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CONDUCTOMETRIC BIOSENSORS FOR FORMALDEHYDE QUANTITATIVE AND QUALITATIVE ANALYSIS

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Формальдегід класифікується як мутаген і канцероген, що підтверджується в експериментах на біологічних об'єктах (індукція ракових пухлин). Останні аналітичні дослідження спрямовані на розроблення біологічного методу визначення формальдегіду за допомогою біосенсора.

Ключові слова: кондуктометричні біосенсори, формальдегід.

Формальдегид классифицируется как мутаген и канцероген, что подтверждается в экспериментах на биологических объектах (индукция раковых опухолей). Новейшие аналитические исследования направлены на разработку биологического метода определения формальдегида с помощью биосенсора.

Ключевые слова: кондуктометрические биосенсоры, формальдегид.

Formaldehyde is classified as a mutagen and carcinogen, which is confirmed with the experiments on biological objects (induction of cancer). The newest directions of analytical researches are directed on the development of a biological method of definition of formaldehyde with the help of biosensors.

Keywords: conductometric biosensors, formaldehyde.

Introduction. A lot of people may be not aware that formaldehyde – toxin and carcinogen – is commonly used in the embalming process, it is also one of the most common ingredients in many materials that make up a normal home. As a result, many household items – from furniture to bed sheets – emit formaldehyde fumes that are harmful to health of families and children.

Formaldehyde is better known for its use as tissue preservative, such as in animals' preservation for dissection in schools. It is the chemical very toxic nature that makes it such effective preservative: it quickly kills bacteria or fungi that may otherwise begin process of decomposition. But formaldehyde is also an ingredient in a wide variety of resins used to make permanent adhesives for plywood and carpeting, causing it to be present in furniture and building materials (particularly those made with pressed wood products) and certain melded plastics.

Formaldehyde resins are used to make textiles crease-resistant and may be found in everything – from curtains to sheets and clothing. These resins are also used in dishwashing liquids, fabric softeners, carpet cleaners, glues, cardboard and paper products (including wallpaper) and certain latex paints. They are also used in products intended to be used on body, such as cosmetics (including nail polish and nail hardener) and paper products (facial tissues, napkins and paper towels).

All of these products outgas small quantities of formaldehyde, as do certain insulating foams that are no longer in use in new home construction but that may be present in older homes. Burning of most materials also releases formaldehyde, so fireplaces, wood stoves and smoking can also be a source of indoor formaldehyde.

The problem set and its relationship to important scientific and practical tasks. Formaldehyde is highly toxic in high concentrations – such as that may result from a workplace accident – and carcinogenic in smaller doses. Even in doses considered as safe for cancer risk, the chemical is still a potent irritant and allergen that may result in serious health problems. In toxic concentrations (25,000 ppb or more), formaldehyde may severely irritate the upper respiratory tract, potentially resulting in swelling or fluid accumulation in the lungs known as pulmonary edema. These symptoms may not manifest until hours after exposure, but may be potentially fatal due to oxygen deprivation. This is not a hazard in normal household exposure, but may be a risk for workers in factories or other workplaces that use formaldehyde.

Formaldehyde getting into people's organism may also cause convulsions, intense pain in mouth and stomach, nausea, vomiting, signs of shock, vertigo, stupor, and diarrhoea. Direct contact of eyes with formaldehyde causes permanent eye damage or loss of vision.

Exposure to high levels of formaldehyde is known to build-up of fluid in lungs, severe shortness of breath, bronchitis, and rapid heart rate. Prolonged effect also causes severe allergic reactions of skin and eyes, skin allergies and rashes, and asthma-like allergies with coughing, wheezing, chest tightness, and a drop in body temperature.

Exposure to low levels of formaldehyde may irritate and burn eyes, nose, throat and skin. In women, such exposure may cause menstrual disorders. People with asthma may be more sensitive to exposure to formaldehyde.

The most of people, working in professions where there is regular exposure to formaldehyde, are at risk, so a diverse range of professions with such exposure appears. Carpenters and builders are exposed working with particle board and other wood products as well as glues; lab technicians, beauticians amongst others are exposed regularly to the chemical.

Formaldehyde may cause skin and nasal irritations, asthma, and other respiratory problems, and has been linked to lung cancer and leukaemia. It may be inhaled through tobacco smoke, gas fires and cookers. When formaldehyde is present in air at levels exceeding 0,1 ppm, some individuals may experience adverse effects such as watery eyes; burning sensations in eyes, nose, and throat; coughing; wheezing; nausea; and skin irritation. Some people are very sensitive to formaldehyde, whereas others have no reaction to the same level of exposure.

Analysis of recent studies and publications that started the problem solution by other scientists. Formaldehyde undergoes rapid chemical changes immediately after absorption. Therefore, some scientists think that formaldehyde is unlikely to have effects at sites other than the upper respiratory tract. However, some laboratory studies suggest that formaldehyde may affect the lymphatic and haematopoietic systems [1].

All these facts convincingly demonstrate the requirement of reliable analytical devices for an accurate FA determination in testing consumer goods, environment, as well as biological samples.

Some standard methods are used for quantitative and qualitative analysis of formaldehyde, namely, gas chromatography [1], fluid chromatography [2], fluorometry, refractometry, and spectrophotometry [3–5]. These methods have some serious drawbacks, such as necessity of using relatively expensive and complicated equipment, use of toxic reagents, and presence of highly-qualified personnel. Moreover, the major part of them is not sensitive, selective and specific enough, and not suitable for the express analysis of a great number of samples. Thus, formaldehyde analysis requires development of some alternative approaches that would be deprived of the majority of drawbacks (ideally – all of them), peculiar to routine methods of its determination. It seems that only bio- and chemosensors, a new generation of analytical devices based on electrochemical or optical transducers and selective molecules of biological and chemical origin, merit these requirements. Recently, a number of bio- and chemosensors [6, 7], including biosensors based on alcohol oxidase [1–5], intact, permeabilized and lyophilized yeast cells [5], bi-enzyme system based on formaldehyde dehydrogenase and diaphorase [1, 7] and formaldehyde dehydrogenises [5] were proposed for quantitative and selective determination of formaldehyde in model and real samples.

Marking out of questions that where not decided earlier and research aim formulation. The greatest drawback of the most formaldehyde dehydrogenase-based sensors is the usage of exogenous nicotinamide adenine dinucleotide (NAD) in analyzed sample, insertion into a bio-selective membrane (BSM) of covalently bounded NAD and necessity of cofactor's regeneration for conducting recurrent analysis using its electrochemical or enzymatic recovering of NADH. It is important noteworthily, that in [1–4] authors propose the original bi-layer architecture for the BSM creation, but these formaldehyde-sensitive biosensors, except of the amperometric biosensor proposed in [3], may only be used for the detection of formaldehyde concentration in solutions with rather low content of formaldehyde.

The main purpose of work is the creation of novel formaldehyde-sensitive biosensors.

Statement of basic materials of research. Novel formaldehyde sensitive conductometric biosensors, based on commercial bacterial formaldehyde dehydrogenase (FDH) from *Pseudomonas putida* and recombinant formaldehyde dehydrogenase (rFDH) from the yeast *Hansenula polymorpha* as the bio-recognition elements, have been developed. The bio-recognition membranes have mono-layer architecture and consist of enzyme cross-linked with albumin and of the cofactors NAD (for FDH-based sensor) or NAD and glutathione (for rFDH-based sensor). Such bio-recognition elements' architecture allows detecting formaldehyde concentration without adding exogenous NAD and glutathione to the analyzed sample and conducting measurements on the same transducer without cofactors regeneration since the bio-membrane contains it at high concentration (100 mM for NAD and 20 mM for glutathione).

The linear dynamic range of the sensor output signals corresponds to 10-200 mM of formaldehyde concentration depending on the enzyme used (Fig.1). The measurements were conducted in 10 mM borate buffer, pH 8,7, at formaldehyde concentration 10 mM (1, 3) and 25 mM (2, 4). The RSD is 10 % (n = 10) and 4 % (n = 10) for FDH-based and rFDH-based conductometric sensor, respectively.

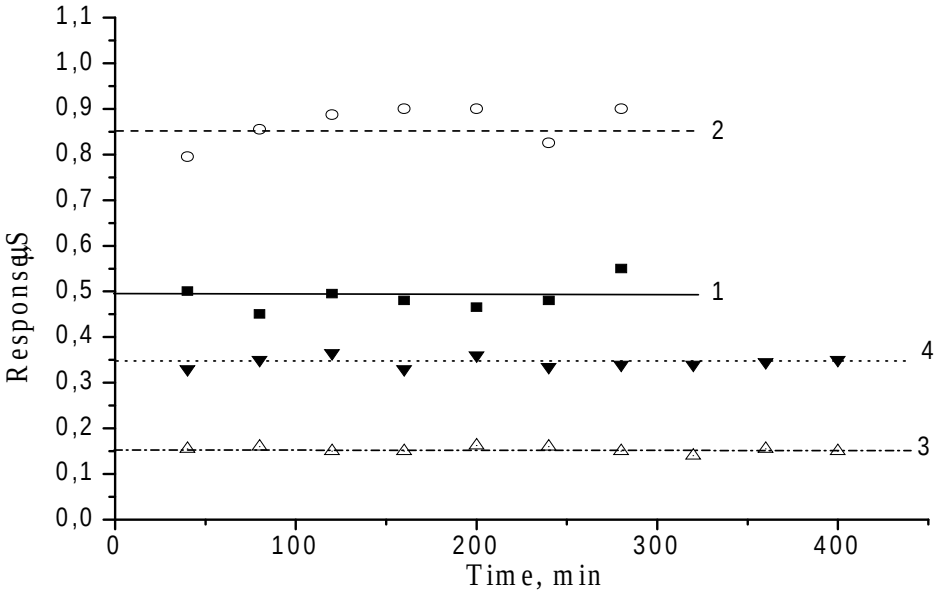

Fig. 1. Operational stability of the formaldehyde-sensitive conductometric sensors: *1, 2* – FDH-based; *3, 4* – rFDH-based

The dependence of the biosensor output signals on pH and buffer concentration as well as operational/storage stability and selectivity/specificity of the developed conductometric biosensors have been investigated (Fig. 2). The biosensors were stored in 10 mM borate buffer, pH 8,7, at 4 °C (1, 3) and in dry state at +20 °C (2, 4).

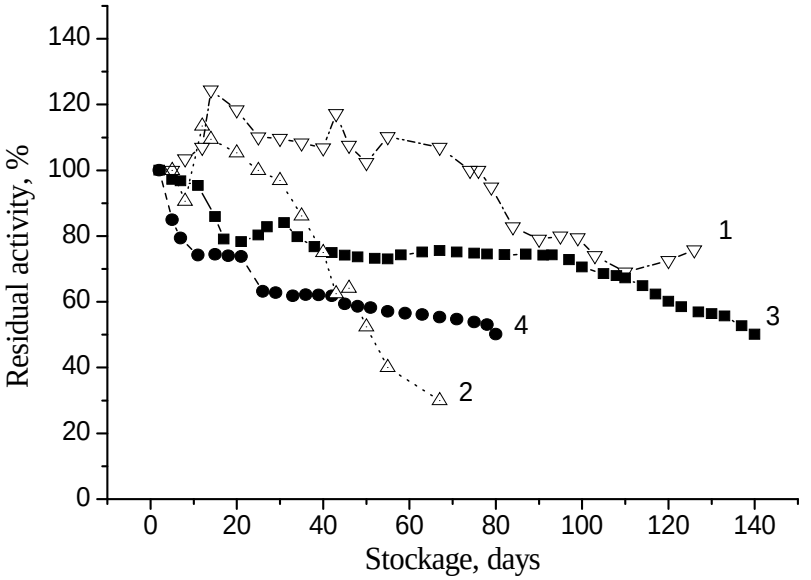

Fig. 2. Storage stability of the formaldehyde-sensitive conductometric sensors: *1, 2* – FDH based; *3, 4* – rFDH-based

The developed biosensors have been validated for formaldehyde detection in some real samples of pharmaceutical (formidron), disinfectant (descoton forte) and industrial product (formalin). A good correlation has been revealed between the concentration values measured by the developed conductometric biosensor, an enzymatic method, amperometric biosensors developed earlier, and standard chemical methods of formaldehyde determination.

Conclusions of the research and perspectives of further developments in this direction. Conductometric biosensors for formaldehyde detection based on commercially available and novel recombinant formaldehyde dehydrogenases have been developed and investigated by the scientists of Institute of Molecular Biology & Genetics NAS (Kyiv, Ukraine) and the authors. An original methodology has been proposed to create a bio-selective element on the transducer surface, where desired enzymes and high concentration of NAD and glutathione are incorporated into a glutaraldehyde cross-linked protein membrane. A high level of NAD and glutathione in BSM has been achieved by using of positively charged polymeric carrier, DEAE-Dextran, which bind with NAD and glutathione non-covalently. A good correlation has been revealed between the concentration values measured by the developed conductometric biosensor, an enzymatic method, amperometric biosensors, developed earlier, and standard chemical methods of formaldehyde determination. Such original method of determination of formaldehyde is the newest and perspective.

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