Analyst

PAPER

View Article Online
View Journal | View Issue

Cite this: *Analyst*, 2015, **140**, 3225

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

Borys Snopok* and Ivanna Kruglenko

Received 18th November 2014,
Accepted 23rd February 2015

DOI: 10.1039/c4an02121e

www.rsc.org/analyst

An ultra-sensitive gas phase biosensor/tracer/bio-sniffer is an emerging technology platform designed to provide real-time information on air-borne analytes, or those in liquids, through classical headspace analysis. The desired bio-sniffer measures gaseous 17α – ethinylestradiol (ETED) as frequency changes on a quartz crystal microbalance (QCM), which is a result of the interactions of liquid sample components in the headspace (ETED and water) with a biorecognition layer. The latter was constructed by immobilization of polyclonal antiserum against a phenolic A-ring of estrogenic receptors through protein A. The QCM response exhibited stretched exponential kinetics 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^{-1}. It was shown that the variations in the QCM response characteristics are due to the change of the water-binding capacity of the sensing layer induced by protein transformations initiated by the binding of ETED molecules. This result is well correlated with the natural physiological function of estrogens in controlling the homeostasis of body fluids in living beings.

Introduction

Biochemical analyses in modern medicine and biology have become progressively more and more sophisticated. This factor, as well as environmental pollution, the need for high-purity pharmaceuticals, requirements for food control, *etc.* means that there is particular interest from 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 to date, exclusive selectivity profiles are only possible to find within biological systems. A well-known natural candidate that may be utilized as a specific receptor molecule is the antibody, a dedicated protein (*e.g.* immunoglobulin, IgG) which is specific against a target antigen (the 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.* ref. 5). This knowledge has led to the accepted convention for their use as bioreceptors for chemical sensing purposes in the 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] Antibodies were initiated to use as sensing agent for QCM based GPB 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 demonstrated that the 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

V. Lashkaryov Institute of Semiconductor Physics, NAS Ukraine, 41 Prospect Nauki, Kyiv, 03028, Ukraine. E-mail: snopok@isp.kiev.ua; Fax: +380 44 5258342

sites on the antibodies will lose their prescribed structure required for molecular recognition. Now, the most important examples of GPB involving 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]

Despite the practical achievements of GPB,[25] both the sensing mechanisms and the driving forces of the interfacial processes are still unclear. To realize the potential of gas-phase biosensors it would be of interest to specify what determines the sensor performance. Is it the native structure of recognition sites, surface reconstruction, or the transduction 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 to air-borne analytes.

The present study deals with the fate of an important environmental xeno-estrogen, 17α – 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 has been detected in surface water all over the world, mostly in the ng L^{-1} 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 the GPB concept for the detection of ethinylestradiol in the 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 paratope will not be so drastically changed outside the aquatic environment to prevent the molecular recognition of the antigen.[27]

The paper is organized as follows: it begins by discussing the methodological aspects of the measurements, an interface modification protocol, 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 a static headspace environment. Finally, the mechanism of sensor response induced by the antigen is discussed.

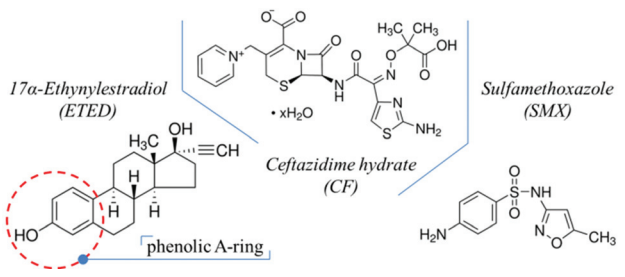

Fig. 1 Molecular structure of the compounds to be analysed.

Materials and methods

Materials

Protein A *Staphylococcus aureus*, 17α – ethinylestradiol (ethinyl estradiol, ETED, EE2, mw 296.4 Da), ceftazidime hydrate (CF), sulfamethoxasole (SMX) and guanidine thiocyanate 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 a concentration of 10 μg ml^{-1} was prepared by dissolution of 2 mg of ETED powder in 200 ml distilled water (the water solubility of ETED is *ca.* 4–11 μg ml^{-1}).[28] Ceftazidime and sulfamethoxasole solutions with concentrations of 1 μg ml^{-1} were prepared in distilled water immediately before measurements (the water solubility of CF and SMX are *ca.* 1–2 mg ml^{-1} and 200–300 μg ml^{-1}, respectively).[29,30]

Functionalization of the QCM transducers

To construct the GPB, antibodies were immobilized onto the surface of a QCM device through protein A using a 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 the 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) then the QCM transducer was immersed in a guanidine thiocya-

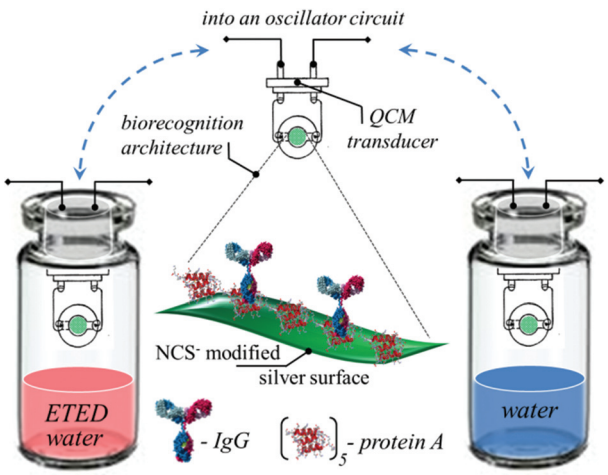

Fig. 2 A schematic diagram of the core procedures, illustrating the analysis of ETED water solutions using a static headspace configuration. Insert: scheme of the interfacial architecture on the QCM transducer surface. The silver electrode on the quartz was modified by isothiocyanate, then protein A and finally anti-ETED antibody molecules (IgG) were immobilized on the surface with recognition paratopes directed into the solution.

nate 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 μg ml^{-1} 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) the QCM sensor was placed in a weighing bottle above water level at room temperature before the measurements. Usually the measurements were performed within 48 h after the sensor's preparation.

Measurement concept and experimental procedure

Acoustic sensors represent a 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 a complex with the surface, and results in a decrease in the resonance frequency as defined by Sauerbrey's equation.[34,35] The typical mass sensitivity of uncoated QCM resonators at 10 MHz is in the range of 0.1–1.0 Hz cm^2 ng^{-1}, 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 Sauerbrey 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 the sensor surface, so, the Sauerbrey model only describes correctly when the interfacial molecular complexes are rigidly attached to the surface.[37] However, variations in the mechanical properties (changes in the viscosity, the mechanical stiffness *etc.*) of interfacial architecture on the surface can also lead to resonance frequency changes of the 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 was used.[40] Briefly, the QCM analyzer contains: (i) a temperature controlled measurement camera with a sensor array of the flowing type; (ii) a quartz generators block; (iii) a block for the frequency measurement and an RS232 interface constructed using a specialized microprocessor (AT89C2051); (iv) a generator of the gas mixtures; (v) system collection and processing of the information on a personal computer.[40–43] To maximize the correctness of the measurements and prevent possible adsorption of components of gaseous mixtures on construction inside the sampling systems, the simplest possible measurement configuration based on a static headspace procedure was used (Fig. 2). Moreover, in this case the headspace is humidified to the limit at a given temperature because the biologically active coatings require a certain amount of moisture to behave as they normally would in solution. The QCM sensor was mounted in a ground-in stopper of a 30 ml weighing bottle containing clean water or ETED solution in the same water with a given concentration and this setup was then connected into an oscillator circuit of the measurement device. A typical measurement procedure involved the following stages at 20 ± 1 °C temperature: (I) measurement of the sensor response in static headspace with the clean water sample (10 ml) until the transducer frequency is stabilized (*ca.* 2 Hz); (II) changing the water sample with a freshly prepared aqueous solution of ETED (10 ml) with a given concentration and monitoring the frequency change for *ca.* 2 minutes; (III) changing to the clean water sample again and recording 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 bottles above water level at room temperature.

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

Data processing

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

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

where $\Delta\mathrm{QCM}_{\max}$ 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. OriginPro 7.5 (OriginLab Corporation) was used to approximate the curves.

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 concentration in the water solution was performed with a competition model using the Morgan–Mercer–Flodin equation or the logistic curve for the data in the steady-state regime:[44]

$$I_S/I_0 \sim \frac{1}{1 + \left(\frac{[\mathrm{ETED}]}{\gamma_0}\right)^{p}} \quad (2)$$

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 mechanisms of the processes occurring in the system.

The typical kinetics are shown in Fig. 3b and illustrate the contribution of the processes of reversible and "irreversible" sorption. It must be emphasized that for baseline recovery after experiments with observed "irreversible" sorption, the QCM sensor must be kept under saturated water vapor for at least 30 minutes.

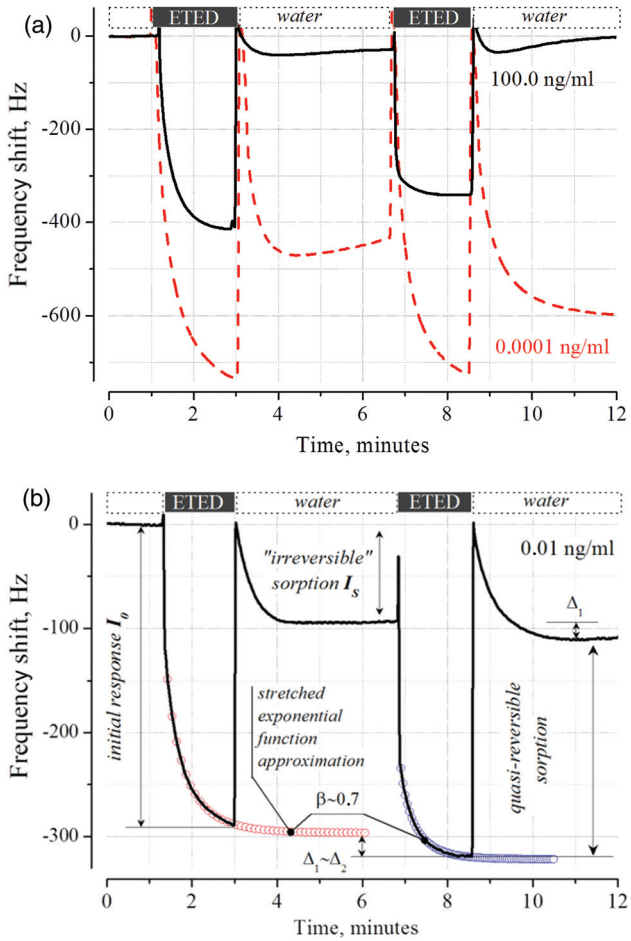

Fig. 3 Typical dependencies of the QCM frequency change on time for two serial repetitions of the same water solution of ETED at different concentrations (a, b) and approximation of the response kinetics by the stretched exponential function (b).

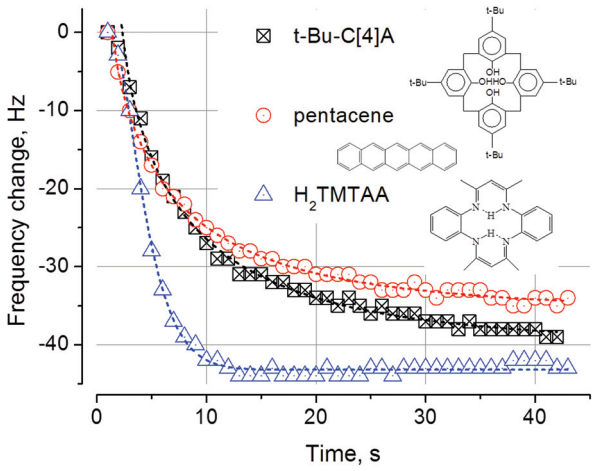

Fig. 4 Typical experimental dependencies of the frequency change on time for QCM transducers covered by *tert*-butyl-calyx[4]arene, pentacene and tetramethyl-dibenzotetraazaannulene for ETED powder.

Results and discussion

Mass uptake estimation

The typical QCM frequency variations for the antibody modified sensitive elements *versus* time taken for a few ETED concentrations in water are shown in Fig. 3. An analysis of the sensor response allows us to conclude that for all samples the responses are monotonic and relatively quickly achieve a 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 when varying the concentration of ETED in an aqueous solution from 100 to 10^{-5} ng ml^{-1} (10^{-10} to 10^{-17} g L^{-1}; *ca.* 10^{-13} to 10^{-20} M L^{-1}), and demonstrates a trend of increasing response with decreasing concentrations below 1 ng ml^{-1}. Despite the fact that some deviations of the sensor response can be induced by the measuring procedure and variations in the experimental conditions, there is no doubt that for such a small concentration of ETED in solution there is an easily observed frequency change with a signal to noise ratio greater than 100. Given the sensitivity of the QCM transducers (*ca.* 1 ng Hz^{-1} for the transducer used), we can easily estimate the mass of the substance adsorbed on the sensor surface at various concentrations of 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 there being such a low concentration of the ETED, the amount of the substance adsorbed on the surface of the QCM sensor is in the picogram range. Comparison of these values with the amount of analyte in a solution (10 ml) shows that at a concentration of ETED of less than 10 ng ml^{-1} the absorbed mass on the QCM surface exceeds the total amount of ETED in the sample. This estimate clearly indicates that the 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 different chemical functionalities with respect to the saturated vapor of ETED. ETED powder was placed in a sealed chamber with an eight channel sensor array using similar QCM transducers at the same temperature (Fig. 4). The saturation levels of the typical responses indicate that the increase in mass on the surface of the transducer as a result of the interactions of different organic materials with the vapor ETED did not exceed 40–50 ng (40–50 Hz). This is approximately an order of magnitude less than that obtained for ETED solutions.

Mechanisms that can generate a response

Among the possible reasons for such a high response of the antibody modified QCM transducers in the headspace of ETED solutions in water, we 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 the elastic properties of the interfacial surface resulted in distortion of the Sauerbrey equation, and (3) changes of the water-binding

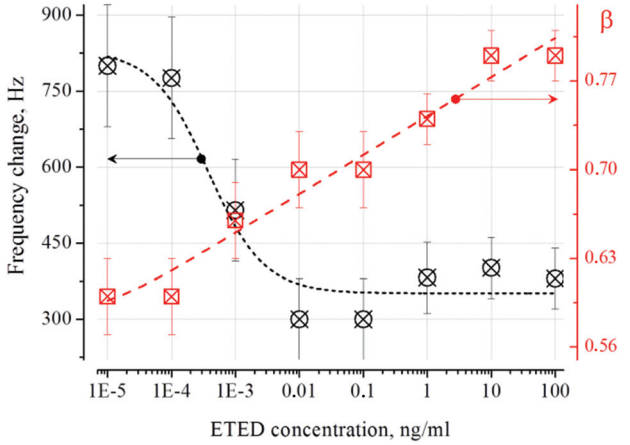

Fig. 5 Dependencies of QCM response magnitude in equilibrium (left axes) and parameter β (right axes) on ETED concentration in water.

capacity of the interfacial sensing architecture as a 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 the headspace as ETED in the sample decreases. However, the non-monotonic change in the response (Fig. 5) suggests that at such low concentrations of dissolved ETED, the change in vapor pressure of the solvent can be neglected.

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

Evolution of QCM response

The kinetics of the QCM response are well described by a 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 the recovery of the initial value after the exposure. Despite the fact that we have not studied this question in detail, 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; this is the situation when the adsorption process is well described by the Langmuir model for a spatially and energetically isotropic surface. If the magnitude of the initial response is greater than 600 Hz, the value of β tends toward the value of 0.5, which indicates that diffusive transport at or near the surface dominates in the response formation.

A decrease of the concentration of ETED in the solution induces a transition from reversible to partially "irreversible" sorption with increasing magnitude of the initial response (Fig. 3 and 5). Reducing the concentration to less than 10^{-18} g L^{-1} (10^{-5} ng ml^{-1}) leads to a drop in response that is probably due to there being too low a concentration of ETED in the gas phase, at this concentration the number of ETED molecules in solution roughly equals the amount of antibody paratopes on the surface.

Model of interfacial transformations

Summarizing the above-mentioned discussion, it is reasonable to conclude that the variations in the QCM response are 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). This is not so surprising because it is well known that water molecules can bind to the backbone and the polar and charged side chains of a protein. Depending on the nature of their side chains directed outward, proteins may bind various amounts of water – they have a water-binding capacity.[45] So, as ETEDs coupled to proteins at the interface, they tend to bind more water, because increased charge or conformational changes resulted 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.

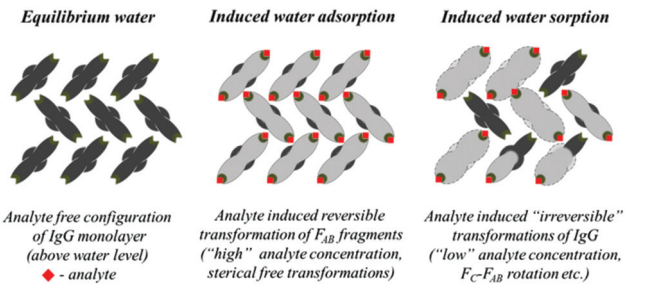

Fig. 6 Schematic diagram of the core processes illustrating the diversity of the supramolecular architectures formed by IgGs at different concentrations of ETED in water.

One of the possible models of the interfacial processes may be as follows. At high concentrations of ETED in the gas phase, ETED induced structural reorganization of a large community of antibodies limited by steric conditions in a monomolecular layer of IgGs immobilized through protein A. According to previous work,[7,8] the biologically inspired protein A based self-assembling procedure leads to close-packed layers. As a 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 has been described in depth in the literature.[47]

Indeed, it was revealed that biological interactions such as analyte–receptor binding events are followed by a conformation change in the receptor molecule.[48] Further work revealed high order (~10–12 Å) 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 was involved in the majority of conformational changes during a binding event, resulting in rotation between the heavy and light chains of IgG.

When the concentration of ETED is less than 1 ng ml^{-1}, 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 which become available due to ETED binding. This process dominates at high concentrations of ETED and is not accompanied by significant structural changes within the bio-recognition layer (Fig. 6). When the concentration of ETED drops, steric blocking of part of the IgGs decreases, allowing stronger 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 by the spatial transformations helps to prevent the association of IgGs due to the fact that the bound water shields the protein molecules 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 the "irreversible" (I_S) and full (I_0) components of its initial value – I_S/I_0 (Fig. 7). Using this as an informative parameter allows, among other things, to reduce the influence of changes in 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

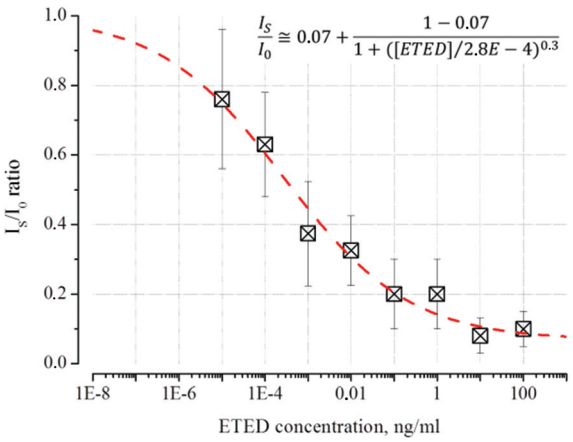

Fig. 7 Dependence of the relative response of the QCM sensor with immobilized IgG for aqueous solutions of ETED at various concentrations. The best approximation according to eqn (2) is shown by the dashed line; the parameters of the theoretical curve according to eqn (2) are given in the insert.

of water modulated by interactions of ETED with proteins on the surface.

Selectivity testing

As 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 a nonsteroidal nature, but with fragments of "similar" structure. Sulfamethoxazole (SMX) and ceftazidime (CF) were chosen as examples, which are frequently used antibacterial drugs. Exposure of the surface architecture to SMX and CF solutions with a concentration of *ca.* 1 μg ml^{-1} showed that in these cases there was

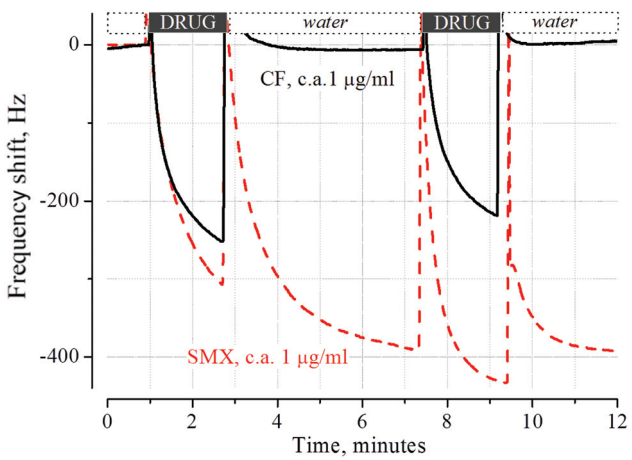

Fig. 8 Typical dependencies of the QCM frequency change on time for two serial repetitions of the same water solutions of SMX and CF (*ca.*1 μg ml^{-1}).

also a response observed, which in the case of CF is reversible and for SMX – irreversible (Fig. 8). Analysis of the kinetics indicates that in the case of CF the adsorption is limited by diffusion processes (β = 0.5), probably because the complex molecular structure of CF requires numerous acts of re-adsorption to achieve the necessary spatial orientation.[42] In the case of SMX, interaction is similar to ETED ($\beta \sim 0.7$) but initially irreversible, resulting in an irreversible blocking of the recognizing antibody, probably due to the presence of SO_2 groups.

Conclusions

A decrease in activity in the field of molecular biosystems for gas analysis in recent years is due to the lack of a clear understanding of the mechanisms of interaction of gaseous analytes with biological receptors, when the conditions of functioning of the latter differ significantly from their natural environment. Indeed, the kinetic energy of the analyte, the viscosity, density, and 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 that there are differences in their behaviour in variable environments. This work makes it possible to reveal several possible mechanisms of this process, allowing some new prospects for the further development of GPB.

However, a lot of questions are still open, like the relationship between the processes of specific and non-specific binding of the analyte on the elements of the interfacial architecture (protein A, IgG *etc.*) with or without transformations of protein conformations. It is 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 these can induce molecular transformations. Therefore it is reasonable to expect new experimental approaches devoted to solve this problem.

It is reasonable to stress that the observed effect of ETED induced water sorption is well correlated with the 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 organization specific for animate nature.

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

In conclusion, it is necessary to stress once again that the use of a very simple registration system allows a 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 potential biological or chemical hazards using the principles inherent in the functioning of wildlife.

Acknowledgements

The authors thank Stewart Armstrong and Frederick Rowell (Centre for Pharmaceutical and Environmental Analysis, School of Science, University of Sunderland, UK) for antiserum against ETED. This work has been supported by a grant of the European Community (INTAS Projects 00-00870) and projects of the National Academy of Sciences of Ukraine.

Notes and references

1 E. Vulliet and C. Cren-Olivé, *Environ. Pollut.*, 2011, **159**, 2929–2934.
2 J. Aufartováa, C. Mahugo-Santanab, Z. Sosa-Ferrerab, J. Santana-Rodríguezb, L. Nováková and P. Solicha, *Anal. Chim. Acta*, 2011, **704**, 33–46.
3 B. Snopok and I. Kruglenko, *Thin Solid Films*, 2002, **418**, 21–41.
4 T. Fodey, P. Leonard, J. O'Mahony, R. O'Kennedy and M. Danaher, *TrAC, Trends Anal. Chem.*, 2011, **30**, 254–269.
5 R. Schirhagl, J. Qian and F. Dickert, *Sens. Actuators, B*, 2012, **173**, 585–590.
6 B. Snopok, S. Darekar and E. Kashuba, *Analyst*, 2012, **137**, 3767–3772.
7 P. Boltovets, O. Polischuk, O. Kovalenko and B. Snopok, *Analyst*, 2013, **138**, 480–486.
8 K. Mitsubayashi, *Biosensors and Biochips*, John Wiley & Sons, 2008.
9 K. J. Mattias Sandström, A.-L. Sunesson, J.-O. Levina and A. P. F. Turner, *J. Environ. Monit.*, 2003, **5**, 477–482.
10 D. R. P. Morrisa, J. Fatissona, A. L. J. Olssona, N. Tufenkjia and A. R. Ferroc, *Sens. Actuators, B*, 2014, **190**, 851–857.
11 K. Otsuka, T. Goto, H. Amagai, N. Ishii, H. Endo and K. Mitsubayashi, *Int. J. Environ. Anal. Chem.*, 2006, **86**, 1049–1056.
12 M. Hulko, I. Hospach, N. Krasteva and G. Nelles, *Sensors*, 2011, **11**, 5968–5980.

13 M. Farre, L. Kantiani, S. Pérez and D. Barcelo, *TrAC, Trends Anal. Chem.*, 2009, **28**, 170–185.
14 W. D. Hunt, D. D. Stubbs and S. H. Lee, *Proc. IEEE*, 2003, **91**, 890–901.
15 F. J. Ngeh-Ngwainbi, P. H. Kuan and G. G. Guilbault, *J. Am. Chem. Soc.*, 1986, **108**(18), 5444–5447.
16 A. A. Suleiman and G. G. Guilbault, *Analyst*, 1994, **119**, 2279–2282.
17 A. A. Suleiman and G. G. Guilbault, *Anal. Lett.*, 1991, **24**, 1283–1291.
18 J. H. T. Luong and G. G. Guilbault, *Biosensor principles and applications*, Marcel Dekker, Inc, New York, 1991.
19 G. G. Guilbault, *Anal. Chem.*, 1983, **55**, 1682–1684.
20 D. Stubbs, *Development of an Acoustic Wave Based Biosensor for Vapor Phase Detection of Small Molecules*, PhD Dissertation, Georgia Institute of Technology, December, 2005.
21 D. D. Stubbs, W. D. Hunt, S. H. Lee and D. F. Doyle, *Biosens. Bioelectron.*, 2002, **17**, 471–477.
22 D. D. Stubbs, S. H. Lee and W. D. Hunt, *Anal. Chem.*, 2003, **75**, 6231–6235.
23 D. D. Stubbs, S. H. Lee and W. D. Hunt, *IEEE Sens. J.*, 2002, **2**, 294–300.
24 X. J. Wu and M. M. F. Choi, *Anal. Chem.*, 2004, **76**(15), 4279–4285.
25 J. M. Bowen, L. J. Noe and P. Sullivan, Immunochemical detection of an explosive substance in the gas phase through surface plasmon resonance spectroscopy, *U.S. Patent* 6, 573,107, 3 June 2003.
26 H. Maes, *Fate of ethinylestradiol in the aquatic environment and the associated effects on organisms of different trophiclevels*, PhD dissertation, Aachen University, Brugge, Belgien.
27 S. Armstrong, Z.-F. Miao, F. J. Rowell and Z. Ali, *Anal. Chim. Acta*, 2001, **444**, 79–86.
28 N. K. Nagpal and C. L. Meays, Water Quality Guidelines for Pharmaceutically-active-Compounds (PhACs): 17α-ethinylestradiol (EE2), Technical Appendix. Science and Information Branch Water Stewardship Division, Ministry of Environment, province of British Columbia.
29 S. H. Yalkowsky and R. M. Dannenfelser, *Aquasol Database of Aqueous Solubility. Version 5*, College of Pharmacy, University of Arizona - Tucson, AZ. PC Version, 1992.
30 D. Zhang, Y. Wang, S. Ma, S. Wu and H. Hao, *J. Chem. Eng. Data*, 2013, **58**, 176–182.
31 A. Savchenko, E. Kashuba, V. Kashuba and B. Snopok, *Sens. Lett.*, 2008, **6**, 705–713.
32 P. Boltovets, B. Snopok, Y. Shirshov and N. Dyachenko, Reports of the National Academy of Science of Ukraine, 2001, 11, 137–144.
33 B. Snopok, M. Yurchenko, L. Szekely, G. Klein and E. Kasuba, *Anal. Bioanal. Chem.*, 2006, **386**, 2063–2073.
34 C. Steinem, *Piezoelectric Sensors: 5 (Springer Series on Chemical Sensors and Biosensors)*, Springer, Berlin Heidelberg, 2007, 484.
35 C. I. Cheng, Y.-P. Chang and Y.-H. Chu, *Chem. Soc. Rev.*, 2012, **41**, 1947–1971.
36 M. Penza, P. Aversa, R. Rossi, M. Alvisi, G. Cassano, D. Suriano and E. Serra, *Sensors and Microsystems: AISEM 2010 Proceedings*, Springer, Netherlands, 2011, vol. 91, pp. 271–277.
37 R. Lucklum and P. Hauptmann, *Sens. Actuators,B*, 2000, **70**, 30–36.
38 U. Latif, S. Can, O. Hayden, P. Grillberger and F. L. Dickert, *Sens. Actuators*, 2013, **176**, 825–830.
39 T. Schäfer, F. D. Francesco and R. Fuoco, *Microchem. J.*, 2007, **85**, 52–56.
40 I. Kruglenko, B. Snopok, Y. Shirshov and E. Venger, *Semicond. Phys., Quantum Electron. Optoelectron.*, 2000, **3**, 529–541.
41 I. Kruglenko, B. Snopok, Y. Shirshov and F. Rowell, *Semicond. Phys., Quantum Electron. Optoelectron.*, 2004, **7**, 207–216.
42 B. A. Snopok, *Theor. Exp. Chem.*, 2014, **50**, 67–95.
43 B. Snopok and I. Kruglenko, *Sens. Actuators B*, 2005, **106**, 101–113.
44 B. Snopok, P. Boltovets and F. Rowell, *Theor. Exp. Chem.*, 2006, **42**, 106–112.
45 V. Vaclavik and E. W. Christian, *Essentials of Food Science*, Springer Science & Business Media, Series: Food Science Text Series, 4th edn, 2014, vol. XXIV, p. 495.
46 R. T. Marshall, H. D. Goff and R. W. Hartel, *Ice Cream*, Springer Science & Business Media, Technology & Engineering, 2003, p. 371.
47 J. M. Rini, U. Schulze-Gahmen and I. A. Wilson, *Science*, 1992, **255**, 959–965.
48 R. L. Stanfield and I. A. Wilson, *Curr. Opin. Struct. Biol.*, 1994, **4**, 857–867.
49 E. A. Padlan, *Mol. Immunol.*, 1994, **31**, 169–217.
50 S. J. Somponpun, *J. Neuroendocrinol.*, 2007, **19**, 809–818.
51 T. W. Hutchens and J. O. Porath, *Clin. Chem.*, 1987, **33**, 1502–1508.
52 G. Almeida-Pereira, R. Roratoa, L. C. Reis, L. L. K. Elias and J. Antunes-Rodrigues, *Horm. Behav.*, 2013, **64**, 847–855.
53 Z. Ali, K. Pavey and E. Robens, *J. Therm. Anal. Calorim.*, 2003, **71**, 31–35.
54 K. C. Persaud, *IEEE Sens. J.*, 2012, **12**, 3108–3112.
55 R. Baronas, J. Kulys, A. Žilinskas, A. Lančinskas and D. Baronas, *Chemom. Intell. Lab. Syst.*, 2013, **126**, 108–116.

