Optical Biosensors for the Determination of Biologically Active Compounds in Samples with Complex Matrices

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This investigation was financially supported by RFBR (Project 12-03-00249-a) and RF Ministry of Education and Science (contracts 8425 and 8448).

Introduction

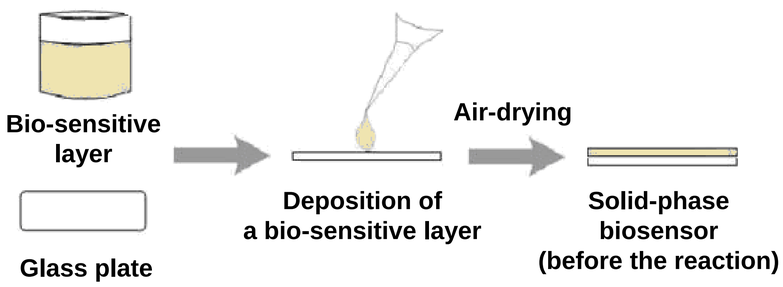
The creation of highly sensitive and selective sensor systems for the determination of different biologically active compounds (such us phenolic compounds, catecholamines, dibenzothiophene and the products of its oxidation, organic peroxides) without preliminary pretreatment of samples with complex matrices is one of the promising directions of modern biochemical analysis. The determination of such compounds is necessary to control the quality of pharmaceutical and food products, plant materials, diesel oil, and etc.

In the present work the constructions of solid-phase optical biosensors with photometric and fluorescent detection of an analytical signal were developed. The action of the proposed biosensors is based on the molecular recognition of the above mentioned analytes by the enzyme - horseradish peroxidase - HRP (or hemoglobin from bovine blood - Hb) , which are included into a self-assembled optical transparent film or gel of a biopolymer (chitosan).

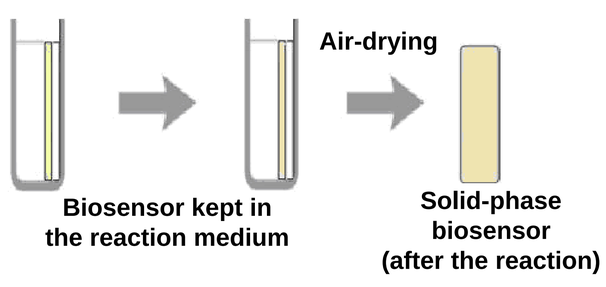
A novel feature

of the proposed biosensors is the measurement of the analytical signal (absorbance or emission) of a glass slide with biorecognizing films or gels {polyelectrolyte-enzyme-photometric or fluorescent reagents} rather than absorbance or emission of the same indicator reaction solution. The solid-phase photometric and fluorescent indicator systems for the determination of each group of the above mentioned analytes were elaborated. As a result, it is possible to analyze emulsions and non-transparent solutions and solutions with a high content of polar organic solvents without preliminary separation of a matrix. The developed optical biosensors were applied for the analysis of a wide range of real samples, including those with water insoluble matrices.

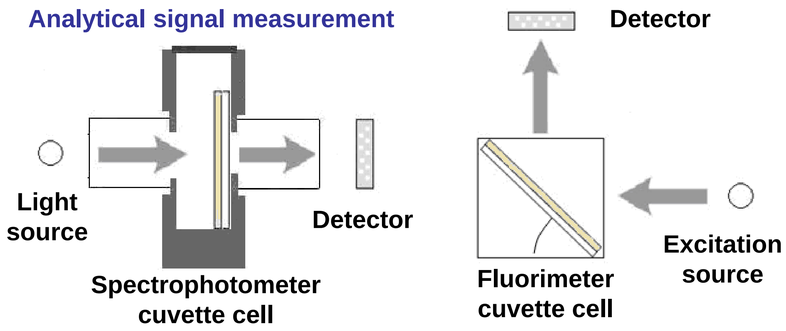
Optical biosensor production



Analysis procedure employing optical biosensor

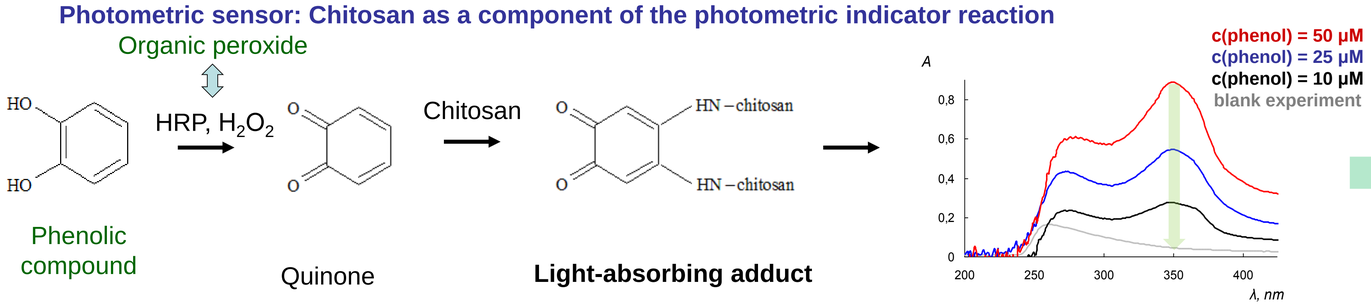


Analytical signal measurement



Indicator systems for solid-phase detection of the analytical signal

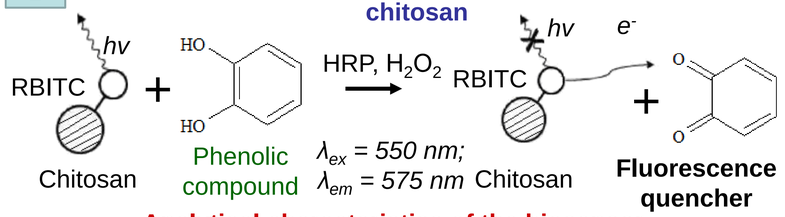
1 Photometric sensor: Chitosan as a component of the photometric indicator reaction



Analytical characteristics of the biosensor

Analyte	Concentration range, μM	c_{min}, nM	RSD, % (n=3, P=0,95)
Hydroquenone	20.0 – 250.0	7	3
Quercetin	10.0 – 100.0	3	3
Rutin	10.0 – 100.0	3	4
2-Butanone peroxide	50.0 – 1000.0	15	3
Benzoyl peroxide	50.0 – 250.0	15	3

2 Fluorescent sensor: Biosensor based on RBITC-labeled chitosan



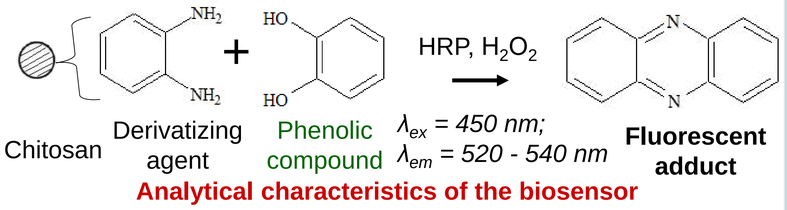
Analytical characteristics of the biosensor

Phenolic compound	Concentration range, μM	c_{min}, μM	RSD, % (n=3, P=0,95)
Catechol	0.5 – 50.0	0.10	4
Resorcinol	0.5 – 25.0	0.15	3
Hydroquinone	0.5 – 15.0	0.20	3
Dopamine	5.0 – 150.0	2.0	5
Epinephrine		1.5	6

Real-samples analysis

Pharmaceutical preparation/analyte	Analytical form	Added (μM)	Found (μM)
Achromine / hydroquinone	Water-insoluble skin ointment	1.0	1.1 ± 0.1
Epinephrine	Injections for intravenous infusion	5.0	5.4 ± 0.6
Dopamine		10.0	10.5 ± 0.8

3 Fluorescent sensor: Biosensor based on the derivatization with *o*-phenilenediamine



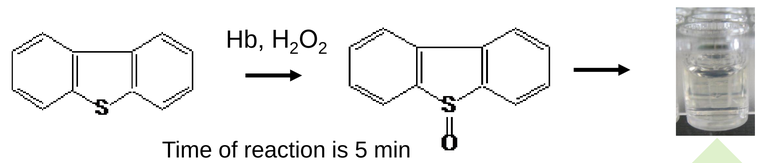
Analytical characteristics of the biosensor

Phenolic compound	Concentration range, μM	c_{min}, nM	RSD, % (n=3, P=0,95)
Catechol	50.0 – 250.0	200	3
Dopamine	25.0 – 200.0	70	3
Epinephrine	10.0 – 100.0	50	4
Homovanillic acid	1.0 – 10.0	5	3
Vanillylmandelic	0.5 – 7.5	3	3

Real-samples analysis

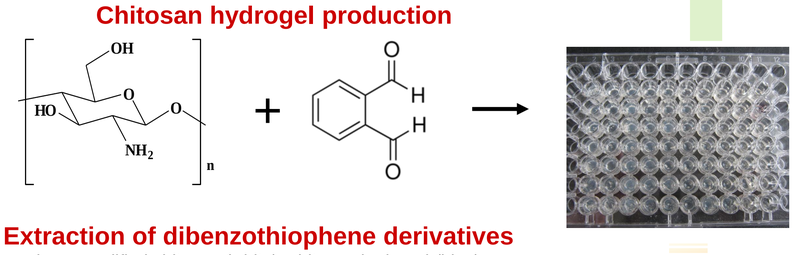
Pharmaceutical preparation/analyte	Analytical form	Added (μM)	Found (μM)
Xylocaine/ epinephrine	Injections for intravenous infusion	0.50	0.49 ± 0.06
Epinephrine		0.20	0.21 ± 0.03
Dopamine		0.10	0.11 ± 0.02

4 Fluorescent sensor: Hydrogel of chitosan as a matrix for the extraction and determination of the products of dibenzothiophene biooxidation



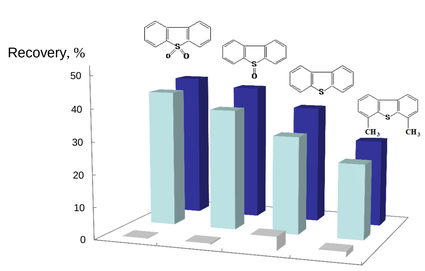
Time of reaction is 5 min
Degree of conversion is more than 95 %

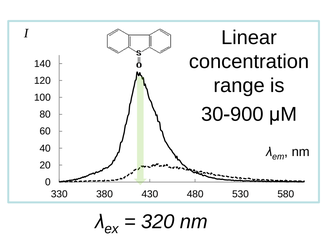
Chitosan hydrogel production



Extraction of dibenzothiophene derivatives

by unmodified chitosan (white), chitosan hydrogel (blue), hydrogel with the molecular imprints (light blue)





λ_{ex} = 320 nm