Nanoliter detectors for flow systems

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Abstract

The new fiber-based optical microfluidic detectors, designed for nano-volume sample measurement, are presented. The silicon-glass microdetectors were fabricated by use of micromechanical techniques. Spectrofluorimetric and spectrophotometric methods of detection were applied. Optical fibers, integrated into microchannels, ensured very low dead-volume (17 nl for spectrofluorimetric and 900 nl for spectrophotometric detector). The microdevices were made as separate modules and may be connected to any microanalytical system, in which the fluorescence or absorbance is measured. The fabrication methods of microdetectors are compatible with micro-total analysis system (μTAS) technology. The preliminary tests showed the linear detection range 0–0.2 mg/ml of fluorescein and erythrosine (spectrofluorimetric detector), and 0.2–1.6 g P/ml of phosphate in water solutions (spectrophotometric detector).

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1. Introduction

Optical sensors have been used in analytical chemistry for several years. There are different types of such sensors, typically light absorption or emission may be used as the valuable measure of analyte properties. Rapid development of liquid micro-total analysis systems (μTASs), fabricated by use of microelectronic born, microengineering techniques, involves increased interest of researches and users in microsensors compatible to μTASs, able to analyze samples in nanoliters regime [1,2].

This is well-known fact that standard optical sensors, which seem to be the most attractive detectors for chemical microsystems, are not compatible neither to structure of μTASs (dimensions, materials used, connections, etc.) nor to a very low quantity of maintained chemicals, which is far below the dead-volume (microliters) of standard sensors. Therefore, several approaches to apply the micro-optical detection in microfluidic systems have recently been proposed in literature. The presented solutions utilize different passive and active optical components including optical fibers [3–6], planar waveguides [7–9] and built-in mesa photodiodes [10,11]. The detection systems based on optical fibers have been demonstrated in many works due to its relative simple integration into microfluidic system and easy connection to analytical instruments.

In this paper, two new micromachined fiber-based optical microdetectors have been presented. The silicon-glass detectors are characterized by a very low nano-picoliters dead-volume.

In the first type of a microdetector, well-known spectrofluorimetric method has been applied, based on the fluorescence effect. In this detector, small amount of fluorimetric indicator (fluorescein, erythrosine) is added to an analyte. Excitation light beam, illuminating an analyte, induces the emission of fluorescent light, which intensity may be a measure of a quantitative and/or qualitative analysis [12].

In the second type of microdetector, spectrophotometric method has been applied due to its simplicity and good sensitivity. In this method, absorption of light in miniaturized flow-through cell is measured. Both sensors, equipped with light guiding optical fibers, have been manufactured as separate, single chip modules. They may be connected to μTAS by use of mechanically resistant, clean inlet/outlet connections. The construction and parameters of the sensors have been designed to ensure reliable use in analytical laboratories as microdevices with parameters as good as standard detectors.

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2. Experiment

2.1. Spectrofluorimetric detector

2.1.1. Detector design

The detector consisted of a silicon chip with etched channels, glass cover, two fibers and glass connections (Fig. 1). Sample (fluid) flowed through the trapezoid-shaped, 500 μm wide and 200 μm deep channel. A standard telecommunication multimode glass fiber (fiber 1), with outer diameter \( \phi = 125 \mu m \) and core size 62.5 μm, was used to illuminate a sample. The fiber was positioned in a V-groove, etched perpendicular to the sample channel. The dimensions of V-groove were carefully adjusted to the fiber 1. Thus, a sample was precisely illuminated near-by semi stream of a flow. The sample volume illuminated by an excitation light was about 17 nl.

Fiber 2, made of polymethyl methacrylate (PMMA), \( \phi = 0.75 \) mm, was used to collect the fluorescent light. The diameter of the fiber 2 was bigger than dimensions of planar area, in which fluorescence takes place. Therefore, most of the fluorescent light, emitted by the sample, was guided to external photodetector.

2.1.2. Fabrication

The fabrication process of the sensor consisted of several steps. The silicon chip was fabricated on n-type, (100)-oriented silicon wafer. Channels were formed in one-step wet silicon micromachining procedure by use of 10 M KOH in 80 °C through SiO\textsubscript{2} wet thermally grown mask layer. After etching, SiO\textsubscript{2} mask layer was removed and a new 0.3 μm thick thermally grown SiO\textsubscript{2} layer was formed.

Next, in the 1 mm thick Borofloat 35 mm × 35 mm glass substrate (Schott, Germany), two φ = 0.9 mm openings were mechanically drilled. Afterwards, the substrate was anodically bonded to the silicon chip through thermal SiO\textsubscript{2} layer [13,14]. Fiber 1 was positioned in the V-groove and small drop of UV-curable glue (Epo-Tek, UV-114) was added. Thanks to capillary forces the glue was transported through V-groove, filling completely a free space. Three glass tubes (0.8 mm inside diameter, 4 mm outside diameter and 5 mm high) were glued to the glass cover. Two of them formed inlet/outlet port for fluid, the third was used as a mechanical support for protection of the fiber 2 (Fig. 2).
End-faces of all used fibers were carefully prepared. Inlet of fiber 1 and outlet of fiber 2 were equipped with connectors compatible to the Fluoromax 3 (Jobin Yvon) spectrophotometer. Next, the assembled detector has been tested.

2.1.3. Test measurements

First, the leakproofness of the microstructure was checked. During pumping (with the use of a peristaltic pump; Minipuls 3, Gilson), a liquid medium through the channel of microdetector, leakage was not observed even when the flow rate was high (3 ml/min). The maximum flow rate value is comparable with total flow rate in the flow injection analysis (FIA) systems, but in the case of presented construction is only the safety parameter. Generally, the spectrophotometric microdetector was adopted for flow chemical analysis at low flow rates.

Next, optical quality of the device was checked. First, the light from an ultra-bright blue LED was coupled to the fiber 1 through microscope objective. An efficient excited fluorescent light was well observed (Fig. 3).

In the further tests, the microdetector was connected to the conventional Fluoromax 3 spectrophotometer. Water solutions of popular fluorimetric indicator (fluorescein), with concentration of 0.1 and 0.2 mg/ml, were prepared. The flow rate of a fluid was 100–150 µl/min.

Fig. 3. Detection of the microdetector: (a) fluidic channel and fiber 1 in a V-groove; (b) fluorescence of an analyte (fluorescein), note the 17 nl light emitting volume.

In order to find the analytical wavelength of the excitation light, for which the detector had maximum sensitivity, the spectral measurements were performed. As shown in Fig. 4a, the maximum output signal of the sensor for fluorescein was observed at λEM = 509 nm for the excitation light λEX = 490 nm. Therefore, this wavelength of excitation light was chosen to measure the dependence of the detector response on the fluorescein concentration. It has been found that increasing the concentration of fluorescein two times (from 0.1 to 0.2 mg/ml) doubled the output signal (Fig. 4b). The response of the detector to the change of concentration was stable and fast.

![Graph](image-url)
Similar measurements were performed for water solutions of erythrosine with concentration of 0.07 and 0.14 mg/ml. The peak wavelength of emitted fluorescent light was found at $\lambda_{EM} = 544$ nm for the excitation light $\lambda_{EX} = 509$ nm (Fig. 5a). The flow pulses from the peristaltic pump caused the noise recorded during single wavelength measurements (Fig. 5b).

The obtained results indicated linear response of the microdetector to the changes in analyte concentration ranging 0–0.2 mg/ml for fluorescein and 0–0.14 mg/ml for erythrosine. The peak wavelengths of emitted fluorescent light $\lambda_{EM}$ (509 nm for fluorescein and 554 nm for erythrosine) were comparable with results obtained for standard Fluka solutions (514 and 556 nm, respectively) [15].

It also appeared that the detection sensitivity for erythrosine is about two times lower in comparison with fluorescein (Fig. 6). The high signal/noise (S/N) ratio was observed during the measurements.

2.2. Spectrophotometric detector

2.2.1. Detector design

The detector was composed of a silicon chip with fluidic channels, glass cover and two fibers for input and output of the light (Fig. 7).

The sample channel and two V-grooves for fibers positioning were wet anisotropically etched in the one-step silicon micromachining procedure. In order to increase the way of light propagation through the analyte, the C-shaped fluidic channel was formed. Two standard multimode fibers ($\phi = 125/62.5 \mu m$) were located at the opposite corners of the fluidic channel to ensure 10 mm optical path length. Thus, for 150 $\mu m$ deep and 700 $\mu m$ wide fluidic channel, the volume of optical cell was about 900 nl.

2.2.2. Fabrication

The fabrication process of the spectrophotometric detector included three main steps: silicon micromachining, glass cover bonding, assembling of the fluidic ports and optical fibers.

In the first step, the fluidic channel and two V-grooves (150 $\mu m$ deep both) were wet anisotropically etched on a 3 in., n-type, (100)-oriented silicon wafer. The one-step etching procedure was performed using standard 10 M KOH solution at 80°C and 0.6 $\mu m$ thermal SiO$_2$ layer as a mask. After etching of silicon and removing of SiO$_2$ mask, a new 0.3 $\mu m$ thick silicon dioxide layer was formed. The silicon wafer was then divided into 25 mm x 25 mm x 0.36 mm chips.
In the second step, two in/out vias (φ = 0.9 mm) were mechanically drilled in 1 mm thick Borofloat glass cover (Schott, Germany) in order to allow the fluid circulation. The fluidic channel was then closed as a result of anodic bonding of the silicon structure to the 35 mm × 35 mm glass cover.

In the last step, two glass capillaries (0.8 mm inside diameter, 4 mm outside diameter and 5 mm high) were positioned over vias and bonded to the glass cover using UV-curable epoxy. Next, the optical fibers were positioned in V-grooves and immobilized by small drop of UV glue. The top view of the assembled spectrophotometric microdetector and enlarged view of detection area are shown in Fig. 8.

2.2.3. Test measurements

The leakproofness of the spectrophotometric microdetector was confirmed by pumping of DI water (Minipuls 3, Gilson) through the channel at 2 ml/min. As mentioned earlier, the construction of the detector as a separate module allowed using it as an alternative “optical cuvette” in conventional spectrophotometers. Therefore, the microdetector was connected to the CDI and miniaturized fiber optic PC-2000 (Ocean Optics, USA) spectrophotometers during the tests. The spectra of test water solution of potassium permanganate (KMnO₄) was measured in a flow mode (100–150 ml/min). The characteristic peak positions were compared with corresponding spectra obtained using DA (Zeiss, Germany) spectrophotometer, equipped with classical cuvette (Fig. 9).

The results of KMnO₄ spectra measurements indicated good compatibility of the microdetector with conventional spectrophotometers.

The analytical usefulness of the microdetector was also tested by the single wavelength measurements of phosphates concentration in water. Standard KH₂PO₄ solution (Fluka) was used to prepare test solutions with different phosphate concentrations.
concentrations (0.2–3.0 g P/ml). The flow rate (100 l/min) of a fluid was set by the peristaltic pump. The calibration curve of detector response versus phosphate concentration was determined by use of the PC-2000 spectrophotometer (Fig. 10). The linear detection range of microdetector was found for concentrations of the phosphate ranging from 0.2 to 1.6 g P/ml.

3. Conclusions

The new spectrofluorimetric and spectrophotometric microdetectors, designed for nano-volume sample measurements, have been presented. Micromechanical techniques such as silicon-glass micromachining and anodizing bonding were used for the fabrication of the detectors. Optical fiber system integrated into fluidic microchannel ensured very low detection volume: 17 nl for spectrofluorimetric detector and 900 nl for spectrophotometric detector. Preliminary results have indicated a good sensitivity and reproducibility of the microdetectors. Linear detection range of the spectrofluorimetric microdetector was noticed for concentrations of fluorescein and erythrosine ranging from 0 to 0.2 mg/ml. Analytical usefulness of the spectrophotometric detector was confirmed by the determination of phosphate concentration in water. The response of the detector was linear for concentration of analyte from 0.2 to 1.6 g P/ml.

The microdetectors were designed as separate modules, thus they can be connected to the conventional analytical equipment to measure absorbance or fluorescence of nano-volume samples. Although no additional optical elements have been used to enhance the detection, high S/N ratio was observed during the test measurements. The parameters of the spectrofluorimetric microdetector may be improve by use of the microlenses [16], fabricated on the glass cover, for focusing the fluorescent light on the plastic fiber. The fabrication methods of presented microsensors are compatible with µTAS technology. Therefore, their integration with miniaturized chemical microsystems will be realized in future.

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