A New Optic Fiber Sensor for Measuring the Concentration of Ethanol in Wine

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This paper presents the development of a new optic fiber sensor for measuring ethanol concentration in real time, obtained by simultaneous immobilization of alcohol dehydrogenase and NAD+ onto optical fiber by sol-gel technique in MTMOS/TEOS matrix. Biosensor was tested in a concentration range of 2-18% ethanol and the results were compared with standard spectrophotometric enzyme assay determinations, indicating that the sensor has a good functionality in the range of the analyzed concentrations.

Keywords: optical fiber sensor, sol-gel, ethanol

Ethanol is one of the most important substances with different application including food industry, biotechnologies, fermenters, pharmaceutical and medicine and many other new fields, like bioethanol production for its application in fuel technology [1, 2]. For any application of ethanol it is necessary to detect and quantify ethanol with high accuracy, but all of these applications have different requirements, including sensitivities, detection limit and assay time. In order to determine the concentration of ethanol many methods were used, such as electrochemical, Raman and mass spectroscopies, polarography, chromatography and enzymatic assay, etc. [3, 4], but most of these methods are usually time consumers and require the use of expensive instrumentation. In enzymatic methods, most enzyme catalyzed reactions can be followed by simple, widely available spectroscopic or electrochemical methods, but they cannot be applied for turbid samples, such as blood or food samples.

Sensors involving light measurements associated with fiber-optics are particularly attractive [5, 6]. The different configurations for fiber optic based sensors, their characteristics and their potential applications have been recently studied [7-9].

The optic fiber function is to transmit light to and from an immobilized reagent phase consisting generally of a fluorescent dye or of a colorimetric indicator secured at the end of the fiber optic. For biochemical analysis, substrates or enzymes can be immobilized, allowing the determination of enzyme activities or substrates [10]. The enzyme immobilization procedure itself is one of the main factors that affect the performance of a biosensor is [11, 12, 13].

An ethanol enzyme assay may use either alcohol oxidase (AOD) or alcohol dehydrogenase (ADH) [14]. In the reaction involving alcohol dehydrogenase (ADH) ethanol is converted to acetaldehyde with the reduction of the nicotinamide adenine dinucleotide cofactor (NAD+) to NADH.

Et-OH + NAD+ + ADH → acetaldehyde + NADH + H+ (1)

Using the enzyme reaction (1), ethanol concentration can be determined by measuring the absorption of NADH at a wavelength of 340 nm [15, 16].

A proper method for enzyme immobilization on optical fiber is sol-gel method [17-31]. We present in this paper the development of a novel absorption fiber optic biosensor for ethanol based on the use of reaction (1) in order to determine the alcoholic concentration. The optical fiber biosensor consisted of enzyme alcohol dehydrogenase entrapped with NAD+ in a sol-gel MTMOS/TEOS matrix. Changes in alcohol concentration affects the amount of NADH produced, resulting in a change of absorbance of 340 nm.

Experimental part

Materials, equipment and methods

All materials used for determinations were purchased from Sigma-Aldrich Company (Germany).

- β-nicotinamide-adeninedinucleotide (NAD+) solution 0,063 mM;
- Alcohol dehydrogenase enzyme ADH (EC 1.1.1.1) 60 U/mL;
- Alcohol absolute (99.95 %);
- Tetraethoxysilane 98% (TEOS);
- Methyltrimethoxysilane 98% (MTMOS);
- Solution HCl 1N;
- Solution NH4OH 1N;
- Phosphate buffer solution pH = 7;

Equipment

For the experiments the following equipment were used:
- Portable laser spectrophotometer, Jazz from Ocean Optics, with two channels, one channel of spectrometric type “master” for UV-Vis range (1025-200 nm) and a “slave” channel for the Vis-NIR range (360-1100 nm). The spectrophotometer is equipped with a built-in microprocessor and OLED display for independent operation (without PC), with 2-channel and source excitation deuterium-halogen-tungsten, 210-1100nm, 7W, with integrated power supply lithium-ion battery, included in the platform.
- Optical Fiber Bifurcated - for the 210-1100 nm, consisting of 2 fibers, one for enlightenment, the other for reading, fiber length 2 m, SMA 905 connectors;
- SMA 905 fiber Multimode connector, prepared for enzyme deposition: the cutting end of the optical fiber was

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polished and a portion of 30-40 mm of the cladding has been removed for the next enzyme applying steps.
- Software Spectrasuite for acquisition and real time analysis of data, as well as for data processing.
- UV-Vis Spectrophotometer Specord 210 Plus (Analytik Jena, Germany).

The experimental setup used to measure the spectral response of the interrogator system used for determinations is presented in figure 1 and is consisting of:
- Jaz Modular Spectroscopy Suite, from Ocean Optics;
- PC on which Spectrasuite software runs for data acquisition and real time analysis;
- Bifurcated optical fiber;
- Optical fiber biosensor.

The instrumental setup is made so that the incident beam generated by the light source of the Jaz Modular Spectroscopy Suite reaches the sensitive layer of the optical fiber biosensor and the reflected beam is collected and guided through the other end of the fiber coupler, the output being acquired by Jaz Spectrometer Module and transferred to the PC where by the mean of Spectrasuite software is processed, saved and displayed.

The developed sensor is based on a plastic optical fiber with the core diameter of 1 mm terminated with a sensing element. The fiber end was cut, polished and a portion of 30-40 mm of the cladding has been removed for the next enzyme applying steps. The sensing matrix is made from MTMOS/TEOS hybrids obtained from sol-gel technique.

Basic working principle is based on a classic colorimetric approach. The interrogator light is scattered and absorbed by the sensing matrix that include ADH and NAD+. The NADH produced in biochemical reaction induces a change of the optical spectrum of the outgoing light collected by the fiber. Analyzing the spectrum of this optical signal the concentration of the ethanol can be measured.

The technique involved the sol-gel enzyme immobilization under mild conditions of temperature and pressure without altering the biological activity. A certain amount of MTMOS: TEOS was mixed by magnetic stirring in a glass bottle in the ratio 1:1 with solution HCl 1N and the mixture was homogenized for 2-3 h. After obtaining the homogenous solution the pH was adjusted to 6 with 25 % ammonium hydroxide solution. It was added the NAD+ solution and ADH solution. When the mixture had a proper consistence the fiber optic was immersed into it and stirred it with a certain speed assuring a uniform film matrix deposition onto optical fiber. The deposited layer was dried at room temperature for 24 h prior to use and kept immersed in phosphate buffer pH = 7. The measurements were performed at room temperature.

To evaluate the performance of the developed sensor, the spectral properties of the sensor have been measured using Spectrasuite software for data acquisition and analysis in real time. The results were compared with spectral analysis realized on the UV-Vis spectrophotometer Specord 210 plus (Analytik Jena, Germany) performed after the standard spectrophotometric enzyme assay [15, 16].

In order to test the optical fiber sensor and to determine the alcohol content the standard curve was set up, using ethanol solutions in the concentration range of 2% to 18 %, as presented in figure 2. The sensor was immersed in ethanol solutions and the optical density at 340 nm was determined. Figure 2 shows the calibration curve set up for the range of linearity.

This figure shows a linear relationship which can be used in quantification of ethanol concentration.

Results and discussions

In order to test the applicability of the sensors to beverages, the biosensor was tested on white wine samples obtained from alcoholic fermentation of the grape must in different stages of fermentation realized at Bay Zoltan Institute of Biotechnology from Szeged, Hungary and the results were compared with the standard spectrophotometric enzyme assay. The ethanol samples were frozen before the analysis and after melting, they were centrifuged for 5 min at 13000 rpm. The supernatant was used for ethanol determinations.

Using the experimental setup, the spectral response of the interrogator system in real time for the wine samples was measured, as presented in figure 3.

All the spectra show a well visible maximum at the wavelength at about 340 nm. The samples were also analyzed using the spectrophotometric standard addition method [15, 16] by the mean of an UV-VIS spectrophotometer Specord 210 plus (Analytik Jena, Germany). Results obtained from three repetitive measurements are shown in table 1.

The data obtained are in good agreement with the colorimetric enzyme test kit measurements based on the standard spectrophotometric method.

These results indicate the possibility for a good application of the optical fiber biosensor for ethanol

![Fig. 2. Standard curve](image)

Fig. 2. Standard curve

![Fig. 3. Absorbance spectra of wine samples measured by optical fiber sensor](image)

Fig. 3. Absorbance spectra of wine samples measured by optical fiber sensor
monitoring in grape must during the fermentation process as well as in wines and other products.

For sample 2, using the fiber optic biosensor the spectra in real time and ADH activity at the wavelength 340 nm was determined, as presented in figure 4 and 5. It is well observed the maximum of absorbance at 340 nm corresponding to NADH obtained in reaction. The absorbance data allow to show how the enzymatic reaction occurs in real time and to determine the ADH activity.

Conclusions

The new developed optical fiber biosensor obtained by simultaneous immobilization of alcohol dehydrogenase (ADH) and NAD+ onto optical fiber by sol-gel technique in MTMOS/TEOS matrix is suitable for the ethanol concentration determination for the range of 2-18% ethanol and it allows experimental determinations in real time. A good correlation between ethanol concentration measured with optical fiber sensor and classical spectrophotometric method has been noticed. The further optical fiber sensor characterization is referred to the stability, the sensitivity and the time response of the sensor.

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References


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