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Proceeding Paper

Interaction of the fluorescent cell labeling dye Rhodamine 6G with low molecular weight compounds: a comparative QCM study of adsorption capacity of R6G for gaseous analytes †

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Abstract: Rhodamine 6G is widely used in biochemistry, cell imaging, as a sensitive layers of chemical sensors. At the same time, the features of the interaction of Rh6G with low molecular weight analytes present in most biochemical preparations have not been studied. In this work, we studied the interaction of Rh6G thin films with water vapor, acetic acid, ethyl alcohol, ammonia, benzene, pyridine, nitrobenzene, acetone, and acetonitrile. The kinetic features and adsorption capacity of the sensitive layer were compared with those of other sensitive layer materials (macrocyclic dibenzotetraazaanulenes, phthalocyanines, and their metal complexes). The response values of the Rh6G-based sensor significantly exceed the responses of other sensors, regardless of the type of analyte. This means that this material is promising for multisensor arrays, where the issue of cross-selectivity is a prerequisite. However, the high sensitivity of Rh6G to a wide variety of low molecular weight analytes must be taken into account when developing selective sensors.

Keywords: QCM sensor; Rhodamine 6G; adsorption; absorbance; low molecular weight analytes.

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1. Introduction

Chemosensors based on rhodamine and its derivatives are potentially attractive tools for analytical chemistry, biology, medicine, and environmental protection areas. Such sensor systems play an important role as simple, fast, effective, and low-cost monitoring and diagnostic tools. High biocompatibility and fluorescence in the near-infrared range of rhodamine derivatives make them an excellent choice for the creation of biosensors.

Rhodamine 6G is a highly effective luminescent xanthene dye of the rhodamine family. The building blocks common to all rhodamines are a dibenzopyrene chromophore (xanthene) and a derivative of the benzoic acid molecule (carboxyphenyl group). Rhodamines are singly charged positive ions with a size of s.a. 0.7x1.4 nm; the positive charge is mainly located on two amino groups (due to tautomerism of two energetically equivalent states with rearrangement of the aromatic ring and a -C=N$^+$- double bond). An interesting feature of its molecular structure is the perpendicularity of the plane of the xanthene chromophore and that of the aromatic carboxyphenyl group.

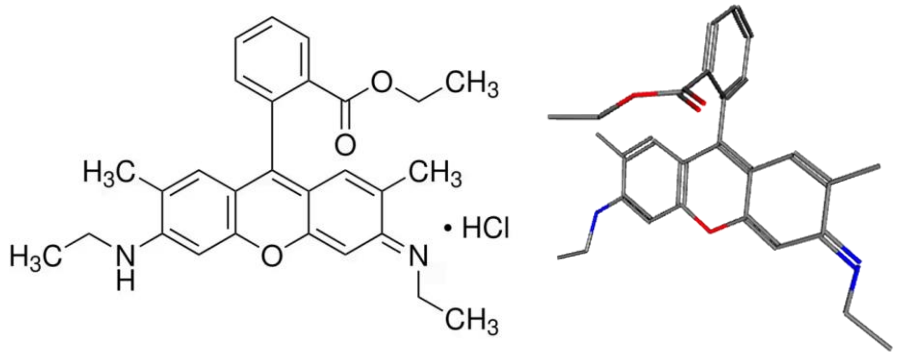

Figure 1. Chemical structure (left) and 3D conformation (right) of Rhodamine 6G. The spatial structure is presented according to PubChem modeling data (https://pubchem.ncbi.nlm.nih.gov/compound/Rhodamine-6G#section=Names-and-Identifiers).

In recent years, the development of sensor systems based on Rhodamine has been observed, as well as increased interest in these systems [1]. In particular, the use of Rhodamine-based luminescent chemosensors that emit in the near-infrared range (NIR) is considered for the detection of metal ions such as Al(III), Cu(II), Hg(II), Co(II), Fe(III), Au(III), Cr(III) [2]. Such systems are also used in SERS structures [3].

Sensors based on Rhodamine 6G can be used for water vapor detection [4], as hydrogel sensors capable of detecting Hg^{2+} in flowing or stagnant water [5]; to detect physiologically important ions of transition metals, such as Cu(II) and Fe(III) [6]. The idea of detecting heavy and transition metal ions using different characteristics the same Rh6G sensors in living organisms is particularly attractive [7, 8].

In general, it can be said that Rhodamine 6G is widely used for labeling oligonucleotides in biochemistry, cell imaging, as a sensitive layers of chemical sensors. At the same time, the features of the interaction of Rh6G with low molecular weight analytes present in most biochemical preparations have not been studied. This is important not only for understanding the possibility of the influence of non-target components on the analytical signal during analysis, but is also of great practical importance, since it allows developing (bio)chemical sensors for specific applications. In this work, we studied the features of the interaction of Rh6G thin films with a number of low molecular weight analytes in the gas phase (to exclude cross-interaction etc.), namely, water vapor, acetic acid, ethyl alcohol, ammonia, benzene, pyridine, nitrobenzene, acetone, and acetonitrile. The kinetic features and adsorption capacity of the sensitive layer were compared with those for other sensitive layer materials (macrocyclic dibenzotetraazaanulenes, phthalocyanines, and their metal complexes).

2. Materials and Methods

H_2TAA, its derivative (EGTAA), and their metal complexes with copper were kindly provided by Prof. Lampeka Ya.D. Phthalocyanine, its metal complexes with copper and lead, as well as Rhodamine 6G, were obtained from SigmaAldrich Inc and were used without additional purification (Figure 2). Planar molecules of H_2TAA and its metal complexes with copper form dimers in the crystalline state; H_2EGTAA molecules and their metal complexes lose their planar conformation due to the steric effects of peripheral substituents. Phthalocyanines do not form dimers, both in the case of planar (H_2Pc CuPc) and structures distorted by the large size of the cation (PbPc).

Thin oriented films of all compounds used as sensitive layers were obtained using thermal sputtering in vacuum (VUP-5M, pressure $5x10^{-4}$ Pa, growth rate 0.1 nm/min) at a temperature of 297±2 ^{o}K. 100 nm thick films were deposited on one of metal electrodes of quartz piezoelectric resonators (quartz crystal microbalance, QCM) of RK169 type with a resonant frequency of 10 MHz.

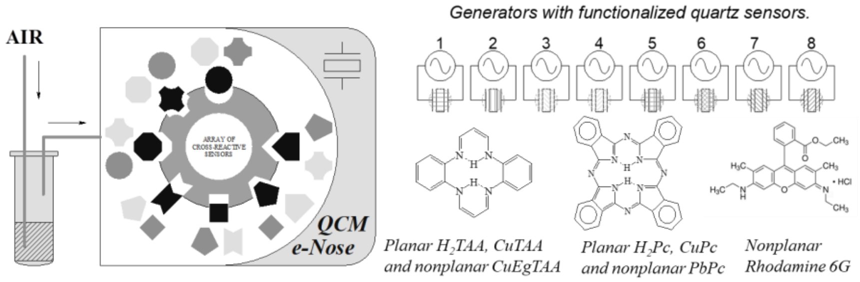

Figure 2. Illustration of the measurement procedure using an "electronic nose" type system based on quartz microbalance transducers with sensitive layers of organic molecular crystals.

To prepare the samples, we used double-distilled water and chemically pure acetic acid, ethyl alcohol, ammonia, benzene, pyridine, nitrobenzene, and acetone acids obtained from the Khimlaborreaktiv (UA) and Makrokhim (UA) companies. Liquids (5 ml) were collected immediately before measurements. To form a sample (headspace), the gas phase formed above the surface of an liquids due to bubbling was mixed with a flow (60 ml/min) of carrier gas (argon) at a constant temperature (297±2 K) (Figure 2).

The measurement procedure included stabilization of the baseline in an argon flow (frequency deviation +/- 2 Hz), exposure to a gas sample of analyte vapor (5-15 min) and subsequent purging with a carrier gas. To minimize the influence of previous experiments, a break of at least 4-5 hours was taken between individual experiments, during which the sensor array was in an atmosphere of dried argon.

The equipment and software developed in the V. Lashkaryov Institute of Semiconductor Physics NAS of Ukraine were used for QCM oscillations excitation and for the measurements of their frequency shifts, which is proportional to the change of mass due to adsorption of analyte's molecules (Figure 2) [9, 10].

3. Results and Discussion

3.1. Features of the optical characteristics of thin films of Rhodamine 6G

Despite the widespread use of Rh6G as a material for many optical devices (active medium of tunable lasers, waveguide substrates, etc.), many features of its absorption spectrum remain unclear (Figure 3). Indeed, the lowest energy absorption band consists of a large peak (c.a. 545 nm, , the bathachromic shift relative to the 520 nm position in solution is due to interaction in a solid [11]) with the smaller peak at 514 nm (c.a. 498 nm in solution) and shoulder around 475 nm. According to the results of quantum chemical calculations, absorption in this region is due to π-π transition corresponding (HOMO-LUMO), whose electron density is localized predominantly in the xanthene ring of Rh6G [12]. A characteristic feature of this transition is a vibronic structure as a shoulder at smaller wavelengths [13] due to electron-vibrational interaction (as, for example, in planar dibenzotetraazaannulene and its complexes) [14, 15]. The results of spectral analysis in the gas phase [16], where the formation of intermolecular aggregates is unlikely, confirm the presence of a shoulder on the short-wavelength side of the main transition. This allows us to conclude that for Rh6G there is a process of interaction between the lower electronic transition and the intramolecular vibration.

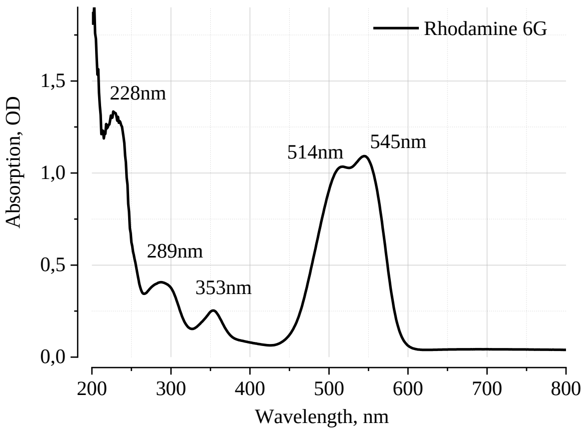

Figure 3. The absorption spectrum of the 100 nm thick Rhodamine 6G film obtained by thermal sputtering in vacuum onto a glass substrate.

At the same time, numerous results of experimental studies show that upon transition from the gas phase to a solvent environment, Rh6G molecules form stable dimeric structures (H-dimer, which form in the ground state), the equilibrium of which with single molecules depends on their concentration in the solution and on the type of solvent [17]. The absorption band of the dimer practically coincides with the position of the first vibronic band of the monomer, which makes their separation difficult. Theoretical calculations confirm the possibility of self-assembly of this kind of Rh6G aggregates [18]: calculation results predict substantial redshifts or blueshifts of the optical absorption spectra of Rh6G dye molecules after aggregation in J or H dimers, respectively. Experimental results confirm theoretical expectations. For example, in thin films it is dimers that are credited with the main contribution to high luminescence quantum yields [19]. Despite some ambiguity in the interpretation of the role of Rh6G dimers in different aggregative states, the need to take into account the characteristic features of excimer aggregates (dimers of the same compound formed in the ground state) when considering the effects with their participation is beyond doubt.

Presented in Figure 3 absorption spectrum of the Rhodamine 6G film demonstrates not only the typical bathochromic shift of the spectrum of organic molecular crystals upon transition to a solid, but also shows changes in the shape of the spectrum compared to the spectrum in solution [20]. Taking into account the short discussion above, it can be assumed that the increase of the band intensity in the 514 nm region is associated with the formation of Rh6G dimeric structures in the solid.

Summarizing all mentioned above it is reasonable to highlight that Rh6G thin films may contane several types of reactive centers, the properties of which differ due to a special local structural organization, namely: 1) several nucleophilic centers of different nature in the region of different types of side groups with the possibility of rotation and the ability to specifically interact with other molecules, (including through the formation of hydrogen bonds); 2) a conjugated parts of molecule, suitable for the formation of various configurations with aromatic fragments of other molecules and stacking interactions, 3) centers whose energy is modulated by the vibrational modes of the molecule; 4) dimeric structures with different energy levels due to local effects of neighboring closely located molecules; 5) supramolecular structures caused by the structural feature of Rhodamine 6G due to the specific configuration of the molecule, namely, the perpendicular arrangement of the plane of the central fragment and a separate aromatic ring.

As noted above, in this work we are interested in analyzing the diversity of binding centers for gaseous molecules in thin films of Rhodamine 6G. The most promising way is direct adsorption measurements using analytes of various natures, which allow the formation of various configurations of the surface-bound organic phase, and, accordingly, obtaining information about the cros-reactive centers are present in this system.

3.2. The ratio between the magnitude of the responses of QCM sensors based on Rhodamine 6G sensitive layers and macrocyclic heterocycles

The analysis of the obtained results unambiguously indicates that, among the used sensitive materials mentioned above, Rh6G has the maximum value of responce with respect to all studied analytes in the gas phase (Figure 4). Despite the fact that the kinetics of the interaction of Rh6G with analytes significantly depends on the nature of the analyte, the interaction is controlled by the process of physical (ad)sorption and is a completely reversible process.

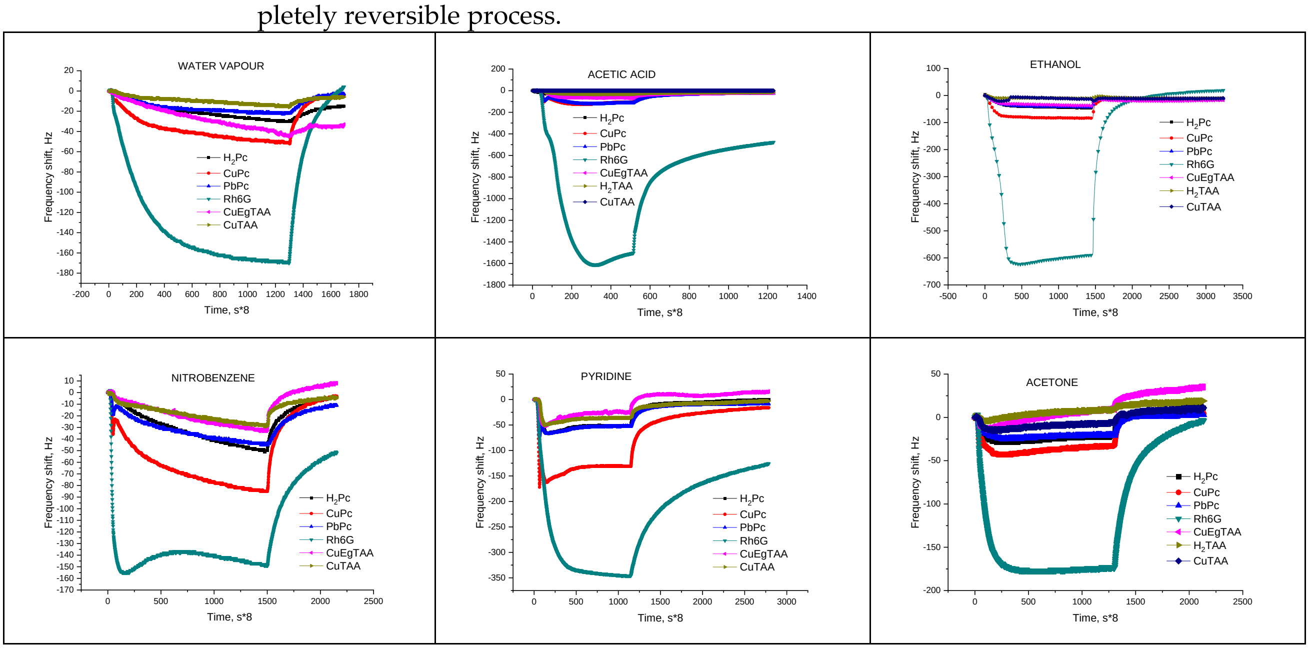

Figure 4. Typical adsorption curves for various analytes (acetic acid, ethyl alcohol, pyridine, nitrobenzene, acetone) for a sensor array with sensitive coatings based on heterocyclic macrocycles (annulenes and phthalocyanines, including their complexes with copper and lead) and Rhodamine 6G.

It should be especially emphasized that the response values for the Rh6G-based sensor significantly exceed the responses of other sensors, regardless of the type of analyte. This means that this material is promising for multisensor arrays, where the issue of cross-selectivity is a prerequisite. However, this is an unacceptable property for selective and, especially, specific sensors, since the surface of the coating based on Rhodamine 6G has a sufficiently large adsorption capacity for compounds of various natures. Let us only note that we are talking about the equilibrium filling of the surface in the presence of the analyte, i.e. reversible processes caused by interactions based on surface binding reactions typical of physical adsorption.

3.3. Features of the response of a sensor with a sensitive coating based on Rhodamine 6G in relation to analytes of various nature

The response of sensors coated by Rhodamine 6G shows that adsorption of all analytes is characterized by monotonic dependences with reaching a saturation level. The magnitude of the frequency shift is determined by the nature of the analyte (Figure 5). As can be seen from the figure, this coating has the greatest adsorption capacity for acetic acid and ethyl alcohol, and the smallest one - for acetonitrile, acetone and nitrobenzene. The rather small response to water (about 200 Hz), in which Rh6G is highly soluble, is apparently due to the short exposure time. Indeed, as can be seen from Figure 4, the adsorption process did not reach a steady state, suggesting a higher adsorption capacity.

Moreover, as was shown, for example in [21], in such cases there are changes in the morphology of the coating and, as a consequence, changes in the selectivity profile of the sensor element. Such sensors are unsuitable for practical use.

Acetonitrile, acetone, and nitrobenzene have in common their limited ability to form hydrogen bonds and the fact that their interactions are usually associated with the polar nature of their molecules (large dipole moments, tendency towards nucleophilic reactions etc.). The smaller response values for these analytes mean that centers for hydrogen bonds may be widely present on the surface, and the ability to form them increases the response. The response to ethyl alcohol confirms this statement. Moreover, the presence of methyl and hydroxyl fragments in the structure of the ethyl alcohol molecule can facilitate its binding, since similar bi-functional fragments are present in the Rhodamine 6G molecule, its dimers, or more complex aggregates. The same principle of multifunctional coincidence according to the somewhat simplified “guest-host” principle can explain the rather strong difference in the adsorption capacity in relation to the analytes listed above.

As can be seen from the presented data, the greatest response is observed for acetic acid vapor. First of all, it should be noted that the response in this case is non-monotonic: after reaching the minimum frequency value, the response begins to increase. This behavior, in particular, may be associated with an anti-Sauerbrey response, i.e. changes in the mechanical properties of the coating due to swelling, morphological changes, etc. [22]. A similar effect, although to a lesser extent, is observed for alcohol, in which these films can also dissolve.

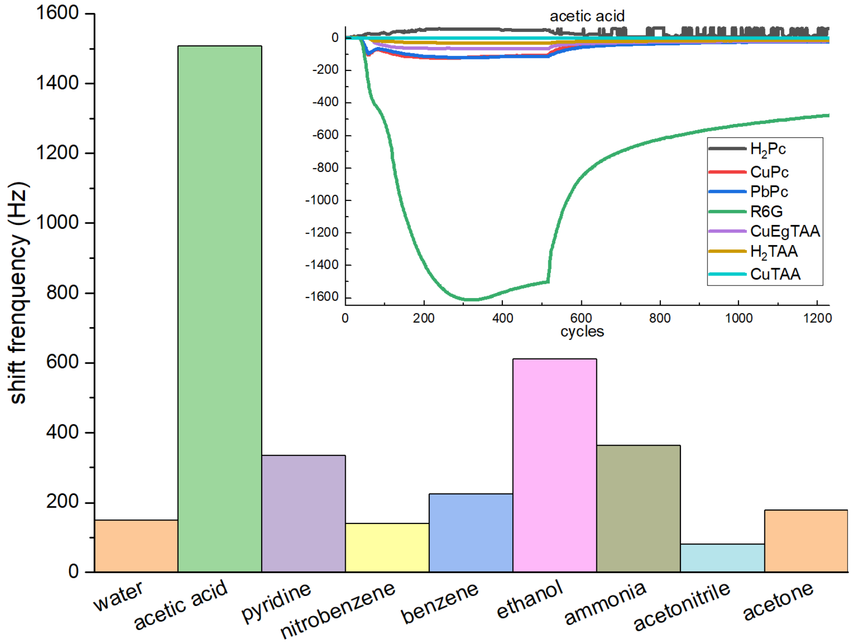

Figure 5. The maximum response amplitudes of a QCM sensor with a sensitive Rh6G-based coating on vapors of different analytes; inset: kinetic dependences of the frequency change on time during the adsorption of acetic acid vapors.

The highest magnitude of adsorption on the surface of acetic acid molecules may be associated with cooperative solvation effects capable of forming multicomponent surface coatings. As is known, in the gas phase, acetic acid is in the form of a dimer, which, when adsorbed on a surface, breaks down into two acid molecules, forming hydrogen bonds with the surface and with each other. In the case of dilute acetic acid, its percentage on the surface decreases due to the presence of water molecules, however, at the same time, conditions are created for the formation of surface networks of a multimolecular coating. According to the classification of solvents, acetic acid belongs to solvents that have acidic properties and are prone to the formation of loosely bound large surface associates [23].

4. Conclusions

The wide variety of low-energy binding sites for gaseous analytes on the surface of Rhodamine 6G films is due to a number of interrelated reasons at different levels of organization of the sensitive materials:

1. The presence of nucleophilic centers of different nature in side groups with the possibility of forming hydrogen bonds;
2. The ability for hydrophobic interactions, including due to aromatic stacking;
3. Binding centers, the energy of which is modulated by the vibrational modes of the molecule;
4. The presence of dimeric structures with different energy levels due to local effects of neighboring closely located molecules;
5. Supramolecular structures that stimulate cooperative surface binding.

All this leads to an increase in the diversity of analytes capable of binding to the surface of Rhodamine 6G. It is the low selectivity, coupled with the difference in response to various analytes, that makes this material promising for cross-reactive sensor systems, provided that the selectivity profile is maintained during operation. At the same time, these effects of physically (ad)sorbed compounds must be taken into account when developing selective sensors.

Finally, it is reasonable to emphasize the promise of Rh6G for the development of composite materials, since the combination of Rh6G with various inorganic nanostructured materials (ZnO, metal nanoparticles, etc.) makes it possible to purposefully change the selectivity profile of sensitive layer. This allows creating efficient sensor arrays optimized for specific application, including environmental monitoring or as a potential bio-sniffer for acute toxicity assays or highly sensitive sensors of low molecular weight biological regulators of vital activity.

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