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FLUORESCENCE INTENSITY RESPONSE OF FUMED SILICA FILMS WITH IMMOBILIZED 7-HYDROXY-4-METHYLCOUMARIN TO ACETONE MICROCONCENTRATIONS IN AIR

The spectral-fluorescence properties of the 7-hydroxy-4-methylcoumarin (Cou4) dye dissolved in ethanol and immobilized in the fumed silica (A-380 aerosil) sorbent in the form of films and their changes upon interaction with acetone molecules have been investigated. The dependence of the change in fluorescence intensity of such film samples on the concentrations of acetone and ethanol in the air in the 3–15 ppm range has been determined. The obtained results indicate the possibility of using the films for the sensory determination of acetone in the air selectively to simple alcohols (ethanol), particularly, for non-invasive diagnosis of diabetes by analyzing the exhaled human air.

Keywords: acetone, adsorption, coumarin dyes, fluorescence, fumed silica, optical chemical sensor.

1. Introduction

Acetone is a volatile organic compound, a polar aprotic solvent widely used in industrial production and everyday life. In case of leaks, acetone poses a danger of explosion and poisoning [1]. Also, acetone is an important metabolite-biomarker that characterizes the body's hydrocarbon and lipid metabolism. An increase in the concentration of exhaled endogenous acetone above 1.8 ppm (parts-per-million) is observed in type 1 diabetes, cardiovascular and some other pathologies, while in healthy people the norm is from 0.3 to 0.9 ppm [2]. The advantage of the diagnosis method by analyzing exhaled air is that it's non-

invasive, and it can detect the disease at an early stage. In addition, the determination of ketone bodies in the blood is used to control the ketogenic diet, which is increasingly used in the complex treatment of epilepsy, cancer, and some other diseases [3]. Considering the high correlation of the concentration of volatile ketones molecules, in particular acetone, in the bloodstream and in alveolar air [3, 4], the transition to non-invasive determination of acetone in exhaled air is of great interest.

Existing methods for determining low concentrations of acetone in exhaled air, such as gas chromatography and mass spectrometry [5, 6], have high sensitivity, selectivity, and accuracy, but aren't widely used in medical diagnostics due to the high cost of devices, difficulty in maintenance, *etc.* Therefore, it's important to create portable and inexpensive sensor devices for determining low acetone concentrations in exhaled air. Such sensors, in addition to the ability to determine low concentrations of the order of 1 ppm, must have high selectivity for acetone, since in human

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exhaled air, in addition to nitrogen, oxygen, carbon dioxide, and argon, more than 3000 different volatile chemical compounds are present in trace amounts [7].

Fluorescent sensors are one of the areas used to detect microconcentrations of substances. They have many advantages: high sensitivity, fast response, low cost, ease of operation and manufacturing, and the physical mechanisms of action of the sensing elements of such sensors allow us to achieve high selectivity for the analyte [6, 8, 9]. In addition, the ability to operate at room temperature, unlike thermocatalytic and metal oxide sensors, significantly increases their stability and service life.

Fluorescent sensors made of a film or a layer of porous sorbent particles functionalized with fluorophore molecules sensitive to the desired analyte can meet the requirements for detecting low concentrations of volatile molecules in the air [10–13]. When interacting with analyte molecules, depending on the physical mechanisms, a shift in the spectrum, quenching, or increase in fluorescence intensity can be observed, the value of which corresponds to the concentration of the molecules. For example, in [8], the fluorescence of nitrogen-doped carbon dots deposited on cellulose paper was studied. When the film interacted with acetone at a concentration of 0.5–150 mM, fluorescence quenching occurred with a linear correlation between the fluorescence quenching amount and the analyte concentration. The detection limit was estimated at 0.5 mM. The fluorescence quenching mechanism is attributed to the internal filter effect (IFE). In [9], the fluorescence of silica gel films with the fluorophore perylene monoimide modified non-planar organoboron derivative (PMI-BQ) immobilized by the drop-coating method was studied. The films showed sensory sensitivity to acetone in the air, which was measured by the fluorescence quenching value. In the concentration range of 50–260 ppm, the fluorescence quenching had a linear correlation with the acetone concentration. The detection limit was 50 ppm. The response time for 2600 ppm was 2 seconds, and the recovery time in clean air was 10 seconds. Such films also had sensitivity to acetone derivative vapors, such as triacetone triperoxide and diacetone diperoxide.

Coumarin derivatives are also used to create fluorescent sensor materials and probes [14–16]. For example, in [17], the fluorescence quenching of a solution of the fluorescent dye 3-methyl 7-hydroxyl

Coumarin (C4) in methanol with a concentration of 2×10^{-5} M in the presence of acetone was studied. The decay of fluorescence intensity was proportional to the concentration of acetone in the solution and was modeled by the Stern–Volmer relationship for dynamic fluorescence quenching

$$\frac{I_f^0}{I_f} = 1 + k_q \tau_0 \cdot Q, \quad (1)$$

where I_f^0 is the fluorescence intensity without quencher, I_f is the fluorescence intensity with quencher, k_q is the quencher rate coefficient, τ_0 is the lifetime of the emissive excited state in the absence of quencher, and Q is the quencher concentration. The Stern–Volmer constant $K_d = k_q \tau_0$ was 46.3 M^{-1} . The fluorescence after quenching was completely recoverable. According to the authors, the mechanism of fluorescence quenching is of dynamic nature, since the absorption spectrum and the position of the fluorescence peak $\lambda_{\text{em}} = 402 \text{ nm}$ of the fluorophore didn't change with the addition of acetone, indicating the absence of a chemical reaction between them. In [18], the dye 7-hydroxy-4-methylcoumarin (Cou4) was used to detect acetone in cosmetic extracts. A significant fluorescence quenching of the Cou4 solution in methanol, ethanol, propanol, butanol, and some other solvents was observed if acetone was present in the samples. The emission wavelength of the dye in ethanol was $\lambda_{\text{em}} = 443 \text{ nm}$ and didn't change with the addition of acetone (also for other alcohols). It's worth noting that the fluorescence intensity of Cou4 in each of these alcohol solvents without acetone was the same within the measurement error, and the fluorescence quenching by acetone was significant. Therefore, studying this dye for sensitivity to acetone and selectivity relative to alcohols in air is important.

The above studies relate to the detection of acetone in the liquid phase using coumarin fluorophores. However, there are practically no publications on the study of fluorescent sensor materials with coumarin dyes that are sensitive to acetone in the air, and the fluorescent and sensory properties of the Cou4 dye in sensor materials under interaction with acetone molecules in the air remain not investigated. The immobilization of Cou4 in a sorbent with a large specific surface area, which is necessary for high sensory sensitivity [6], can solve the problem of determining low

concentrations of acetone in human exhaled air or in the environment. Aerosil, a dispersed pyrogenic silica or fumed silica, can be used as a sorbent. It has such advantages as a high sorption surface, homogeneity of structure, and easy controllability of the size of the samples formed from it [19].

This work aimed to study the spectral-fluorescence characteristics of aerosil films with Cou4 in the interaction with acetone in the air medium and their sensitivity to acetone in the air in the range of low concentrations of about 3–15 ppm, which is typical for exhaled air of a diabetic person. We also wanted to determine the selectivity of such films to acetone relative to simple alcohols using ethanol.

2. Materials and Methods of the Experiment

Aerosil A-380, with a specific surface area of $380 \text{ m}^2/\text{g}$, was used as a sorbent to create film matrices. The sorbent material was chosen due to its high sorption surface, homogeneity of structure, and controllability of the sample size formed from it. The fluorescent organic dye 7-hydroxy-4-methylcoumarin (coumarin 4, Cou4, CAS 90-33-5, manufactured by Avocado, UK) was used as a fluorophore.

The dye was immobilized onto the sorbent by adsorption from a solution in 96% ethanol. 50 mg of aerosil was placed in 1 ml of dye solution and kept for 24 hours. The concentration of the dye in the solution was 10^{-3} mol/L . The formation of film samples was carried out by direct pressing in a cylindrical frame. The diameter of the samples was 6 mm, the weight was 15 mg, and the mass fraction of the dye in the aerosil was 0.35%.

The absorption and fluorescence excitation spectra of the samples were measured on a Specord M40 UV-VIS spectrophotometer with a fluorescence measurement attachment. The fluorescence spectra of the films were measured on a Solar TII SL40-2 spectrometer, excitation was carried out by laser radiation at $\lambda_{\text{ex}} = 405 \text{ nm}$. The fluorescence of the solutions was measured using a BioTek FLx-800T fluorometer.

A dye solution in ethanol with a concentration of 10^{-3} mol/L was used to study the quenching of Cou4 fluorescence by acetone in solution. The volume of the solution in the measuring cell was $200 \mu\text{L}$. The concentrations of acetone were 0, 1.24, 2.27, 3.14, and 3.89 mol/L. Fluorescence excitation was applied at $\lambda_{\text{ex}} = 360 \text{ nm}$, registration was at $\lambda_{\text{em}} = 460 \text{ nm}$.

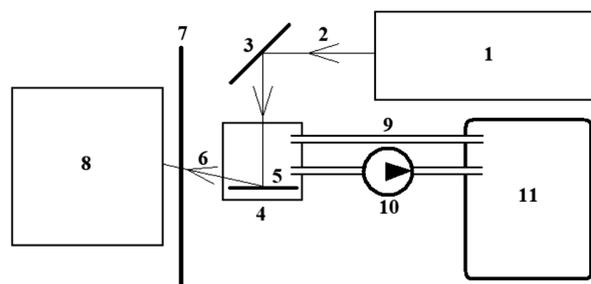


Fig. 1. The experimental setup for studying the sensory properties of film samples to acetone molecules in the air. See the explanation in the text

To study the response of film samples to the presence of acetone in the air, a specially designed experimental setup based on a Specord M40 UV-VIS spectrophotometer with a fluorescence measurement attachment was used (Fig. 1). The excitation radiation 2 with a given wavelength is transmitted from source 1, which is a halogen lamp with a monochromator, to sample 5, which is located in an optically transparent quartz sealed cuvette 4, through the mirror plate 3. The fluorescent radiation 6 of sample 5 passes through the cut-off excitation radiation light filter 7 and is transmitted to the photoelectric multiplier of the spectrophotometer 8. The air mixture containing volatile molecules of acetone or ethanol from a 1 liter glass container 11 was pumped through fluoroplastic tubes 9 using an air pump 10 through a 4 ml quartz cuvette 4.

The sensory properties of the film samples were determined by a method similar to that described in [19] by recording the fluorescence kinetics. The fluorescence excitation wavelength was $\lambda_{\text{ex}} = 335 \text{ nm}$. Fluorescence was registered through a light filter transparent in the range of $\lambda_{\text{em}} = 360\text{--}900 \text{ nm}$. First, the fluorescence intensity signal of the sample was measured for 60 s with the pump turned off, the following 120 s – when pumping an air mixture with a given concentration of impurities through the cuvette with the sample, and the next 300 s – when pumping clean air without impurities. The difference in the averaged fluorescence intensity before pumping (40–60 s) and at the end of pumping the air mixture (160–180 s) was considered a sensory response. The recovery of the fluorescence intensity after pumping clean air was used to assess the recovery of sensory properties. Measurements were performed for concentrations of 0–15 ppm with an interval of 3 ppm.

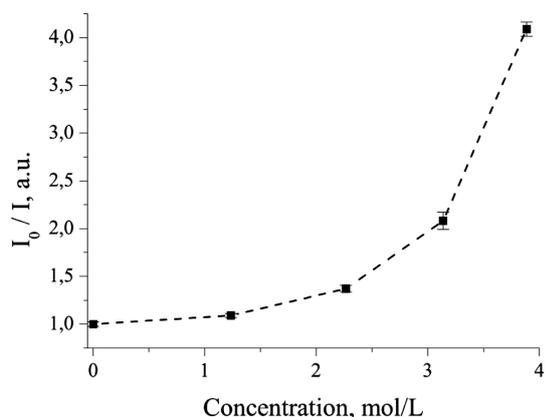


Fig. 2. Stern–Volmer plot: the dependence of I_0/I on the concentration of acetone in solution

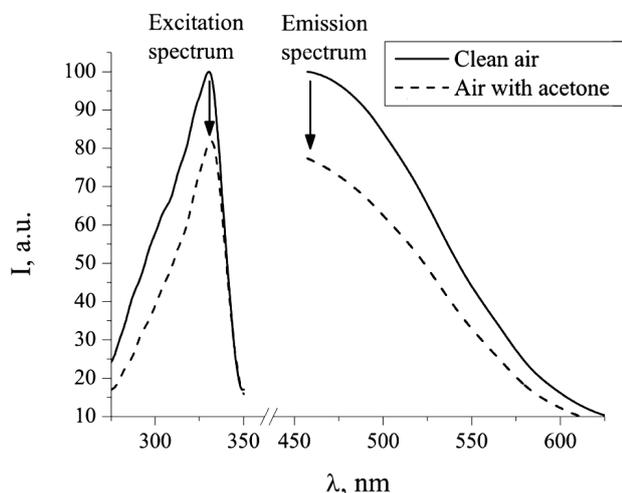


Fig. 3. Fluorescence excitation spectrum (left) and fluorescence spectrum (right) of aerosil film with Cou4 dye. The solid line indicates the spectra measured in pure air, and the dashed line – in air with 150000 ppm of acetone injected into a 4 ml measuring cell with a sample

Air mixtures with impurities of acetone or ethanol were created by the static volumetric method [20] at room ($T \approx 290$ °C) temperature.

3. Results and Discussion

In solutions of Cou4 in ethanol, blue fluorescence with a maximum of $\lambda_{em} = 443$ nm was observed upon excitation by UV radiation. First, we studied the qualitative effect of high concentrations of acetone molecules on the fluorescence of Cou4 in solutions and films. The fluorescence intensity of Cou4 in solution upon interaction with acetone decreased significantly,

i.e., fluorescence quenching, most likely of a dynamic nature [17], occurred. Fig. 2 shows the Stern–Volmer plot – the dependence of I_0/I on the concentration of the quencher (acetone). I_0 is the fluorescence intensity of the solution without a quencher, I is the fluorescence intensity with a quencher. The dependence is nonlinear. For the given wavelengths and the lowest concentration of the quencher, 1.24 mol/L, which is closest to the linear area typical of low quencher concentrations [18, 21], the Stern–Volmer constant $K_d = 0.0725 \pm 0.0017$ mol⁻¹·l was determined. Deviations from the linearity of the Stern–Volmer plot for high quencher concentrations toward higher values may indicate that static fluorescence quenching mechanisms also begin to act for these concentrations [21]. They are often observed at high quenching agent concentrations due to an increase in the number of quencher-fluorophore pairs in which the quenching agent molecule is close enough to the fluorophore [22].

Samples of aerosil films with Cou4 had strong elastic light scattering due to a large number of agglomerated SiO₂ nanoparticles in the aerosil structure [23], so optical absorption spectra couldn't be registered. We studied the fluorescence excitation spectra, which are much less affected by the transparency of the medium, as well as the fluorescence (emission) spectra (Fig. 3). The fluorescence excitation spectrum is characterized by a band with a maximum of $\lambda_{max} = 330$ nm and a full width at half maximum FWHM = 47 nm. Due to the peculiarities of the measurement technique, only a part of the band in the region of 457–625 nm was registered in the fluorescence spectrum. The figure also shows that in an air environment with acetone (concentration of 150000 ppm), there's a significant fluorescence quenching. At the same time, there's practically no shift in the position of the fluorescence excitation spectrum band, while it wasn't possible to determine the presence or absence of a shift from the fluorescence spectrum.

Changes in the fluorescence of aerosil films with Cou4 observed upon interaction with acetone vapor in the surrounding air are associated with two physical processes. Firstly, the adsorption of acetone molecules from the air onto the developed surface of the aerosil sorbent (5.7 m² for the investigated samples with weight 15 mg), and secondly, the quenching of the fluorescence of Cou4 molecules placed on the sorbent surface upon interaction with the adsorbed acetone molecules. The absence of a shift in the spec-

tral band of fluorescence excitation upon interaction with acetone may indicate the lack of complexation of fluorophore molecules in the basic unexcited state with acetone molecules, and hence the dynamic nature of fluorescence quenching. However, in this case, a more important indicator should be the absence of such changes in the optical absorption spectrum [22].

The influence of acetone molecules on the fluorescence of aerosil films with Cou4 during adsorption/desorption in the region of microconcentrations (0–15 ppm) in the air was studied by recording the fluorescence kinetics according to the method described in Section 2. The fluorescence excitation was applied at $\lambda_{\text{ex}} = 335$ nm near the maximum of the fluorescence excitation spectrum band (see Fig. 3) to ensure the highest signal-to-noise ratio of the spectrometer. This is especially important for sensitive measurements where fluorescence signal changes are weak. Fig. 4 shows one of the typical kinetics with a segment of pumping through a cuvette with a sample of an air mixture with 15 ppm acetone (60–180 s), followed by pumping clean air.

At the beginning of the measurement, no fluorescence changes are observed, except for the noise of the measuring device (Fig. 4, time interval 0–60 s). When the air mixture with 15 ppm acetone is pumped (60–180 s), there is a gradual decrease in the fluorescence intensity for nearly 100 s (Fig. 4, 60–160 s), after which it remains at approximately the same level (Fig. 4, 160–180 s). This behavior is associated with a gradual increase in the amount of adsorbed substance (adsorbate) on the surface of the sorbent due to the predominance of the adsorption rate of acetone molecules over the desorption rate, reaching an equilibrium value that corresponds to the equal rate. The value of fluorescence quenching of the Cou4 molecules placed on the sorbent corresponds to the adsorbate concentration in the sorbent. When pumping clean air without acetone was performed (Fig. 4, 180–480 s), the fluorescence intensity was restored to its initial level (for the time of 300 s shown in the chart, the recovery was 78%), which is explained by the gradual desorption of acetone molecules from the sorbent surface into the surrounding air and a corresponding decrease in fluorescence quenching.

The fluorescence quenching under the adsorption of acetone molecules on the sorbent of the film was proportional to the concentration of acetone in the air. We chose the difference ΔI normalized by 100%

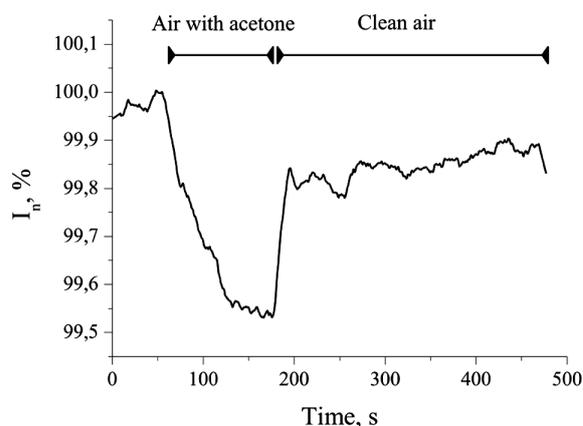


Fig. 4. Fluorescence kinetics (excitation at $\lambda_{\text{ex}} = 335$ nm) of the aerosil film with Cou4: interval 60–180 s – pumping of an air mixture with 15 ppm acetone, 180–480 s – pumping of air without acetone

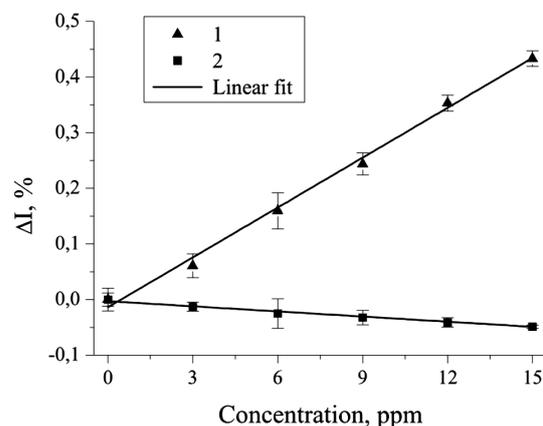


Fig. 5. Dependence of the fluorescent sensing response (in percent) of aerosil films with Cou4 on the concentration of acetone (1) and ethanol (2) in the air. The lines show a linear approximation of the dependencies

of the initial fluorescence intensity in pure air (Fig. 4, 0–60 s) and the fluorescence intensity in the kinetics region of 160–180 s (see Fig. 4) as the value of the sensory fluorescence response to the acetone concentration. The latter (160–180 s) corresponds to the establishment of the equilibrium of the adsorption-desorption rate and the maximum quenching value for a given acetone concentration. The dependence of this sensory response on the concentration of acetone in the air in the range of 0–15 ppm is shown in Fig. 5. Also, Fig. 5 shows the corresponding dependence for similar measurements of ethanol air mixtures of the same concentrations and wave-

lengths. Since the adsorption of ethanol on the film samples, unlike acetone, resulted in an increase in fluorescence intensity, the dependence has an opposite slope in the figure, i.e., negative values of ΔI .

The dependencies were well approximated by linear functions with a slope $k_{ac} = 0.0299 \pm 0.0014$ and a correlation coefficient $R_{ac} = 0.998$ for acetone and, respectively, $k_{et} = -0.00308 \pm 0.00054$ and $R_{et} = 0.998$ for ethanol. The linearity of the dependence may indicate the predominantly monomolecular nature of adsorption with a small degree of sorbent coverage in the studied concentration range. A significantly larger change in fluorescence intensity ΔI upon the adsorption of acetone molecules compared to ethanol $\left| \frac{k_{ac}}{k_{et}} \right| = 9.7$ indicates a high sensory selectivity for acetone.

4. Conclusions

The dependence of the fluorescence intensity of the organic dye Cou4 dissolved in ethanol on the concentration of the acetone quencher in the range of acetone concentrations of 0–3.89 mol/L was determined and a Stern–Volmer plot was obtained. It was found that both dependencies were nonlinear. For the lowest concentration of 1.24 mol/L, closest to the linear region, the Stern–Volmer constant $K_d = 0.0725 \pm \pm 0.0017 \text{ mol}^{-1} \cdot \text{l}$ was determined. The quenching of Cou4 fluorescence by acetone molecules is likely to be dynamic. Still, at high concentrations, a static quenching mechanism can be involved and lead to a nonlinearity of the Stern–Volmer relationship.

Samples of aerosil A-380 films with immobilized Cou4 were prepared. It has been found that upon interaction with acetone molecules in the air environment, the fluorescence of the films is quenched almost without a shift in the band position ($\lambda_{\max} = 330 \text{ nm}$) of the fluorescence excitation spectrum. Such changes in fluorescence are explained by two physical processes: adsorption of acetone molecules from the air onto the developed surface of the aerosil sorbent and probably dynamic quenching of the fluorescence of Cou4 molecules upon the interaction with adsorbed acetone molecules. Changes in the film's fluorescence intensity (sensory response) upon interaction with low acetone concentrations in the air in the range of 3–15 ppm were determined. The response time was nearly 100 seconds and corresponded to the equilibration of adsorption and desorption processes and, as a result, the fluorescence intensity reached a steady

state. It was found that the sensory response was proportional to the concentration of acetone and ethanol with a linear dependence ($R = 0.998$). Still, for acetone it was 9.7 times higher, indicating a high selectivity over simple alcohols such as ethanol. It should be noted that, unlike acetone, an increase in the fluorescence intensity of the films was observed for ethanol. The sensory response was completely and repeatedly recoverable due to desorption of acetone molecules from the sorbent surface into clean air. Thus, after 300 seconds for 15 ppm, it was restored by 78%.

The revealed properties of high fluorescent sensitivity and selectivity to acetone in the low concentration range, and multiple recovery of sensory properties after airing indicate that the obtained sensor films are competitive with thermocatalytic, metal oxide, electrochemical, and gravimetric gas sensors [7–9]. At the same time, in terms of cost, ease of manufacture, and compactness, they are preferable to gas chromatographic and mass spectrometric analyzers [6, 7]. Therefore, the studied aerosil films with Cou4 are promising as sensitive elements of fluorescent sensors for monitoring trace amounts of acetone in human exhaled air for non-invasive medical diagnosis of diabetes mellitus or in the environment air.

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ВІДГУК ІНТЕНСИВНОСТІ
ФЛУОРЕСЦЕНЦІЇ ПЛІВОК ПІРОГЕННОГО
ДІОКСИДУ КРЕМНІЮ З ІММОБІЛІЗОВАНИМ
7-ГІДРОКСИ-4-МЕТИЛКУМАРИНОМ
НА МІКРОКОНЦЕНТРАЦІЇ АЦЕТОНУ В ПОВІТРІ

В роботі досліджено спектрально-флуоресцентні властивості барвника 7-гідрокси-4-метилкумарину (Cou4), розчиненого в етанолі та іммобілізованого в сорбенті – пірогенному діоксиді кремнію (аеросилі А-380), у формі плівок і їх зміни при взаємодії з молекулами ацетону. Визначено залежності зміни інтенсивності флуоресценції таких плівкових зразків від концентрацій ацетону та етанолу в повітрі в діапазоні 3–15 ppm. Отримані результати вказують на можливість застосування досліджуваних плівок для сенсорного визначення концентрації ацетону в повітрі селективно стосовно простих спиртів (етанолу), зокрема, для неінвазивної діагностики діабету шляхом аналізу видихуваного людиною повітря.

Ключові слова: адсорбція, ацетон, кумаринові барвники, оптичний хімічний сенсор, флуоресценція.