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Sensitive coating for water vapors detection based on thermally sputtered calcein thin films

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ABSTRACT

In this paper the adsorption properties of thermally sputtered calcein thin films towards water and other polar molecules vapors are studied by different characterization techniques: quartz crystal microbalance, surface plasmon resonance and visible spectroscopy. Sensitivity of calcein thin films to water vapors resulted much higher as compared with those of a number of dyes whose structure was close to that of calcein. All types of sensors with calcein coatings have demonstrated linear concentration dependences in the wide range of water vapor pressure from low concentrations up to 27,000 ppm (close to saturation). At higher concentrations of water vapor all sensors demonstrate the abrupt increase of the response (up to two orders). A theoretical model is advanced explaining the adsorption properties of calcein thin films taking into account their chemical structure and peculiarities of molecular packing. The possibility of application of thermally sputtered calcein films in sensing technique is discussed.

1. Introduction

Humidity sensors can be found in a variety of applications: environmental monitoring, air conditioning, storage, biotechnologies, agriculture, electronics, and so on. Their wide range use has resulted in numerous sensor designs that typically incorporate capacitive, resistive, gravimetric or optical techniques. At present, there are many various humidity sensors with inorganic as well as organic sensitive coatings. Among those based on inorganic compounds, there are rather highly sensitive ones, with small hysteresis and good reproducibility [1–9]. The most frequently used materials in this area are silicon nanostructures [1], SnO_2 [2], TiO_2 [3], ZnO [4], as well as combined inorganic–inorganic [5] and organic–inorganic [6–8] layers. In recent years, much attention is paid to coatings based on nanostructured materials [3–9]. Among the sensors based on organic materials, the most widespread are those with conductive polymers [10–16].

Current research is directed towards the continual improvement of sensor properties such as sensitivity, durability, reliability, range of humidity and temperature operation, hysteresis, size and cost [17,18].

Calcein, also known as fluorexon, fluorescein complex, is a fluorescent dye that has a number of properties that make it promising for application in chemical sensing. Calcein is a stable compound that decomposes at a rather high temperature (300 °C), so thin films made with this material may be deposited using thermal sputtering.

It is known that calcein and its derivatives were used for detection of calcium [19,20] and other metal ions (Cu^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+}, and Fe^{3+}) [21] and as fluorescent sensor for amino acids [22], as well as fluorescent marker [23] etc. In [24], it was reported on application of calcein-PAN composite [calcein-poly(acrylonitrile)] as well as polymer film of calcein–HMDA (hexamethylene diamine) for ammonia sensor. Nevertheless, the possibilities of application of this material in sensor technique are not studied completely. For example, we did not find in the literature references to application of thermally deposited calcein films for detection of water molecules.

In the same time, due to some features of calcein molecule structure, this material is very promising for the humidity sensing. A calcein molecule contains hydroxyl (–OH), oxy (=O) and carboxyl (–COOH) groups as well as two tertiary atoms of nitrogen ($–N(CH_2COOH)_2$) that can form hydrogen bonds with water molecules as well as various (in)organic compounds. The geminate arrangement of substituents promotes actively formation of hydrogen bonds as well.

The aim of this work is to propose the investigation of adsorption properties of thermally sputtered calcein thin films towards

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water vapors molecules. A comparison with the sensing properties towards different polar molecules such as those of alcohol vapors is also proposed. Both optical and mass-sensitive techniques were used to this purpose, namely quartz crystal microbalance technique, (QCM), surface plasmon resonance (SPR) technique and visible spectroscopy. The sensing properties of calcein thin films were compared with those of number of heterocyclic compounds whose structure is close to that of calcein (such as Rhodamine 6G, thionine, tertbutylcalix/6/arene and lead phthalocyanine). Some theoretical explanation of the possible chemical mechanism involved in the interaction between the investigated sensing layers and the analytes is finally withdrawn.

2. Materials and methods

Thermal sputtering in vacuum was used for the deposition of different kind of materials in thin film form, namely calcein, Rhodamine 6G, thionine, tertbutylcalix/6/arene (C[6]A) and lead phthalocyanine (PbPc) (Sigma–Aldrich). Thin films were deposited at a constant deposition rate of 0.1 nm/min from a Knudsen cell at room temperature; a quartz thickness meter was used to check film thickness in the course of deposition. Each coating (about 30 nm) was deposited in the course of a single cycle of sputtering onto different substrates depending on the characterization to be done: silver electrodes of QCM sensors, SPR chips (namely, 45 nm Au thin films thermally evaporated on a 15 mm × 15 mm × 1 mm glass substrate with a refractive index of 1.61) and 5 mm × 12 mm × 1 mm glass plates for spectral investigations.

Doubly deionized water (Diana, Ukraine), ethanol and methanol (ChemLabourReagent, Ukraine) were used as analytes.

2.1. QCM characterization

A home-made experimental set-up was used. Proper QCM sensors (AT-cut quartz resonators with silver electrodes, resonant frequency 10 MHz), coated with the investigated sensing layers were placed in a measuring flow-through cell and were held in an argon flow until the frequency of quartz resonators stabilized (deviation of ±2 Hz). An analyte in the liquid phase was maintained in the vial till the saturation level in the gas phase over the liquid surface was achieved. Then, the dry argon flow carried the analytes vapor through the measurement cell (the concentration of an analyte was close to the saturated vapor). The shift of sensor oscillation frequency, that was proportional to sensor mass change caused by adsorption of molecules of the substance under investigation, was recorded [25]. The whole volume of the measuring cell was maintained at a constant temperature (22 °C). Concentration dependences were measured in the steady-state cell. The required concentrations were set by injecting the known amount of saturated vapor to the measurement cell with given volume.

2.2. SPR characterization

Angle-resolved SPR measurements were performed using a scanning SPR spectrometer “BioHelper 2.0” (ISP NASU) [26] with a semi-cylindrical prism (refractive index of 1.61). A gas cell (volume of 0.5 ml) operated in the flow mode. The concentration dependence measurement was performed in tandem with QCM.

2.3. Optical absorption characterization

Optical sensing tests were performed using a filtered light source from AVANTES tungsten–halogen lamp, guided into an optical fiber. The absorption spectra (in 350–800 nm range) were collected and analyzed using a commercial spectrophotometer AVANTES mod. MC 2000 [27]. The effect of the analyte vapor on the absorption properties of the active layer was measured in a home-made dynamic pressure system where dry air at ambient pressure was used as carrier and reference gas. The spectra of absorption were measured in dry air as well as under presence of water vapor of preset concentration. The optical response, i.e., an integral I of the absorption spectrum over light wavelength in the 400–600 nm range was measured with the aim to obtain its concentration dependence. After stabilization of signal in the dry air flow and measuring I_{air}, water vapor of preset concentration was supplied to the cell. Then, after time interval required for signal stabilization at a new level, we measured the integral I_{water}. The difference $\Delta I = I_{\mathrm{water}} - I_{\mathrm{air}}$ of the above integrals represented the sensor response.

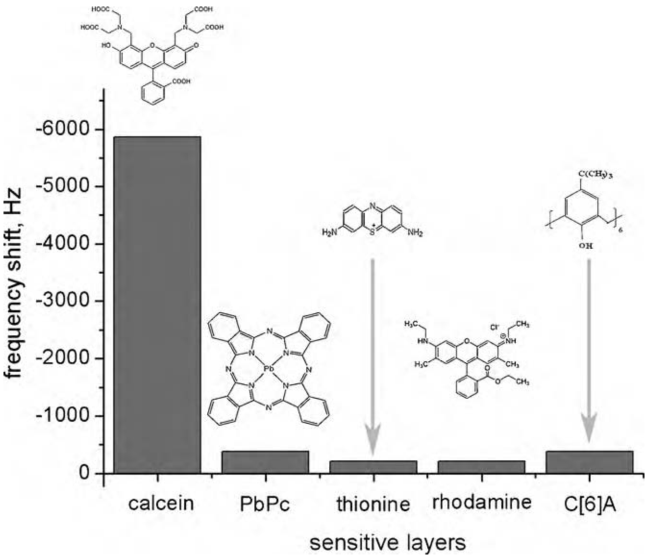

Fig. 1. QCM sensor response on saturated water vapor for different sensors: calcein, PbPc, thionine, rhodamine 6G, and calixarene C[6]A.

2.4. X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) was performed for the QCM sample with 30 nm calcein film using SERIES-8XPS Kratos analytical instrument. Photoelectrons were excited using Al Kα 1486.6 eV X-ray irradiation.

3. Results and discussion

The response of QCM sensor coated by calcein film to saturated water vapor is more than one order higher than that for ethanol and methanol and totals −5860 Hz (the responses for ethanol and methanol are −88 Hz and −116 Hz, respectively).

Fig. 1 presents the responses of QCM sensors with thermally sputtered films of calcein, lead phthalocyanine, thionine, Rhodamine 6G and calixarene C[6]A to saturated water vapors. The structural formulas of adsorbates are placed above the corresponding bars in the diagram.

Calcein thin film demonstrates higher response to water vapor molecules by more than an order of magnitude with respect, in particular, to Rhodamine, that belongs to the same group (fluorenes) as calcein.

SPR curves measured for calcein thin films deposited on proper Au/glass substrates and recorded in dry argon flux and under presence of saturated water vapor (29,400 ppm) are shown in Fig. 2a. The following two features are to be noted: (i) shift of the angle of minimum towards bigger values, and (ii) considerable increase of reflected light intensity in the minimum under adsorption of

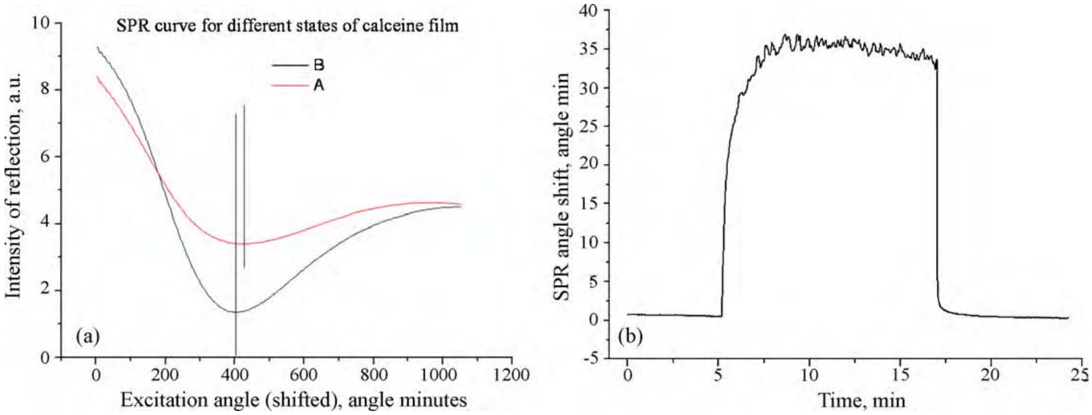

Fig. 2. SPR curves for an SPR chip coated with calcein taken in dry argon and saturated water vapor (a) and kinetics of SPR angle variation at calcein film exposure to saturated water vapor (b).

water molecules. The above effects are reversible: after returning to dry argon, the SPR curve tends to its initial value. (The kinetics of adsorption and desorption is presented in Fig. 2b.)

The concentration dependences for QCM and SPR responses to water vapor were measured parallel (the devices worked in tandem). The dependence for QCM sensor is shown in Fig. 3. The dependence for SPR sensor has similar character and is not presented.

The dependence has linear character up to 27,000 ppm with the abrupt increase of response magnitude in the region close to saturation.

The optical absorption spectra of calcein thin film in dry air and in the saturated water vapor conditions are shown in Fig. 4. When the adsorption of water vapors molecules occurs, absorption increases at wavelengths below 600 nm, while the absorption band width remains unchanged.

The concentration dependence of optical response ΔI of calcein thin film to the water vapors has similar to the QCM and SPR sensors character: linear in the wide concentrations range (5000–25,000 ppm) with abrupt increase of the response at higher concentrations.

The results obtained have demonstrated extremely high sensitivity of calcein thin films to water vapors of high concentration with all investigation techniques. The sensor responses to water vapors molecules obtained with SPR and QCM techniques are one-two orders higher than those to alcohols. For all sensors, the responses are linear functions of water vapor concentration up to 25,000–27,000 ppm while at higher pressures the sensitivity increases abruptly.

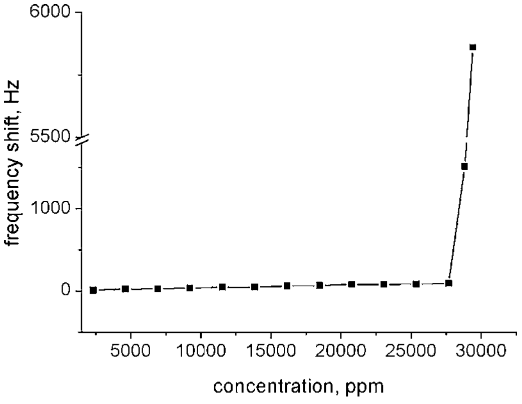

Fig. 3. The response of QCM sensor as function of water vapor concentration.

The response time is about 2 min for QCM as well as SPR sensor. The responses of both sensors are reversible: the signals return to zero level after pure argon flow. In this case, the SPR-based sensor demonstrates the best speed of signal recovery: the SPR curve returns to zero level at replacement of water vapor with dry argon in 5 s (see Fig. 2b).

In the following section the peculiarities of water-sensitive properties of calcein film and possible mechanisms of the effects are discussed.

4. Mechanism of adsorption of water molecules onto calcein thin film

Shown in Fig. 1 are the structural formulas of molecules of the sorbent materials under investigation. Three of them (calcein, Rhodamine 6G and thionine) are flat. In a lead phthalocyanine molecule, the lead atom projects beyond the ligand plane due to its big size. The spatial geometry of calixarene has hexagonal symmetry. Each of the above molecules can form hydrogen bonds. For Rhodamine 6G, this occurs via the oxygen atoms in the substituted aromatic rings, while for thionine and phthalocyanine via the nitrogen atoms, and for calix/6/arene via oxygen in the phenol fragment. Nevertheless, only calcein demonstrates extremely high sensitivity to water vapor of high concentration.

Calcein molecule has several active centers [–OH; –COOH; –N(COOH)$_2$] able to form hydrogen bonds. The arrangement of these centers relative to each other is vicinal. The combination of

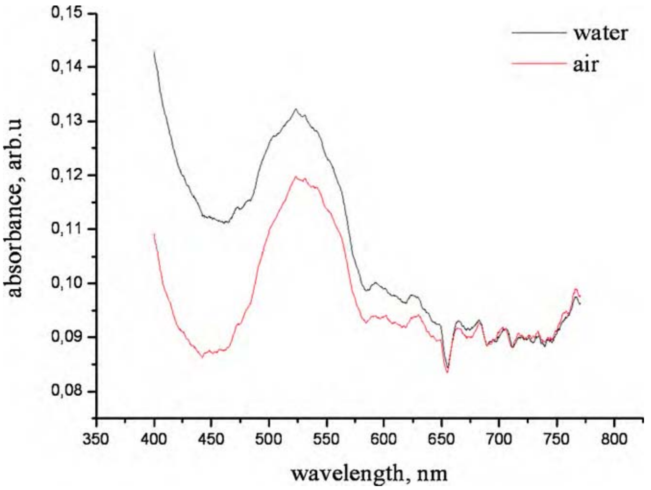

Fig. 4. Optical absorption spectra of calcein film in dry air and in water vapor.

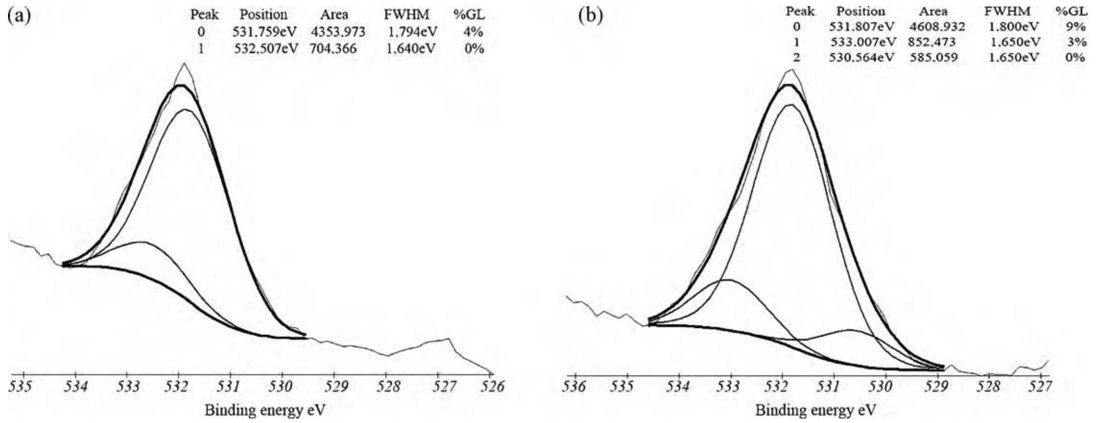

Fig. 5. O 1s XPS spectra of calcein film before (a) and after (b) treatment in saturated water vapor.

these factors creates conditions for water clusters forming. Their size depends on the concentration of water vapor in argon + water mixture. A water vapor molecule, unlike those of methanol and ethanol, is characterized by its smaller size and also by the presence of two hydrogen atoms that can form a hydrogen bond. This feature itself plays decisive role in formation of water clusters in the course of adsorption onto surface.

When comparing the chemical structure of calcein with that of its analog in the heterocyclic series, rhodamine 6G, it is possible to note a number of key distinctions. Thus, rhodamine has no carboxyl and hydroxyl groups, the two tertiary atoms of nitrogen (that are constituents of rhodamine molecule) are inherently aniline ones. This means that their lone electron pair (that is responsible for formation of hydrogen bond) becomes coupled to aromatic system of the heterocyclic nucleus, thus decreasing the hydrogen bonding activity. In addition, one of the nitrogen atoms in a rhodamine molecule is deactivated by cation formation ($=NH^+–C_2H_5$) when interacting with a molecule of hydrochloric acid.

For thionine, along with presence of aniline atoms of nitrogen, the positively charged thiazine fragment leads to appearance of high electron deficiency of the phenothiazine ring. This results in a greater extent of deactivation of active centers owing to considerable delocalization of the lone electron pair of nitrogen atoms in the phenothiazine heterocyclic system. In the case of tert-butyl calixarenes, low adsorption of water may be related to high hydrophobicity inherent in substances with high content of methyl groups [28]. The sensitivity of lead phthalocyanine film to water molecules (that is “the highest of the lowest” see Fig. 1) follows naturally from the property of metal-containing molecules to form coordination bonds with adsorbates [29].

The important role of hydrogen bonds in the calcein–water interaction can be proved by the O 1s XPS spectra presented in Fig. 5.

In the O 1s spectra of calcein film (Fig. 5a) two peaks are observed. One of them, π bond with 531,759 eV energy, can be related to the carbonyl bond C=O, while the second one (σ bond with 532,507 eV) corresponds to the C–O bond. After treatment of the sample in saturated water vapor the additional peak (530,564 eV) appears which can be related to the oxygen bonded with hydrogen (O–H). At the same time, other two peaks are shifted (the new values are 531,807 eV and 533,007 eV).

The formation of hydrogen bonds may occur not only between the calcein and water molecules but between two calcein molecules as well. Thus, several calcein molecules can form a flat cluster. The latter, in its turn, may form multiple-decker clusters because of π–π interaction between the aromatic rings [30]. As a result, in the course of calcein film deposition onto a substrate, the deposited molecules form multiple-decker clusters and held as multilayers.

In the course of water adsorption new hydrogen bonds are formed between the water vapors molecules and active groups of calcein molecules. As a result, the calcein molecules of a cluster get separated, and the hydrogen-bonded groups (mostly COOH) are saturated with water molecules.

Such changes may occur, first of all, in the stressed areas where flat calcein molecules make small angles, and calcein–calcein hydrogen bonding is somewhat weakened. Owing to redirection of hydrogen bonds to water molecules, the calcein fragments may change the above angles and occupy some other position that corresponds to lower value of free energy.

As to the increase of optical absorption in the high-energy optical spectral region, one should take into account that, on the one hand, appearance of water clusters in calcein film voids promotes dissipation of the energy absorbed via its transfer to the surrounding aromatic fragments. On the other hand, spatially nonuniform adsorption of water molecules in nonequilibrium thermally deposited calcein films has to produce fluctuations of refractive index, in both the nanopores (where they replace the argon (air) molecules) and the bulk of the basic material where structural relaxation occurs after thermal sputtering. From the AFM data (not presented) we can suppose that size d of such regions is several nanometers, i.e., much below the wavelength λ: $X = d/\lambda \ll 1$. Therefore, it seems reasonable to consider decrease of calcein film transparency at water adsorption as consequence of light scattering on fluctuations of refractive index of the film.

Thus, high sensitivity of calcein to water vapors can be explained by the presence of several centers capable of forming hydrogen bonds, as well as the features of their arrangement. The structure of thermally sputtered calcein film is of importance too. At adsorption of water molecules in the stressed areas where the calcein–calcein hydrogen bond is somewhat weakened, the spatial arrangement of calcein molecules changes because of hydrogen bonds are redirected to water molecules.

5. Sensing performances of calcein thin films

Thermally sputtered calcein films demonstrate reversibility of response, good repeatability and long-term stability. Fig. 6 demonstrates series of repeatable measurements with QCM sensor (analyte – saturated water vapor). The measurement is performed with 2-year old sensor which has been totally exposed to water and other analytes more than 50 h.

The response to water is 14% lower comparing with a new calcein sensor and totals on average 4990 Hz with standard deviation 60.2 (1.5% from the average response). Since all other properties remain the same (the character of concentration dependence, sensitivity to water of high concentration) we consider this result to be

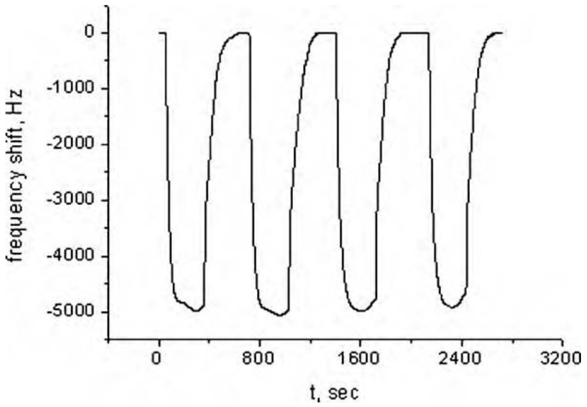

Fig. 6. The kinetics of QCM sensor response on the cyclic replacement of dry argon by saturated water vapor and vice versa.

good from the point of view of long-term stability and the practical use of calcein-based sensors.

The QCM-based sensor demonstrates the highest sensitivity to the water vapors of high concentration. On the other hand, the SPR technique demonstrates the highest operation speed. Replacement of water vapor with dry argon is detected within 5 s (Fig. 2b). This may be related to the features of the optical and gravimetric mechanisms of adsorption detection. Indeed, the gravimetric technique determines directly just weight, i.e., the number of adsorbed molecules. Contrary to this, the optical technique deals with adsorption but indirectly, via variation of either refractive index or thickness of the film-sorbent. Really, contribution of adsorption to the optical response is proportional to the phase thickness $\delta = N \times d \times \cos \Phi$, where N is refraction coefficient, Φ is the angle of incidence, and d is the thickness. Therefore, as quick adsorption or removal of molecules from a calcein film occurs, the film thickness may vary much quicker than the refractive index decreases. Just this effect may be the reason for the fact that optical response is much quicker than that in the QCM technique.

Bearing in mind practical application, the measurement of optical absorption is less attractive because the optical technique has lower sensitivity and requires more time for measurement. Thus, one should choose either the QCM or SPR technique, depending on the specific task.

6. Conclusions

In this work, different physical effects occurring when exposing hydrophilic calcein thin films to water vapors, namely, variations of mass, optical absorption, and SPR angle have been studied. Very high response of calcein-based sensors to high concentration water vapor was observed. The concentration dependences of QCM, SPR and optical sensors responses to water vapor are linear up to 25,000–27,000 ppm with abrupt increase of sensitivity at higher concentrations.

The demonstrated properties of calcein can be related to specific arrangement of hydrogen-bonded groups on the periphery of calcein molecule, as well as to the structure of thermally sputtered films.

The above-mentioned properties of thermally sputtered calcein film are of great scientific and practical interest.

From the point of view of the application of calcein films as sensitive coating of chemical sensors, mass-sensitive and SPR sensors are the most promising. The QCM sensor ensures higher sensitivity than SPR one and may be integrated into sensor arrays. At the same time, the SPR technique ensures higher operation speed.

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