different OBPs used in our measurements possess a different specificity to different odorant molecules indicating the possibility of their simultaneous use for the design of a sensor array.

The response curves to carvone from 9 ppm to 80 ppm are reported in Fig. 3(b). In this case, dmbOBP-based SAW biosensor exhibited the best sensitivity (S=9.2 Hz/ppm) and detection limit (DL=1.1 ppm). The obtained sensitivity and detection limit for wtbOBP-based SAW biosensor and wtpOBP-based SAW biosensors were, respectively, 5.4 Hz/ppm and 1.8 ppm, and 7 Hz/ppm and 1.4 ppm. The higher sensitivity to the carvone was obtained for the

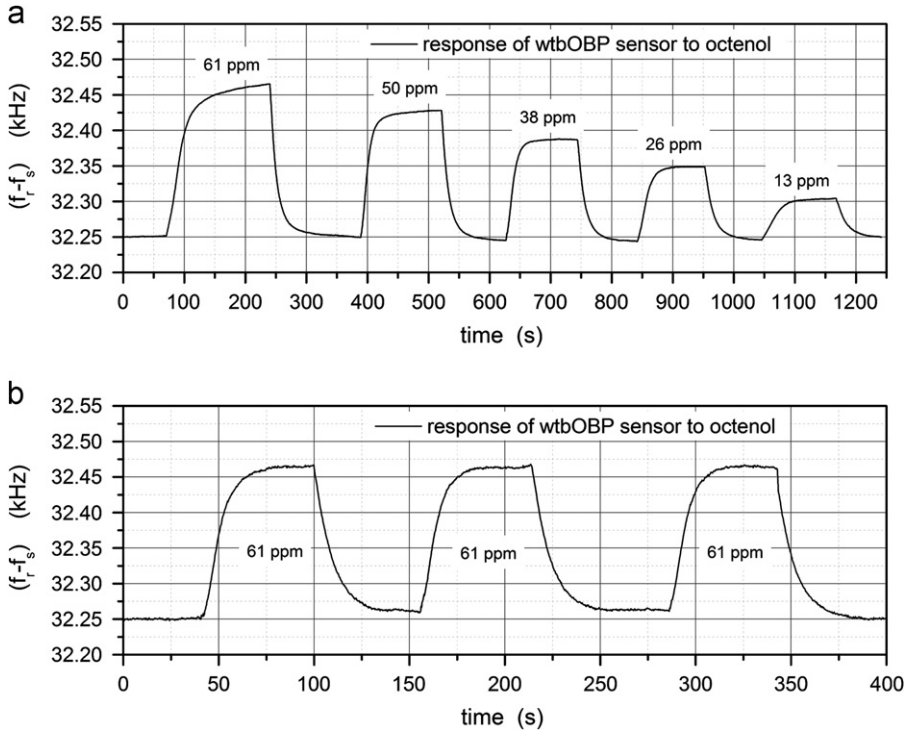


Fig. 2. wtbOBP-based biosensor frequency response vs. time to octenol: (a) decreasing concentrations from 61 ppm to 13 ppm; (b) three repeated cycles of 61 ppm. The response is the difference between the reference and the wtbOBP-based biosensor frequency shifts.

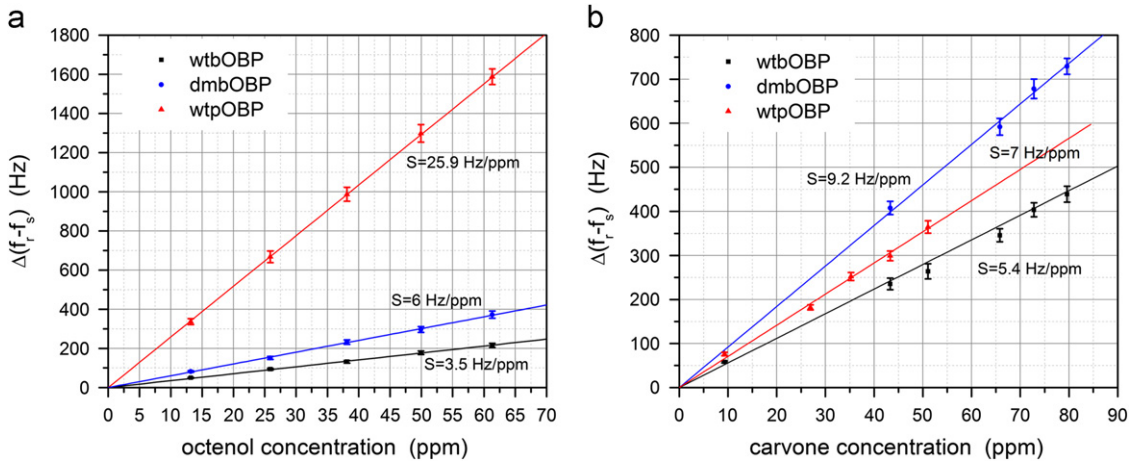


Fig. 3. (a) Response curves for wtbOBP, dmbOBP and wtpOBP based biosensors upon exposure to different concentrations of octenol; (b) response curves for wtbOBP, dmbOBP and wtpOBP based biosensors upon exposure to different concentrations of carvone. Error bars indicate the repeatability.

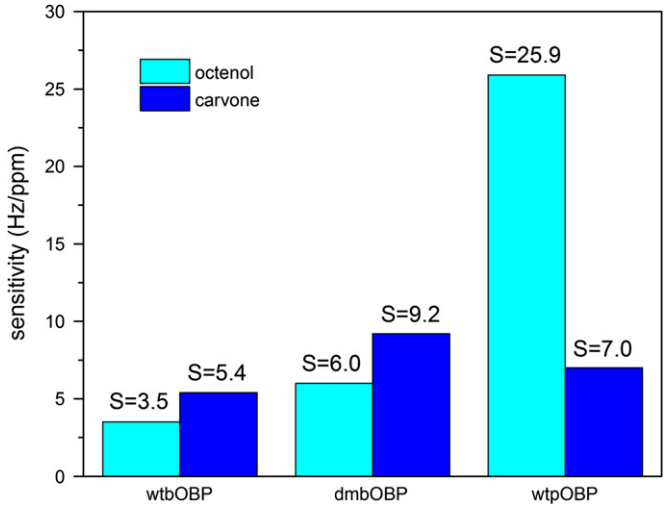


Fig. 4. Comparison between sensitivities to octenol and carvone detection of the wtbOBP, dmbOBP and wtpOBP based biosensors.

dmbOBP-based SAW biosensor. The SAW biosensors showed a good repeatability as indicated by the error bars reported in Fig. 3.

The detection limits of the proposed biosensor system are comparable with those obtained by similar systems based on QCM for the detection of alcohols (Sankaran et al., 2011), but the achieved sensitivities are much higher.

A comparison between the sensitivities of the three SAW biosensors toward the tested odorants is shown in Fig. 4. The wtbOBP-based SAW biosensor and the dmbOBP-based SAW biosensor showed a higher sensitivity to the carvone odorant, respect to the octenol. On the contrary, the wtpOBP-based SAW biosensor showed the higher sensitivity toward octenol. These results pointed out that our SAW biosensor array is able to discriminate octenol from carvone.

The reproducibility of surface densities of the OBP coating achieved by the droplet method was appropriate to test the sensing capabilities of the three selected OBPs even if this deposition technique could not be suitable for the fabrication of the sensors in a large-scale manufacturing process. In this regards, different deposition techniques will be investigated. In particular, one promising deposition method could be the Laser Induced Forward Transfer (LIFT) technique. This process was, in fact, already used to fabricate DNA and biomolecules microarrays obtaining good uniformity and reproducible films (Serra et al., 2004).

4. Conclusions

A novel SAW biosensor array system, exploiting protein based sensitive layers with the capability to detect and discriminate different odorant molecules, has been fabricated and tested. The comparison of the sensing capabilities of three different OBPs for detection of octenol and carvone has been performed.

The used OBPs, selected for the properties to preserve their full functionality when exposed to air environment, were deposited by means of the droplet method on the SAW device surfaces. The immobilization of the proteins on the Au/Al transducers was confirmed by the resonant frequency shifts of the SAW devices after the deposition of the biomolecules. The frequency shift, measured at Network Analyzer, for the three sensors was in the range between 23 and 45 kHz. The obtained surface densities of OBP layers, calculated considering the mass sensitivity of the SAW devices, were 2.31×10^{-6} kg/m^2 for the wtbOPB, 1.23×10^{-6} kg/m^2 for the dmbOBP and 1.18×10^{-6} kg/m^2 for the wtpOBP. The developed OBPs-based SAW biosensors were able to detect low concentrations of octenol (13 ppm) and carvone (9 ppm). The highest sensitivity for octenol detection (25.9 Hz/ppm) was obtained by using the wtpOBP-based SAW biosensor, whereas the highest sensitivity for carvone detection (9.2 Hz/ppm) was obtained using the dmbOBP-based SAW biosensor. The different sensitivities showed by the three SAW biosensors indicated the capability of the SAW biosensor system to discriminate between octenol and carvone. The response curves of the three OBPs-based SAW biosensors were linear for all tested concentrations of the odorant compounds.

Future work will investigate the application of the SAW biosensor system for sensing of food flavour to assess their quality, and for the detection of explosives and drugs. For this purposes, other biomolecules will be tested as sensitive layers for the SAW biosensor systems.

The aging of the system regarding in particular the stability of OBPs on the SAW devices and their functionality will be considered.

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