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ARTICLE TYPE

Analyte induced water adsorbability in gas phase biosensors: the influence of ethynylestradiol on the water binding protein capacity

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An ultrahigh-sensitive gas phase biosensor/tracer/bio-sniffer is an emerging technology platform designed to provide real-time information on the air-borne analyts or ones in liquids through classical headspace analysis. The desired bio-sniffer measures gaseous 17ά – ethynylestradiol (ETED) as frequency change of quartz crystal microbalance (QCM), which is the result of interactions of liquid sample components in headspace (ETED and water) with biorecognition layer. The last one was constructed by immobilization of polyclonal antiserum against a phenolic A-ring of estrogenic receptors through protein A. The QCM response exhibited stretched exponential kinetic of negative frequency shifts with reversible and "irreversible" components of mass uptake onto the sensor surface in static headspace conditions when exposed to water solutions of ETED over the sensor working range, from 10^{-10} to 10^{-17} g/L. It was shown that the variations of QCM response characteristics are due to the change of a water-binding capacity of the sensing layer induced by proteins transformations initiated by binding of the ETED molecules. This result is well correlated with natural physiological function of estrogens in controlling the homeostasis of body fluids in living beings.

Introduction

The biochemical analyses of the modern medicine and biology become progressively more and more sophisticated. This factor, as well as environmental pollution, need in high-purity pharmaceuticals, requirements for food control, *etc.* determine particular interest of both consumers and specialists in problems dealing with sensor technology and alarm instrumentation. The distinguishing feature of the present-day problems in this area is the need for a quick analysis of low-molecular weight compounds demonstrating strong biological influence.[1] One could mention highly potent estrogenic steroids in biological or environmental liquids where water serves usually as their carrier.[2]

It is a common feeling, that the limitations relating to chemical sensor performance are specific to the sensitive layer.[3] This is a keystone in the context of chemical sensors, since the recognition "efficiency" of receptor sites on a certain transducer is the source of sensor functionality. It seems evident that chemists are presently in a strong position to have significant impact on future developments in materials for sensitive layers, but up to date, exclusive selectivity profile is only possible to find within the biological systems. Well-known natural candidate that may be utilized as the specific receptor molecule is antibody, - dedicated protein (*e.g.* immunoglobulin, IgG) specific against a target antigen (analyte).[4] Antibodies have been generated for a wide variety of antigens and are commercially available from numerous industrial sources. It is well understood that to maintain antibodies native structure and hence their prescribed functionality, they must be in an aqueous environment (for immunosensing with artificial antibodies in organic solvents see *e.g.*[5]). This knowledge has led to the accepted convention for their use as bioreceptors for chemical sensing purposes in liquid phase.[6,7]

To date there has been a limited amount of work reported on gas phase biosensors (GPB, "biosniffers") where biomolecules are utilized for the detection of specific air-borne analytes.[8-14] Instead of the single trials practically antibodies were initiated to use as sensing agent by the Guilbault group.[15-18] As an example, he reported the use of biomolecules on Quartz Crystal Microbalance (QCM) devices for vapor phase detection of formaldehyde and organophosphorous pesticides such as parathion.[19] However, subsequent studies demonstrate that situation is essentially more complicated and were unable to confirm the specificity of antibodies against air-borne antigens.[20] One explanation for the nonspecific binding was that in the absence of an aqueous environment, the binding sites on the antibodies will lose their prescribed structure required for molecular recognition. Now, the most important examples of GPB involve a label free detection mechanism are with antibodies entrapped in a semi-aqueous layer (hydrogel) to achieve molecular recognition of relatively small molecules in the vapor phase.[21-25]

Instead of the fact of some practical achievements of GPB[25] both sensing mechanisms and driven forces of the interfacial processes are still unclear. To realize the potential of the gas-phase biosensors it would be of interest to specify what is the carrier of sensor performance? Is it the native structure of recognition site, surface reconstruction, or the transduction

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mechanism itself? To gain greater insight into the response inducing mechanism of QCM based GPB there is a need to define the source of frequency change under exposure of air-borne analytes.

The present study deals with the fate of an important environmental xeno-estrogen, 17ά – ethinylestradiol (ETED). The widespread use of ETED as the active agent of many pharmaceutical formulations (typically in the μg-mg range per pellet) results in continuous release and distribution of this substance in natural waters, - ETED was detected in surface water all over the world, mostly in the ng/L range. Due to its chemical properties, ETED is highly resistant to degradation processes and has the tendency to sorb to organic matter, to accumulate in sediments and to concentrate in organisms along the food chain.[26]

To realize GPB concept for detection of ethinylestradiol in gaseous phase we used polyclonal antibodies reacted with compounds which possessed a phenolic A-ring of estrogenic receptors to modify the surface of QCM transducers (Fig. 1). These antibodies recognize relatively common molecular fragments and, hopefully, their recognition epitope will not be so drastically changed outside the aquatic environment to prevent the molecular recognition of antigen.[27]

The paper is organized as follows: it begins by discussing the methodological aspects of the measurements, an interface modification protocols, experimental equipment *etc*. This is followed by a detailed analysis of our experimental data and peculiarities of the sensor response under various concentrations of ETED as well as selectivity tests with ceftazidime and sulfamethoxasole in static headspace environment. Finally, the mechanism of sensor response induced by antigen is discussed.

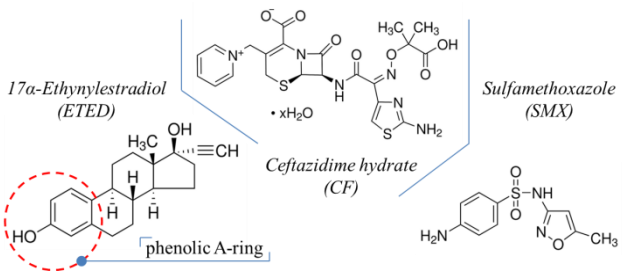

Fig. 1 Molecular structure of compounds to be analysed.

Materials and Methods

Materials

Protein A *Staphylococcus aureus*, 17ά - ethinylestradiol (ethinyl estradiol, ETED, EE2, mw 296.4 Da), ceftazidime hydrate (CF), sulfamethoxasole (SMX) and guanidine thiocyanat were received from Sigma-Aldrich. Antiserum was raised in rabbits to an immunogen of 17-β-estradiol-hemisuccinate-bovine serum albumin following a reported protocol and kindly given to us by Prof. F.Rowell and S. Armstrong from the University of Sunderland.[27]

Sample Preparation

ETED stock solution with concentration 10 μg•ml^{-1} was prepared by dissolution of 2 mg of ETED powder in 200 ml distilled water (water solubility of ETED is *c.a.* 4-11 μg•ml^{-1})[28]. Ceftazidime and sulfamethoxasole solutions with concentration 1 μg•ml^{-1} were prepared in distilled water immediately before measurements (water solubility of CF and SMX are *c.a.* 1-2 mg•ml^{-1} and 200-300 μg•ml^{-1} correspondingly)[29, 30].

Functionalization of the QCM Transducers

To construct GPB antibodies were immobilized onto the surface of a QCM device through protein A using biologically inspired self-assembling process as is shown schematically in Fig. 2. The antibody immobilization technique involved the following stages: (i) the silver surface of QCM transducer was treated with a $HCl/H_2O_2/H_2O$ mixture (15/15/70 v/v/v) to remove possible organic contaminants;[31] (ii) than QCM transducer was immersed in a guanidine thiocyanate solution (0.12 M, 5 min, to protect protein molecules against denaturation)[6,7, 32] followed by washing with water and then PBS; (iii) deposition of a solution of protein A (50 mkg/ml in PBS, 30 min) followed by surface washing with a buffer solution; (iv) deposition of the specific rabbit antiserum with high affinity to protein A in PBS (antiserum 1:1000 dilution, 60 min)[33], followed by further surface washing with a buffer solution and then water; (v) placed QCM sensor in a weighing bottle above water level at room temperature up to the measurements. Usually the measurements were performed during 48 h after the sensor's preparation.

Measurements Concept and Experimental Procedure

Acoustic sensors represent popular analytical tool for high precision chemical and biological sensing with real-time capability. When used in this capacity, often the sensor response is treated as a measure of mass adsorption to the sensor surface. This mass loading effect occurs when the incoming analyte molecule forms complex with the surface, and results in a decrease in the resonance frequency as defined by Sauerbreys' equation.[34,35] Typical mass sensitivities of uncoated QCM resonators at 10 MHz is in the range of 0.1-1.0 Hz•cm^2/ng, which is sufficiently high to detect low-molecular weight analytes[36]. QCM efficiency can be improved by mass amplification using very adsorbent materials which increase the adsorbed mass. The Sauerbreys' equation however, is only applicable if certain conditions are satisfied. These include the presence of a uniform, thin and rigid film that vibrates in phase with sensor surface, - so, the Sauerbreys' model describes correctly only the interfacial molecular complexes rigidly attached to the surface.[37] However, variations of mechanical properties (changes in the viscosity, the mechanical stiffness *etc*.) of interfacial architecture on the surface as well can lead to resonance frequency changes of QCM transducer.[38,39]

For experiments a full-automatic home-made 8-channel QCM-based array system with a time resolution of 1 s utilizing 10 MHz AT-cut RK169 transducers were used.[40] Shortly, QCM analyzer contain: (i) temperature controlled measurement camera with the sensor matrix of the flowing type; (ii) quartz generators block; (iii) block of the frequency measurement and RS232 sequential interface constructed on the base of a specialized microprocessor (AT89C2051); (iv) generator of the gas mixtures; (v) system collection and processing of the information on the base of personal computer[40-43]. To maximize correctness of measurements and prevent possible adsorption of components of gaseous mixtures on construction inside the sampling systems the simplest possible measurement configuration based on static

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headspace procedure was used (Fig. 2). Moreover, in this case the headspace is humidified to the limit at given temperature because the biological active coatings require a certain amount of moisture to behave as they normally would in solution. The QCM sensor was mounting in ground-in stopper of 30 ml weighing bottle containing clean water or ETED solution in the same water with given concentration and this setup is then connected into an oscillator circuit of measurement device. A typical measurement procedure involved the following stages at 20±1 °C temperature: (I) measurement the sensor response in static headspace with the clean water sample (10 ml) until the transducer frequency is stabilized (*c.a.* 2 Hz); (II) changing the water sample by the freshly prepared aqueous solution of ETED (10 ml) with given concentration and monitoring frequency change for *c.a.* 2 minutes; (III) change to clean water sample again and record until the QCM frequency returns to its initial value, if any; (IV) repeat steps (II) and (III). To overcome the problem of dehydration of the biomolecular film, the QCM sensors with biofilms were stored in hermetic weighing bottle above water level at room temperature.

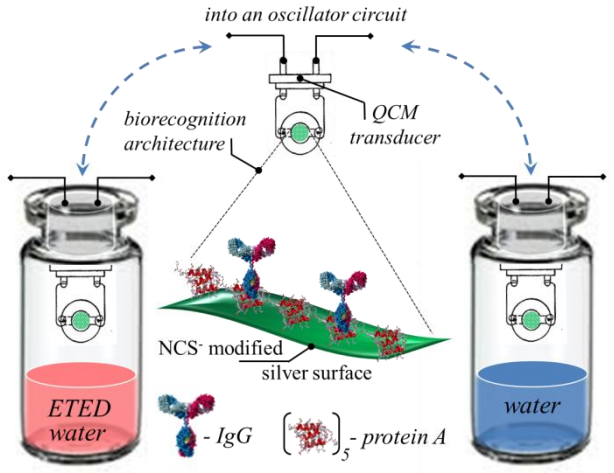

Fig. 2 Schematic diagram of core procedures is illustrating the analysis of ETED water solutions using a static headspace configuration. Insert: Scheme for the interfacial architecture on the QCM transducer surface. The silver electrode on the quartz had been modified by isothiocyanate, then protein A and finally anti-ETED antibody molecules IgG was immobilized on the surface with recognition epitops directed into the solution.

For measurements of ETED powders the same QCM based electronic nose instrument was used with transducers modified by thermally evaporated thin films (100 nm) of annulenes (dibenzotetraazaannylene, H_2TAA and tetramethyl-dibenzotetraazaannulene, H_2TMTAA), calixarenes (tret-butyl-calyx[4, 6, 8]arenes), polyacenes (tetracene and pentacene) as well as metal free phtalocyanine H_2Pc.[41]

Data Processing

The analysis of QCM kinetics was performed in the frame of the model that takes into account heterogeneous processes at the interface, using the stretched exponential function:[42,43]

$$\Delta QCM(t) = \Delta QCM_{max} \cdot \left(1 - exp\left(-\left({}^{t}/_{\tau}\right)^{\beta}\right)\right) \quad (1)$$

where ΔQCM_{max} is the saturation level of the response, τ is the characteristic time, and β is a parameter that indicates the mechanism of surface layer evolution. To approximate the curves the procedures of OriginPro 7.5 (OriginLab Corporation) were used.

The analysis of the I_S/I_0 ratio, where I_0 and I_S are total initial and balanced "irreversible" QCM responses (Fig.3), *versus* ETED's concentration in the water solution was performed in the frame of the competition model using the Morgan–Mercer–Flodin equation or the logistic curve for the data in the steady-state regime:[44]

$$I^* \sim \frac{1}{1+(\frac{[ETED]}{\gamma_0})^p} \quad (2)$$

where γ_0 is the normalizing factor and [ETED] is the concentration of ETED in aqueous solution. Power p represents the effective order of the reactions indicating the mechanism of the processes occurring in the system.

Typical kinetic dependence is shown in Fig.3b illustrates the contribution of the processes of reversible and "irreversible" sorption. It must be emphasized that for baseline recovery after experiments with observed "irreversible" sorption QCM sensor must be under saturated water vapor for at least 30 minutes.

Results and discussions

Mass uptake estimation

The typical QCM frequency variation for the antibody modified sensitive elements versus time taken for few ETED concentrations in water are shown in Fig.3. An analysis of the sensor response allows concluding that for all samples responses are monotonic and relatively quickly achieving the saturation level with absolute values of frequency variation mainly in the range of 300-800 Hz. In general, the response of the sensor remains in the above-mentioned limits by varying the concentration ETED in an aqueous solution of 100 to 10^{-5} ng/ml (10^{-10} to 10^{-17} g/L; *c.a.* 10^{-13} to 10^{-20} M/L), and demonstrates trend of increasing response with decreasing concentrations below 1 ng/ml. Instead of the fact that some deviations of the sensor response can be induced by measuring procedure and variations of the experimental conditions, there is no doubt that for so small concentration of ETED in solution one induces easy observed frequency change with signal to noise ratio greater than 100. Given the sensitivity of the QCM transducers (*c.a.* 1 ng/ Hz for transducer used), easily estimate the mass of the substance adsorbed on the sensor surface at various concentrations ETED in solution. It should be noted that since the QCM is in the gaseous phase, the actual quantity of ETED molecules within the headspace is much less than its concentration in solution. However, despite so low concentration of the ETED, the amount of the substances adsorbed on the surface of the QCM sensor is in picograms range. Comparison of these values with the amount of analyte in a solution (10 ml) shows that at a concentration ETED less than 10 ng/ml the absorbed mass on the QCM surface exceeds the total amount of ETED in the sample. The estimate obtained clearly indicates that observed QCM response cannot be due to the adsorption of only ETED.

In order to confirm this conclusion, we evaluated the adsorption capacity of a number of organic materials with

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different chemical functionality with respect to the saturated vapor of ETED, - ETED powder was placed in a sealed chamber with eight channel sensor array using similar QCM transducers at the same temperature (Fig.4). Saturation levels of the typical responses indicate that the increase in mass on the surface of the transducer as a result of interaction of different organic materials with the vapor ETED not exceed 40-50 ng (40-50 Hz). It is approximately an order of magnitude less than obtained for ETED solutions.

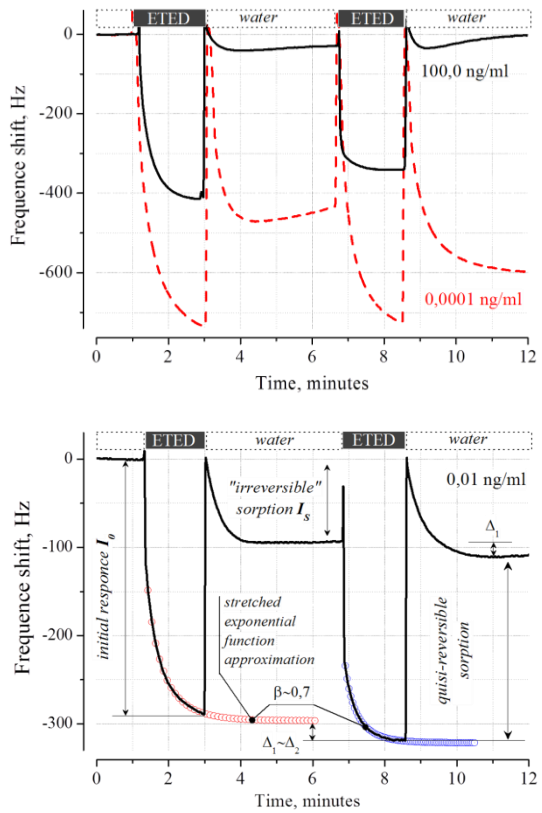

Fig. 3 Typical dependencies of the QCM frequency change on time for two serial repetitions of the same water solution of ETED at different concentrations (a, b) and approximation of response kinetic by stretched exponential function (b).

Mechanisms that can generate a response

Among the possible reasons for so great response of antibody modified QCM transducers in headspace of ETED solutions in water, should consider the following: (1) a change in the vapor pressure of water due to the dissolution of ETED, (2) the effect of ETED adsorption on elastic properties of the interfacial surface resulted in distortion of the Sauerbreys' equation, and (3) changes a water-binding capacity of the interfacial sensing architecture in consequence of binding ETED.

In accordance with the conventional theory of solutions, a dissolving agent reduces the vapor pressure of the solvent above its surface. This should lead to an increase in the water concentration in headspace decreases ETED in the sample. However, the non-monotonic change in the response (Fig. 5) suggests that at such low concentrations of dissolved ETED change in vapor pressure of the solvent can be neglected.

It is known that a change in elastic properties of the coating can also affect the change in the frequency of QCM.[38,39] It is a common expectations, that with the decreasing in the viscosity of the surface layer QCM response tends to decrease in respect to the "hard" structure with the same mass and geometry of the coating. In accordance with the data presented in Fig. 3 and 5 decreasing of the ETED concentration in the solution increases QCM response, - so one induces formation a more rigid structure. However, both values of averaged response and response change over the concentration range simultaneously cannot be explained by variations of mechanical properties of the interfacial structure. Thus, it is reasonable to assume that the adsorption of ETED molecules may change adsorption capacity of coating for water and one, probably, is the main mechanism of formation of the QCM response.

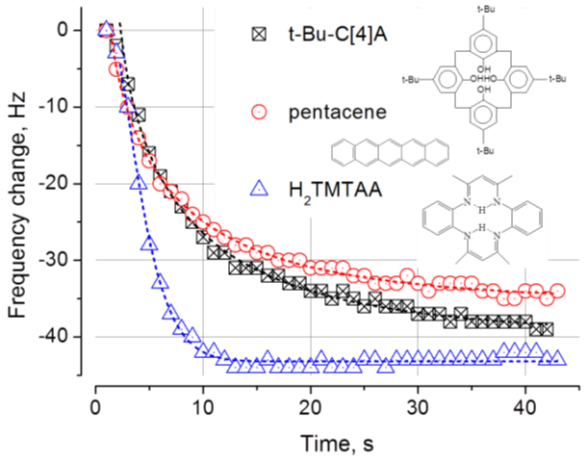

Fig. 4 Typical experimental dependencies of the frequency change on time for QCM transducers covered by tret-butyl-calyx[4]arene, pentacene and tetramethyl-dibenzotetraazaannulene for ETED powder.

Evolution of QCM response

The kinetic of the QCM response is well described by stretched exponential function (Fig. 3) with the value of the parameter β depending on the ETED concentration in solution, - β is successively changed with a decrease in the concentration of the analyte in solution. Decrease of β from 1 (characteristic of the Langmuir model) under ETED treatment indicates the presence of spatial and/or temporal (traps) restrictions on the movement of the analyte (water or ETED) to the adsorption sites on the surface.[42] However, there is a significant dependence of β on the history of the sample, mainly in recovery of the initial value after the exposure. Despite the fact that we have not studied in detail this question, we can formulate the observed trend - the smaller the initial response and higher its reversibility (smaller I_S), the closer the value of β to unity; it is the situation when the adsorption process is well described by the Langmuir model for spatially and energetically isotropic surface. If the magnitude of the initial response is greater than 600 Hz, the value of β tends to the value of 0.5, which indicates that diffusive transport at or near the surface dominate in the response formation.

Decrease of the concentration of the ETED in solution induces a transition from a reversible to the partially "irreversible" sorption with increasing magnitude of the initial response (Fig. 3 and 5). Reducing the concentration of less than 10^{-18} g/L (10^{-5}

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ng/ml) leads to a drop in response is probably due to too low concentration of ETED in gas phase, - at this concentration number of ETED molecules in solution roughly equal the amount of antibody epitopes on the surface.

Model of interfacial transformations

Summarizing the above-mentioned discussion it is reasonable to conclude that the variations of QCM response is due to the change of the sorption capacity of the coating for water induced by surface reorganization initiated by binding of the ETED molecules (Fig. 6). It is not so surprising because it is well known that water molecules can bind to the backbone and to polar and charged side chains of a protein. Depending on the nature of their side chains directed outside, proteins may bind various amounts of water – they have a water-binding capacity.[45] So, as ETEDs coupled to proteins at the interface, ones tend to bind more water, because increased charge or conformation changes results in increased affinity for water molecules. For example, the water binding capacity of a denatured protein is generally greater than that of the native protein.[46] However, if denaturation leads to aggregation of the protein, then its water binding capacity may actually decrease.

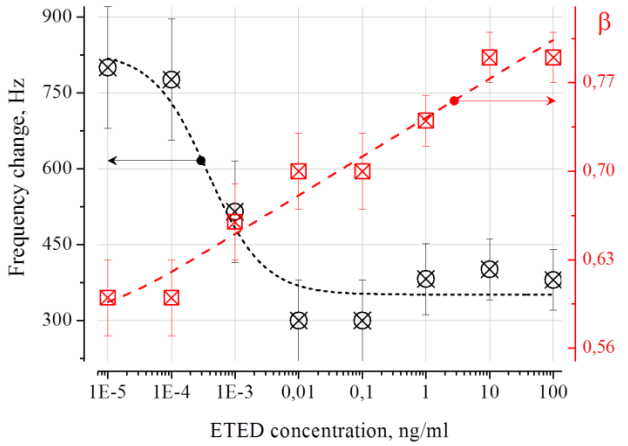

Fig. 5 Dependences of saturation level and parameter β versus ETED concentration in water.

One of the possible models of interfacial processes may be follows. At high concentrations of ETED in the gas phase ETED induced structural reorganization of large community of antibodies limited by steric conditions in a monomolecular layer of IgGs immobilized through the protein A. According to[7,8] biologically inspired protein A based self-assembling procedure get close-packed layers. As the result ETED induced IgG transformations may cause only limited conformational rearrangements of immunoglobulin opening access to only part of the "extra" hydrophilic regions of the protein globules, leading to "excessive" sorption of water molecules. The possibility of such conformational changes in antibodies induced by their binding to a selective antigen deeply described in the literature.[47]

Indeed, it was revealed that biological interactions such as analyte-receptor binding events are followed by a conformation change in receptor molecule .[48] Further work revealed high order (~10-12Å) of conformational changes during specific and non-specific antibody–antigen (specifically small molecules) interactions.[49] It was identified that the hypervariable loop motif responsible for molecular recognition involved in the majority of conformational changes during a binding event resulting in the rotation between heavy and light chains of IgG.

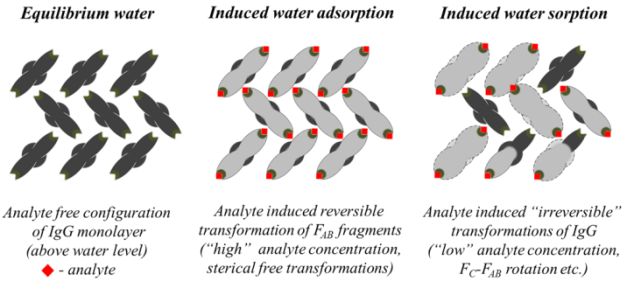

Fig.6 The schematic diagram of the core processes is illustrating the diversity of the supramolecular architectures formed by IgGs at different concentration of ETED in water.

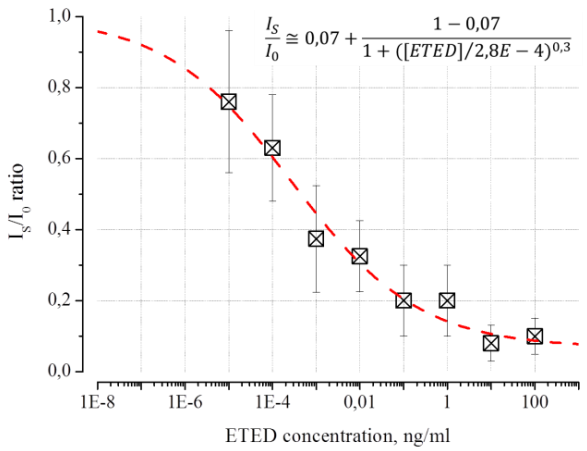

Fig. 7 Dependence of the relative response of the QM sensor with immobilized IgG for aqueous solutions of ETED at various concentrations. The best approximation according to Eq. (2) is shown by the dashed line; the parameters of the theoretical curve according to Eq. (2) are given in the insert.

When the concentration of ETED less than 1 ng/ml, the steric constraints imposed by the transformation of neighboring antibody molecules in the layer are weakened, allowing for a strong conformational rearrangement of part antigen-antibody complexes. Thus it can be assumed that the surface architecture with adsorbed ETED can be in two states, characterized by "high" and "low" adsorption capacity with respect to water molecules. Initially, the process is fast and reversible surface adsorption on sites become available owing to the ETED binding. This process dominates at high concentrations ETED and is not accompanied by significant structural changes within the bio-recognition layer (Fig. 6). When the concentration of ETED drops steric blocking for part of IgGs decreasing allowing more strong conformational changes in the protein components. This in turn stimulates the diffusion fluxes of water molecules inside the structure, which determines the decrease of β (Fig. 5). The presence of bound molecules within the new accessible areas originated owing to spatial transformations helps to prevent association of IgGs due to the fact that the bound water shields the protein molecules

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from each other so the protein dispersion within the interfacial architecture tends to be more stable. In this case, the most informative parameters associated with the concentration of ETED can serve as a ratio of reversible and "irreversible" components of the response, or "irreversible" (I_S) and full (I_0) of its initial value - I_S/I_0. Last choice as an informative parameter allows, among other things, to reduce the influence of the change of the adsorption capacity due to poorly-controlled experimental conditions. In this case there is a natural correlation between β values and the I_S/I_0 ratio so far as both of them represent the same process of interfacial sorption of water modulated by interactions of ETED with proteins on the surface.

Selectivity testing

As it was mentioned above in this study we used polyclonal antibodies which recognize relatively common molecular fragments and are cross-reactive against various steroidal drugs.[27] Accordingly, it was interesting to test the response of the surface architecture against analytes of nonsteroidal nature, but with fragments of "similar" structure. As examples were chosen sulfamethoxazole (SMX) and ceftazidime (CF) frequently used antibacterial drugs. Exposure of the surface architecture of the SMX and CF solutions with a concentration of *c.a.* 1 mkg/ml showed that in these cases there also observed response which in the case of SF is reversible, and for SMX - irreversible (Fig. 8). Analysis of kinetics indicates that in the case of SF the adsorption is limited by diffusion processes (β = 0,5), - probably because of the complex molecular structure of SF to achieve the necessary spatial orientation requires numerous acts of re-adsorption.[42] In the case of SMX interaction is similar to ETED (β ~ 0,7) but initially irreversible, resulting in an irreversible blocking of recognizing antibody, probably due to the presence of SO_2 groups.

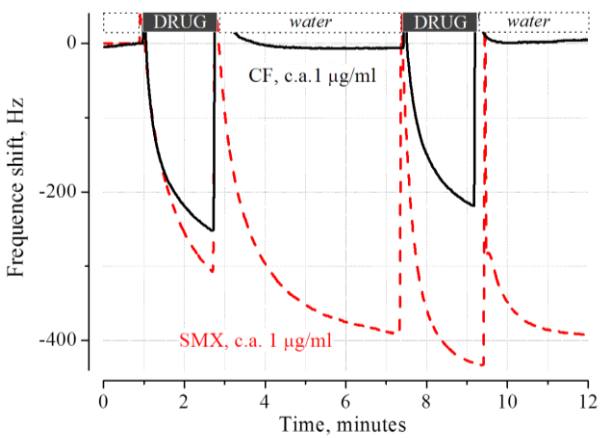

Fig. 8 Typical dependencies of the QCM frequency change on time for two serial repetitions of the same water solution of SMX and CF (c.a.1 μg/ml).

Conclusions

A decrease in activity in the field of molecular biosystems for the gas analysis in recent years is due to the lack of a clear understanding of the mechanisms of interaction of gaseous analyte with the biological receptor, when the conditions of functioning of the latter differ significantly from its natural environment. Indeed, the kinetic energy of the analyte, viscosity, density, dielectric properties of the medium, the number of hydrogen bonds *etc.* in the gas phase are fundamentally different from those in biological fluids. Taking into account the fact that biological structures are inherently adaptive systems, it is not surprising differences of their behaviour in variable environments. This work makes it possible to reveal several possible mechanisms of this process, allowing some new prospects for further development of GPB.

However, a lot of questions are still open like the relationship between the processes of the specific and non-specific binding of the analyte on the elements of interfacial architecture (protein A, IgG etc.) with or without transformations of protein conformations. Understandable that since volatile compounds can penetrate inside the biological films, the surface mass may increase as a result of selective and non-selective sorption / adsorption and both of them can induce molecular transformations. Therefore it is reasonable to expect new experimental approaches devoted to solve this problem.

It is reasonable to stress that observed effect of ETED induced water sorption is well correlated with physiological functions of estrogens in living beings. Indeed, estrogens play an important role in controlling the homeostasis of body fluids. Several studies have reported the involvement of steroids in the homeostatic control of hydromineral balance and the influence of estrogens on the modulation of this system.[50-52] The obtained results help to clarify the situation at the molecular level and bridge the processes observed on the different levels of organizations specific for animate nature.

From a practical standpoint, the use of saturated response of QCM immune-sensors for determination of concentration of low molecular weight regulators limited by nonspecific process of water sorption. However, due to the fact that even in ultra-low concentrations of ETED one capable of initiating the process of binding the excess water by surface architecture, their use is very promising as a threshold indicator like alarm device. Alternatively, the sensing array where each QCM sensor has a relatively wide but unique response/signature can be used to solve the problem. This behaviour suggested that polyclonal/ class specific antibodies/selective receptor/etc. may be used to yield useful information about both a specific and non-specific contributions. So, the using "electronic nose" methodology (*i.e.* cross-selective sensing massifs[3, 53-55]) may be useful for further developments with aim to decrease those unspecific effects. Of course, using kinetic information or multiply measurements can resolve the problem as described above

In conclusion, it is necessary to stress once again that the use of very simple registration system allows, however, obtaining qualitatively correct test for the presence of ultra-low (up to fM) concentrations of potentially hazardous biological xenobiotics in a few minutes. This opens up new possibilities for developing simple, cheap and highly effective alarm systems for potentially biological or chemical hazards using the principles inherent in the functioning of wildlife.

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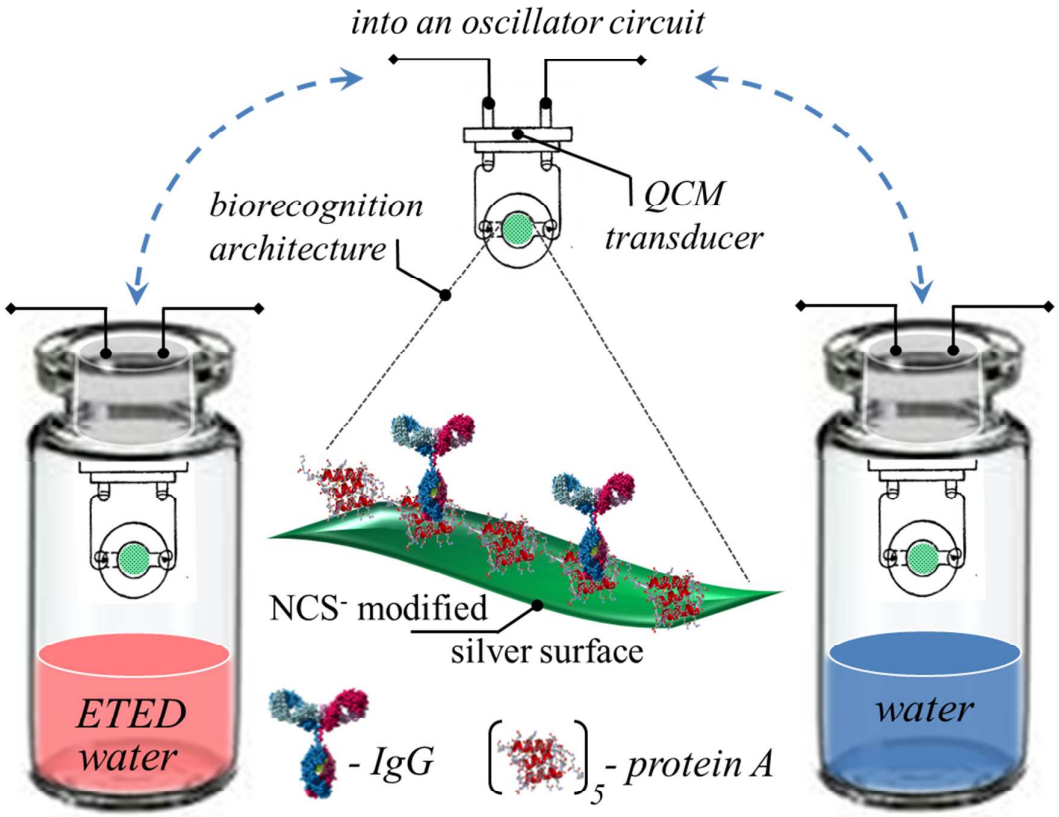

The response of gas phase biosensor for 17ά – ethynylestradiol is due to the change of a water-binding capacity of the proteins induced by binding of the ETED molecules.

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