Optical Sensor receptors for Food Freshness and Pesticides detection

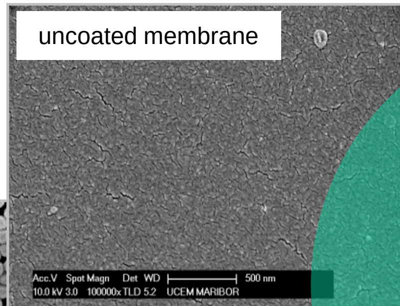
Aleksandra LOBNIK, Luka Popović, Edouardo Dona,V. Šumak, V. V. Siliesarenko

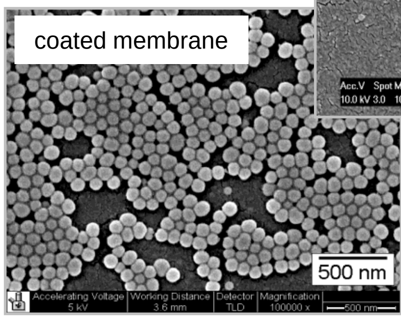
24-26 April 2023, 2nd ISO-FOOD SYMPOSIUM

http://www.ios.si/

IOS, Institute of Environmental Protection and Sensors

PATENT:
KOŠAK Aljoša, BAUMAN Maja, LOBNIK Aleksandra *"A method of surface treatment of thin film composite (TFC) membranes with tetraalkoxysilanes for retention of heavy metal ions in the membrane filtration processes of waste waters", Patent No. SI 23535 A, 2012*, **The Slovenian Intellectual Property Office, Ljubljana**

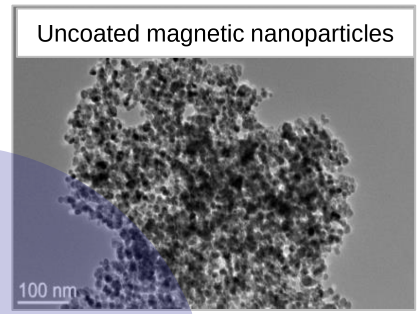


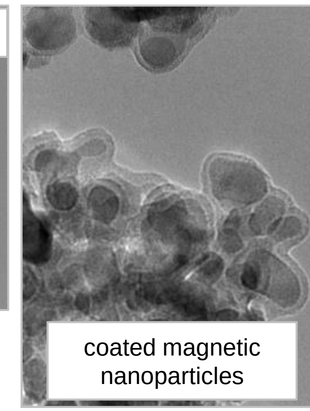


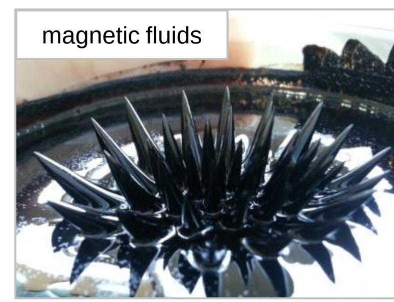
RECYCLING TECHNOLOGIES 4
WATER, TEXTILE & PLASTIC WASTES, METALS

OPTICAL CHEMICAL/BIO SENSORS

NANOTECHNOLOGY & NANOMATERIALS





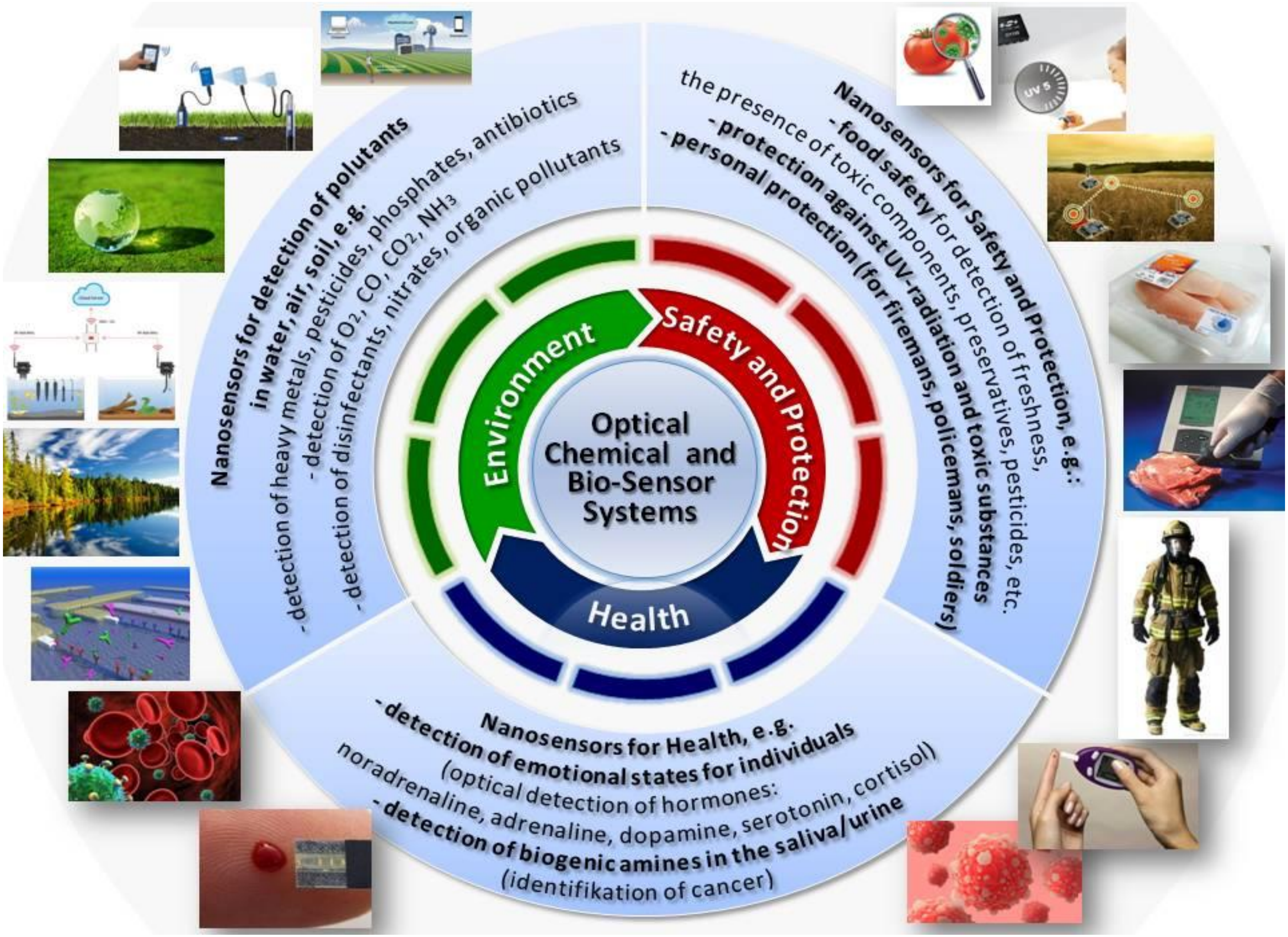


PATENT:
LOBNIK Aleksandra,
KORENT UREK Špela.
"A method and an optical chemical sensor with a sol-gel membrane for the detection of organophosphates",
Patent No. SI 23556 A, 2012,
The Slovenian Intellectual Property Office, Ljubljana

PATENT:
LOBNIK, Aleksandra *"Sol-gel based optical chemical sensor for detection of organophosphates and method for preparation thereof"*:
US Patent 2013/0251594A1; Appl. No.: 13/989,529; PCT/SI2011/000068;
United States and International Intelectual Property Law, 2013

PATENT:
KOŠAK Aljoša, LAKIĆ Marijana, LOBNIK Aleksandra, *"Process for the preparation of superparamagnetic hollow spherical nanostructures", application number P-201400120*, **European Patent Office CA G2**, *25 Mar 2015*.

Sensor Applications



DEFINITION OF SENSORS

Chemical Sensors are miniaturized analytical devices that can deliver real-time and on-line information on the presence of specific compounds or ions in complex samples.

Nanosensors – using nanomaterials or nanotechnologies to prepare nano sensor receptors

Analytical aspects of sensors

- sensitivity in the range of interest
- selectivity for the analyte
- broad dynamic range
- reversibility
- lack of frequent calibration
- fast response
- inertness to sample matrix
- small size

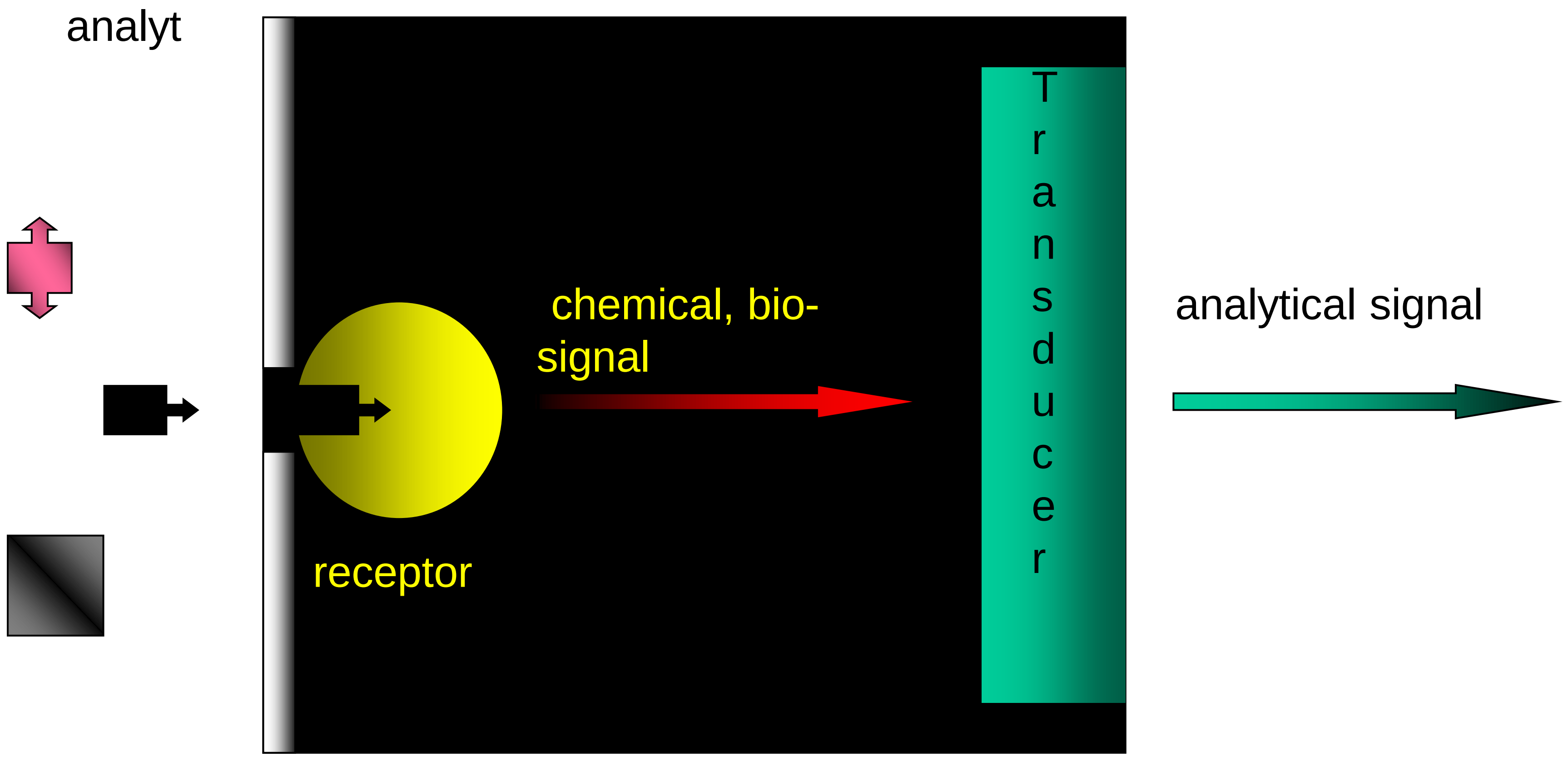
“Optrode” - (from optical electrode) and “optode” (from Greek - the optical way)

- intrinsic optical property of the analyte is utilized for its detection

- indicator (or label) based sensing is used when the analyte has no intrinsic optical property INDICATOR CHEMISTRY

- (FOCSs) represents a subclass of chemical sensors in which an optical fiber is used aspart of the transduction element.

OPTICAL CHEMICAL/BIO- SENSOR system



Design of optical chemical sensor
„Indicator chemistry“

Indicator
Polymer matrix
Immobilization

Sensor characteristics

Indicators

Absorbance based:

Undergo colour change

Detection by “naked "eye

Detection by colorimetry

Luminescence based:

Fluorescence

Phosphorescence

Chemiluminiscence

Electroluminiscence

Detection by:

UV lamp

Intensity change

Lifetime measurements

Polymer carrier

* hydrophobic

PVC, PMMA,
PE, PS, …

Detection of gases

* hydrophilic

polysaccharides,
polyacrilates,
polyamines,
hydrogels…

Ion detection

* hidrophobic-
hydrophilic

sol-gel

Detection of ions and gases

Sol-gel process (Silica nanoparticles)

1. *hydrolysis*

$$Si(OR)_4 + H_2O \rightarrow HO\text{-}Si(OR)_3 + ROH$$

2. condensation*:*

$$HO\text{-}Si(OR)_3 + HO\text{-}Si(OR)_3 \rightarrow HO\text{-}Si(OR)_2\text{-}O\text{-}Si(OR)_2 + HOH$$

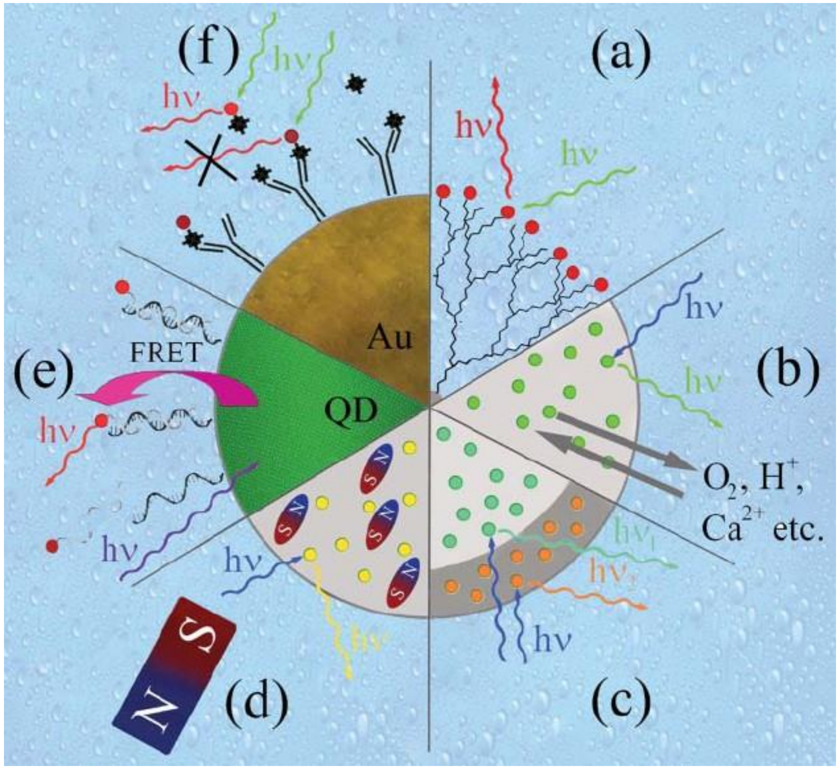
$$HO\text{-}Si(OR)_3 + Si(OR)_4 \rightarrow Si(OR)_3\text{-}O\text{-}Si(OR)_3 + ROH$$

Monomers	Other metals
$Si(OR')_4$	$Zr(OR')_4$
$R_1\text{-}Si(OR')_3$	$R_1\text{-}Zr(OR')_3$
$R_1 R_2\text{-}Si(OR')_2$	$R_1 R_2\text{-}Zr(OR')_2$
R: aliphatic, aromatic	Ti, Sn

ADVANTAGES OF USING NANOMATERIALS FOR SENSORS

- Improved sensor characteristic (response time, sensitivity, etc.)
- In-vivo measurements,
- Small sample volumes,
- Multi-analyte sensing

Design of Optical nanosensor

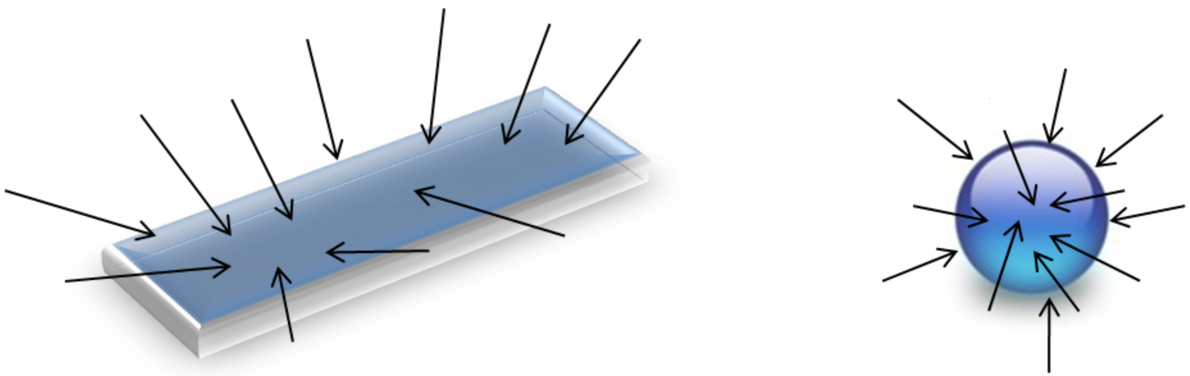


Borisov SM, Klimant I (2008) Analyst 133:1302-1307

a, macromolecular nanosensors (dendrimers); b, NSs based on polymer materials and sol-gels; c, multi-functional core-shell systems; d, multi-functional magnetic beads; e, NSs based on quantum dots; f, NSs based on metal beads

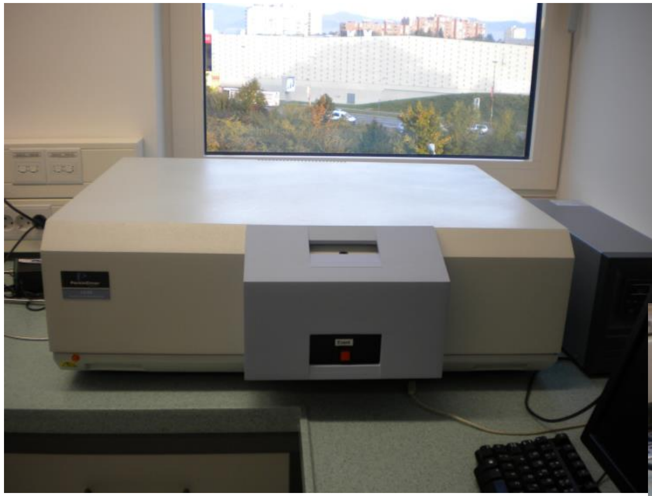
Comparison of OP sensor characteristics

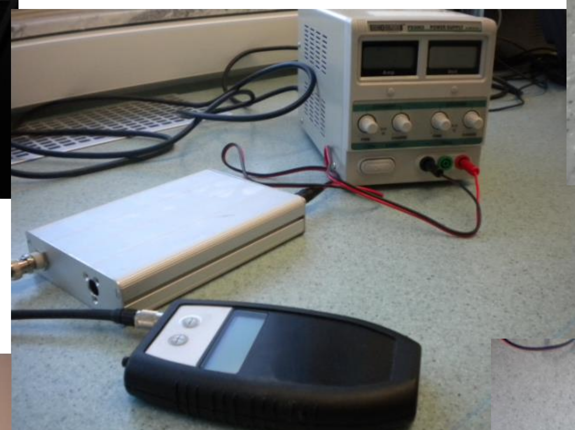
(A. Lobnik, Š. Korent Urek, EU, USA, Russia patents)

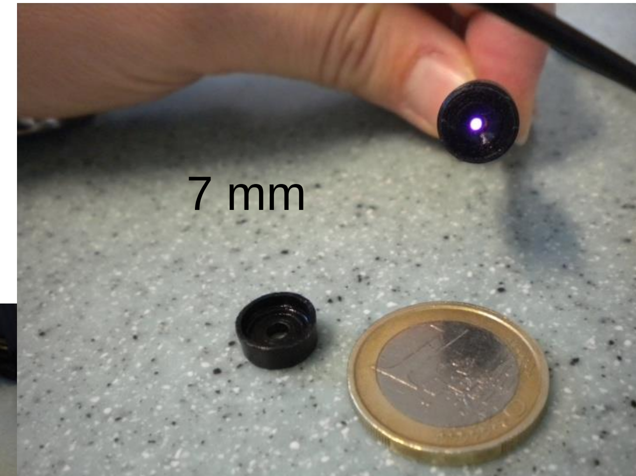


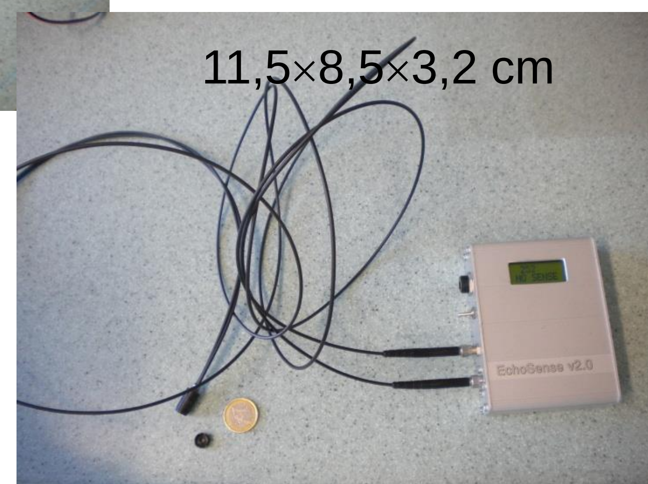
	Dye-doped thin films	Dye-doped nanoparticles
Limit of detection (mol/L)	6.7×10^{-7}	0.17×10^{-9}
Working range (mol/L)	$6.9\times10^{-7} - 6.9\times10^{-3}$	$0.17\times10^{-9} - 2.3\times10^{-7}$
Response time (s)	600	12

Luminiscence measurements of OP









Applications in Food safety: Food freshness

Food freshness depends on microbialogical activity

and

Various Biogenic amines are formed and released

- **Vino**

(histamin, tiramin, triptamin, feniletilamin, agmatin, kadaverin, putrescin, spermidin)

Hrana in pijača

- **Sir**

(putrescin, kadaverin, histamin, tiramin, feniletilamin, spermin, spermidin, agmatin)

- **Riba/Tuna**

(histamin, triptamin, kadaverin, feniletilamin, spermin, spermidin, tiramin, agmatin)

- **Meso/Klobasa**

(triptamin, feniletilamin, putrescin, kadaverin, histamin, serotonin, tiramin, spermin, spermidin, agmatin)

- **Sadje/Zelenjava**

(dopamin, tiramin, putrescin, kadaverin, histamin, serotonin, agmatin, feniletilamin, spermidin, spermin, agmatin)

Amino acid — decarboxylation amino acid → biogenic amine

$$H_2N-\underset{\displaystyle R}{\underset{|}{CH}}-COOH \xrightarrow[-\,CO_2]{} R-CH_2NH_2 + CO_2$$

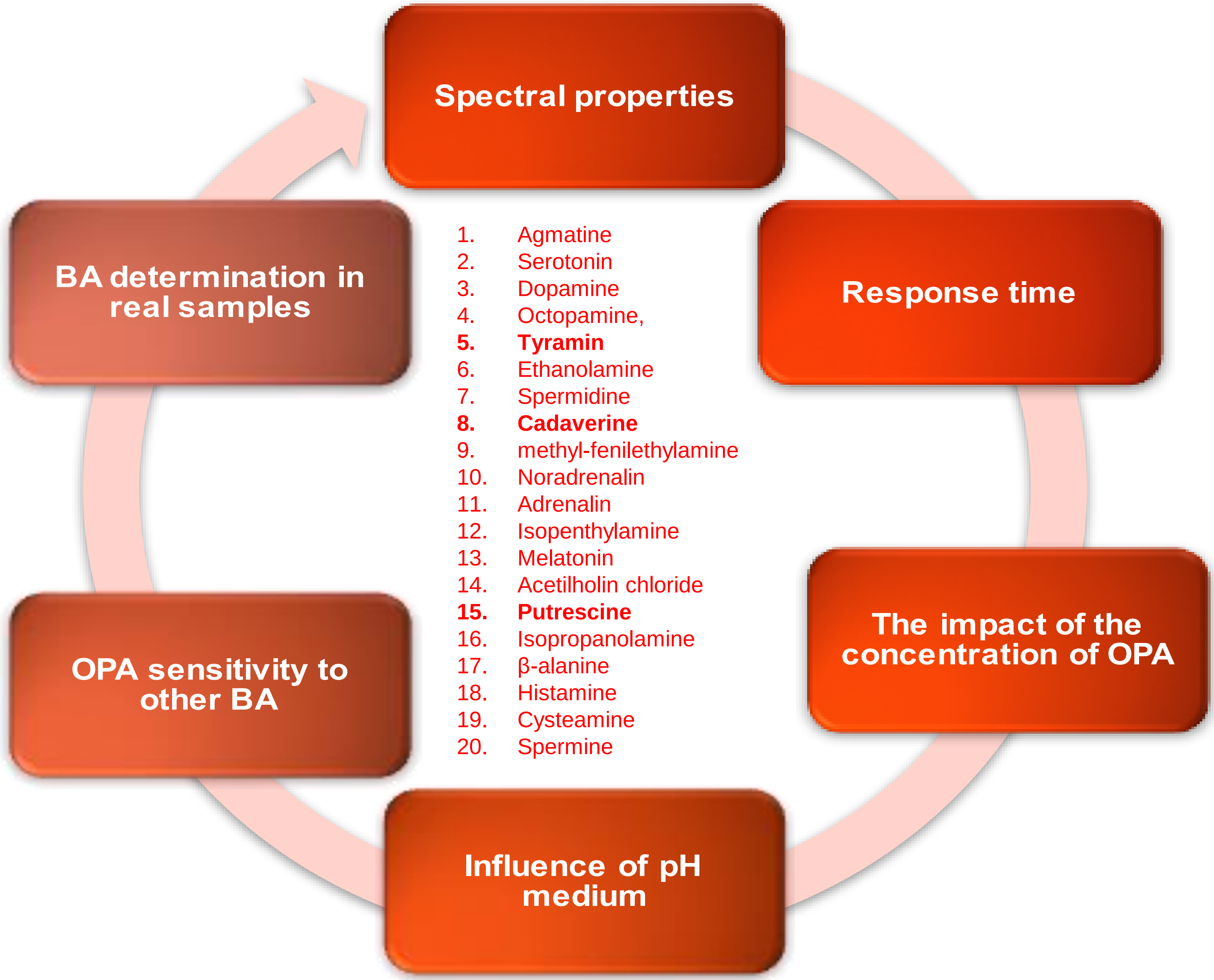
METHODS FOR BIOGENIC AMINE DETERMINATION

- **Instrumental or classical methods**
  - High-performance liquid chromatography (HPLC)
  - Thin-layer chromatography (TLC)
  - Gas chromatography (GC)
  - Micellar electrokinetic chromatography (MEKC)
- **Other methods**
  - electrochemical methods (capillary electrophoresis) (CE)
  - enzymatic methods (biosensors)
- **Optical methods**
  - **Optical chemical sensors (OCS)**

Derivational reagents:

- dansyl chloride,
- benzoyl chloride,
- dansyl chloride,
- fluorescein,
- 9-fluorenylmethyl chloroformate,
- naphthalene-2,3-dicarboxaldehyde
- **orthophthalaldehyde (OPA)**

OPTICAL DETERMINATION OF BA BY O-PHTHALDIALDEHYDE (OPA)



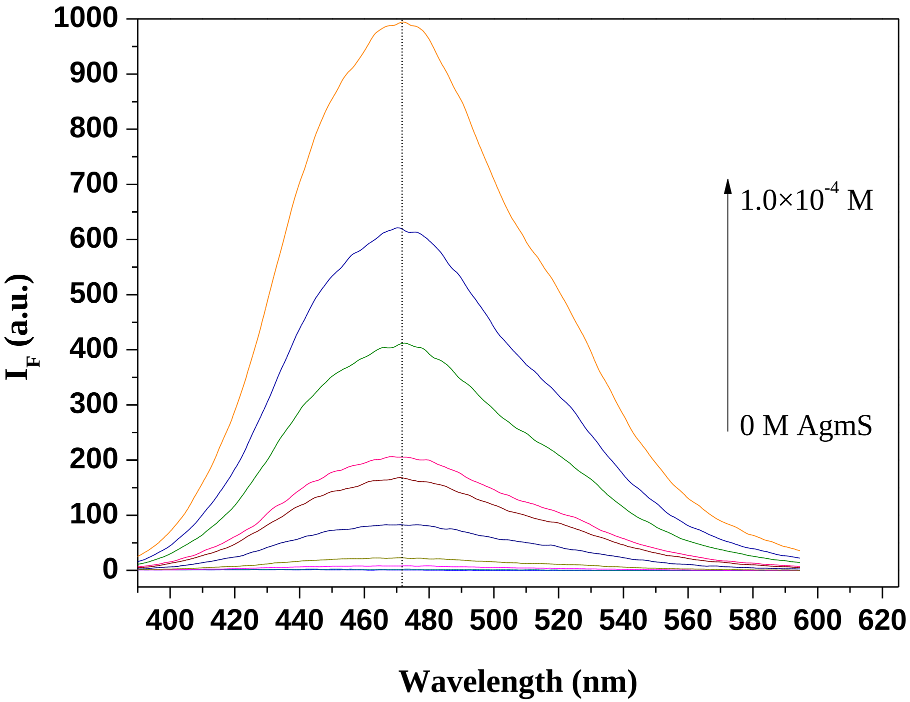
1. Agmatine
2. Serotonin
3. Dopamine
4. Octopamine,
5. **Tyramin**
6. Ethanolamine
7. Spermidine
8. **Cadaverine**
9. methyl-fenilethylamine
10. Noradrenalin
11. Adrenalin
12. Isopenthylamine
13. Melatonin
14. Acetilholin chloride
15. **Putrescine**
16. Isopropanolamine
17. β-alanine
18. Histamine
19. Cysteamine
20. Spermine

OPTICAL DETERMINATION OF BA IN SOLUTION

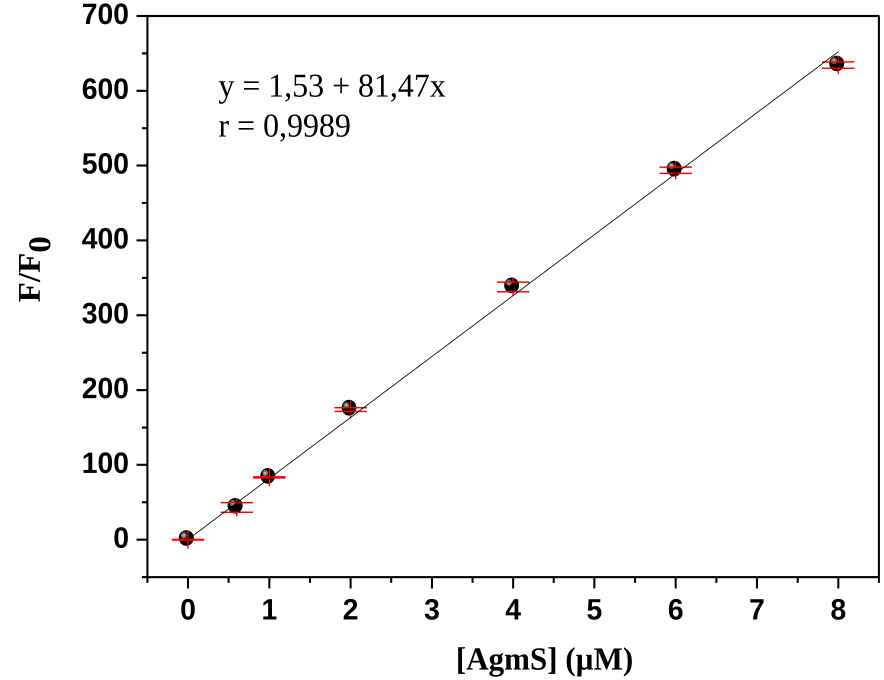
Spectral properties

AGMATINE

Response time

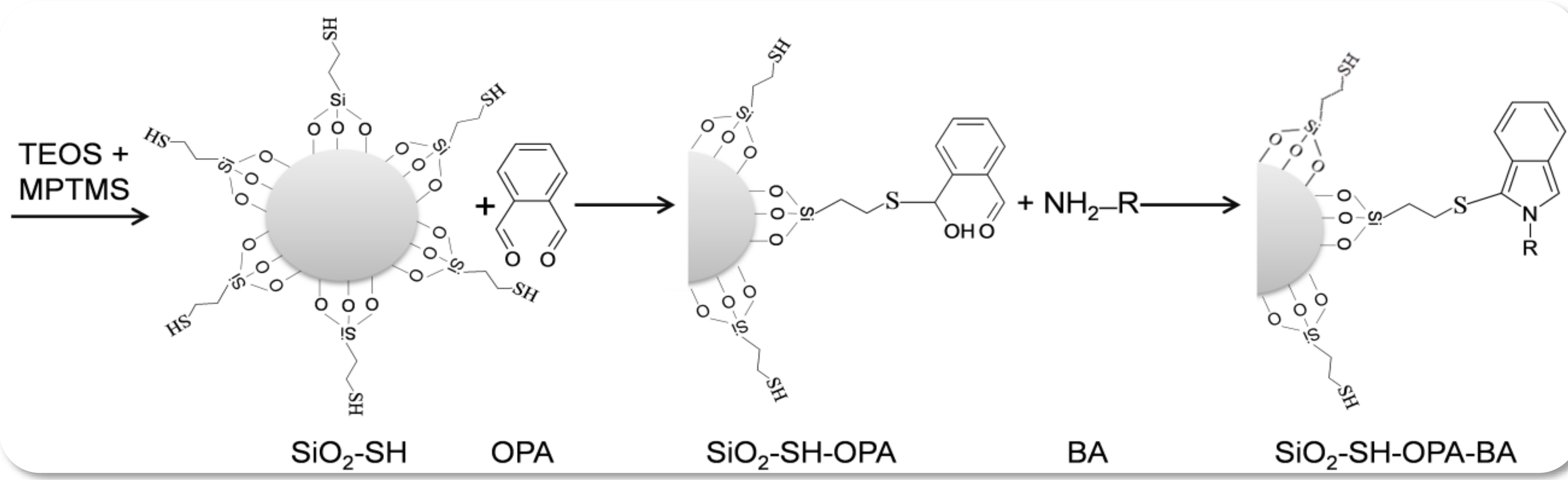


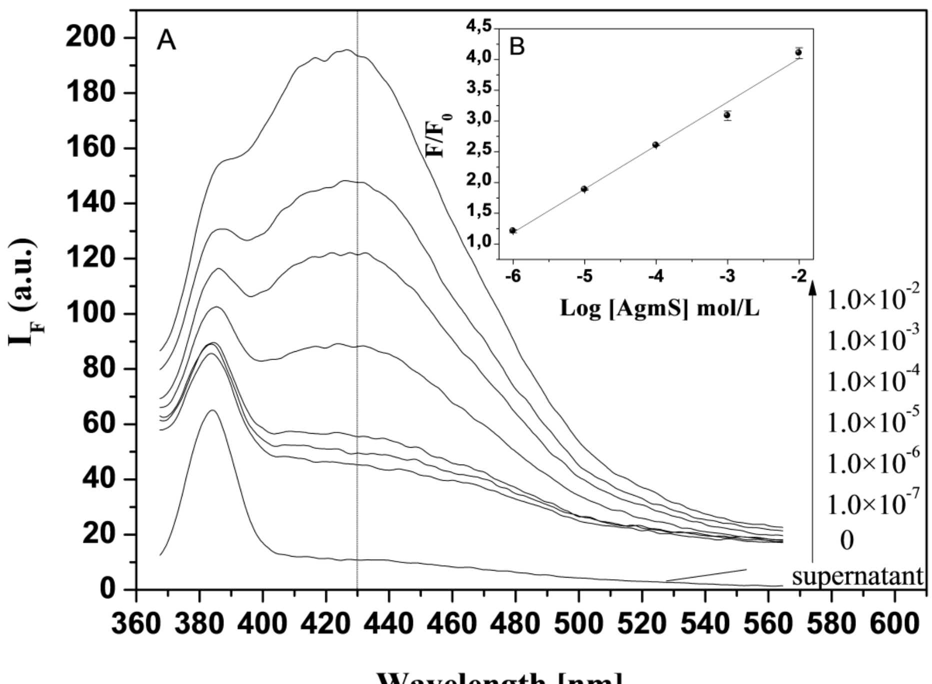
Emission spectra of OPA-AgmS product
$6,0\times10^{-7}$ M – $1,0\times10^{-4}$ M



Calibration curve of fluorescent product OPA-AgmS
$6,0\times10^{-7}$ M – $8,0\times10^{-6}$ M
LOD = $2,5\times10^{-7}$ M

OPTICAL DETERMINATION OF BA BY O-PHTHALDIALDEHYDE (OPA)





OPTICAL DETERMINATION OF BA BASED ON SiO_2 PARTICLES

SUMMARY

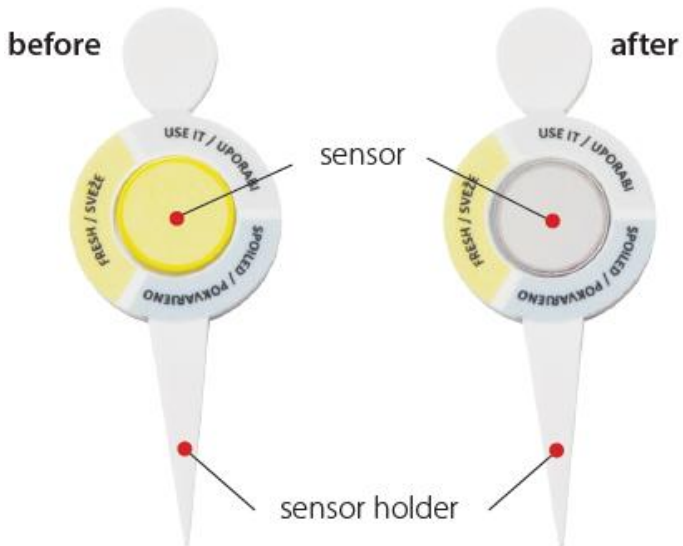
AGMATINE

Comparison of the results based on the optical determination of AgmS in solution with and without SiO_2-SH-OPA particles at pH 13

Parameters	Optical determination of AgmS	
	with OPA	with SiO_2-SH-OPA particles
Fluorescent product	OPA-AgmS	SiO_2-SH-OPA-AgmS
Spectral properties ($\lambda_{ex}/\lambda_{em}$)	340 nm / 473 nm	340 nm / 430 nm
Concentration range	6.0×10^{-7} M – 8.,0 $\times 10^{-6}$ M	1.0×10^{-6} M – 1.0×10^{-2} M
The correlation coefficient r^2	0.9989	0.9989
Linear equation	y = 1.53 + 81.47x	y = 5.43 + 0.71x
LOD	2.5×10^{-7} M	7.3×10^{-7} M
Response time	20 min	2 – 3 min
Buffer	pH 13	pH 13

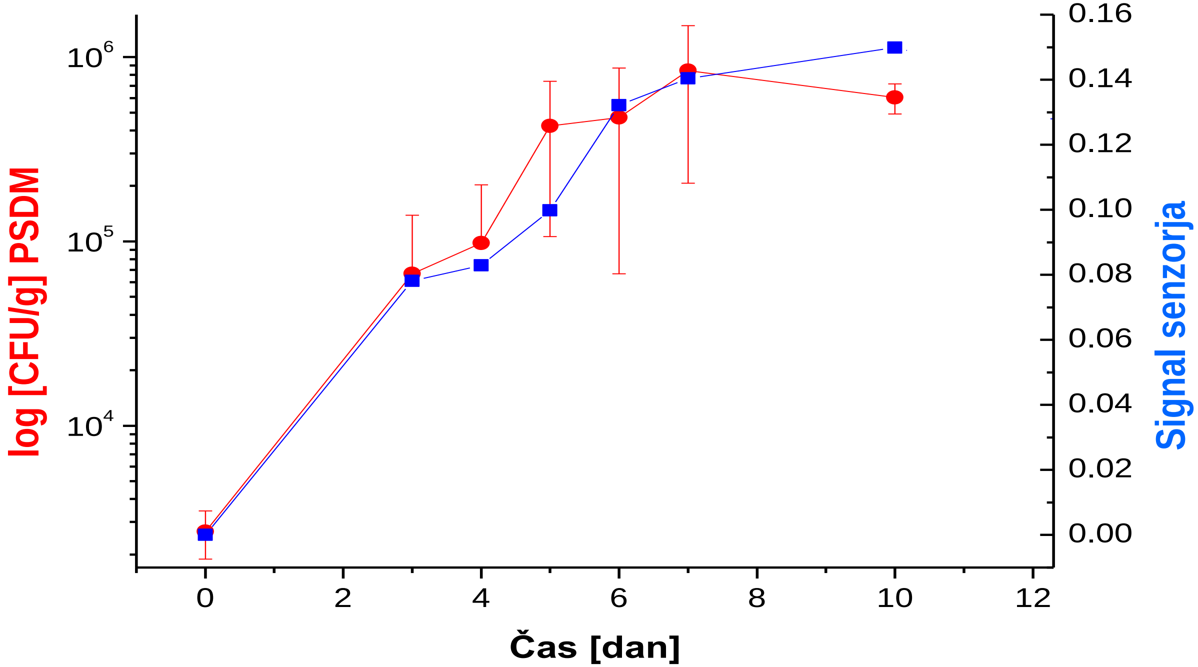
Journal of Sol-Gel Science and Technology, 2016, 1-10

Freshsens



- The sensor is suitable for raw, untreated fish and chicken meat
- Color change is a measure of the usefulness of the meat (see color scale)
- Response time is 30 minutes
- The sensor is useful when blue coloration is reached (spoiled meat) and can be used again if the initial color is yellow

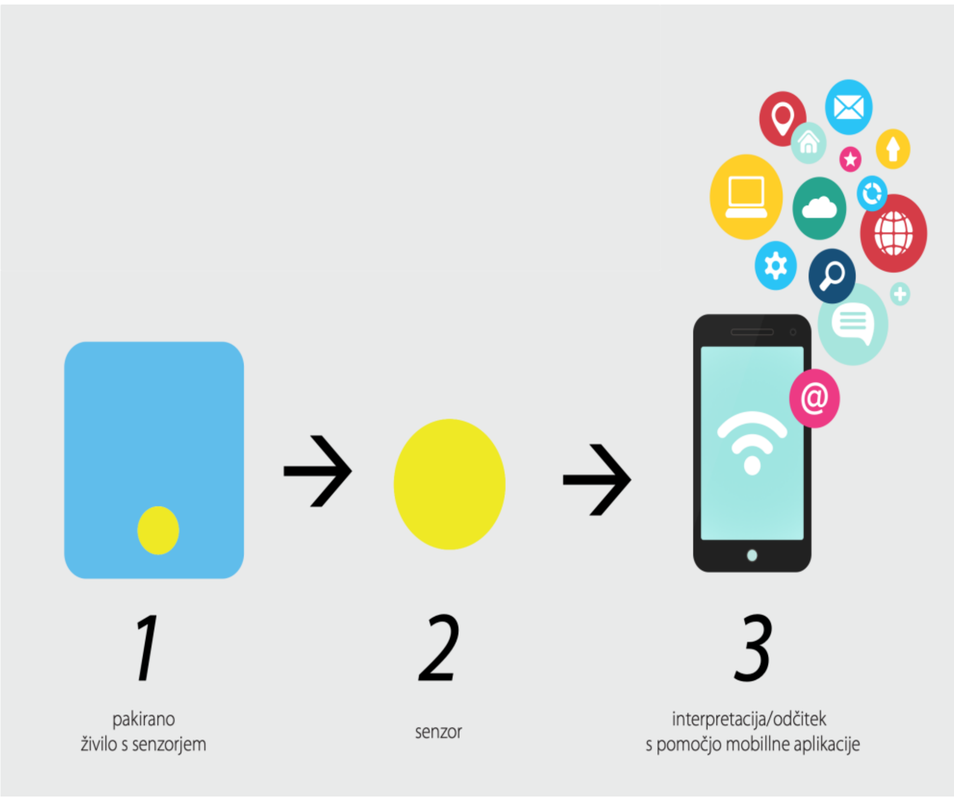
Correlation to the microbiological measurements



- sensor absorption measurements on spectrophotometer (laboratory)
- monitoring of the activity of microbiological parameters - bacteria Pseudomonas spp. (PSDM)
- signal of the sensor in correlation with the increase in the number of bacteria Pseudomonas spp.

meatQ

Natur
PREMIUM
Piščančji file
Svežina mesa
barvna lestvica
Sveže
Uporabno
Neuporabno



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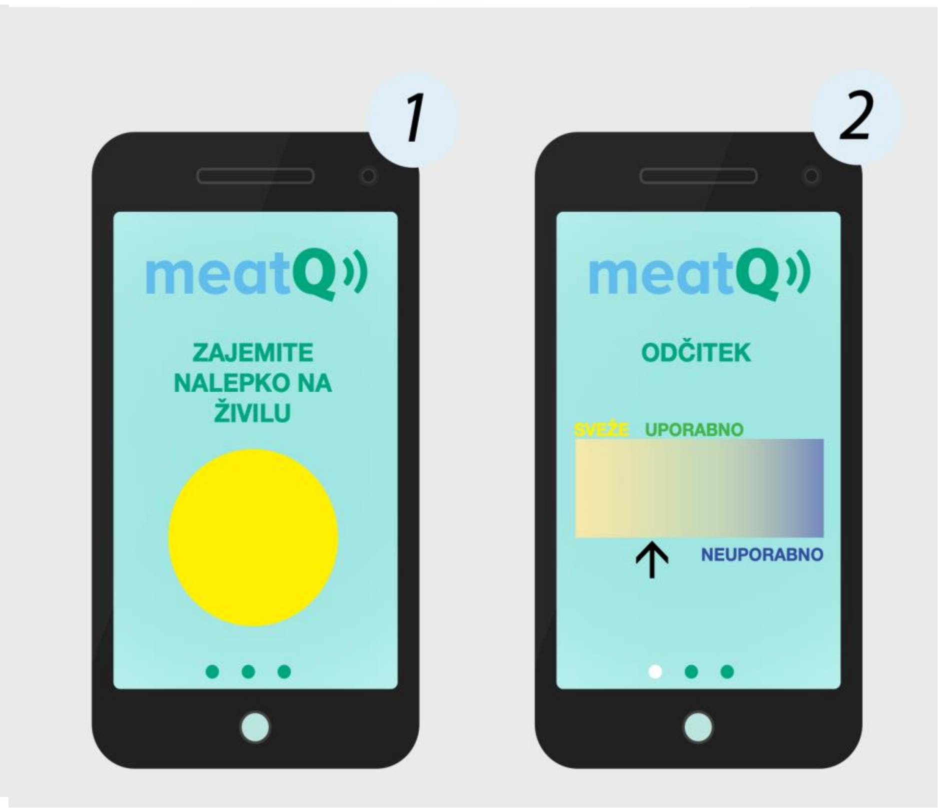


1
meatQ
ZAJEMITE
NALEPKO NA
ŽIVILU
2
meatQ
ODČITEK
SVEŽE
UPORABNO
NEUPORABNO

Biosensing layer for OP determination

(

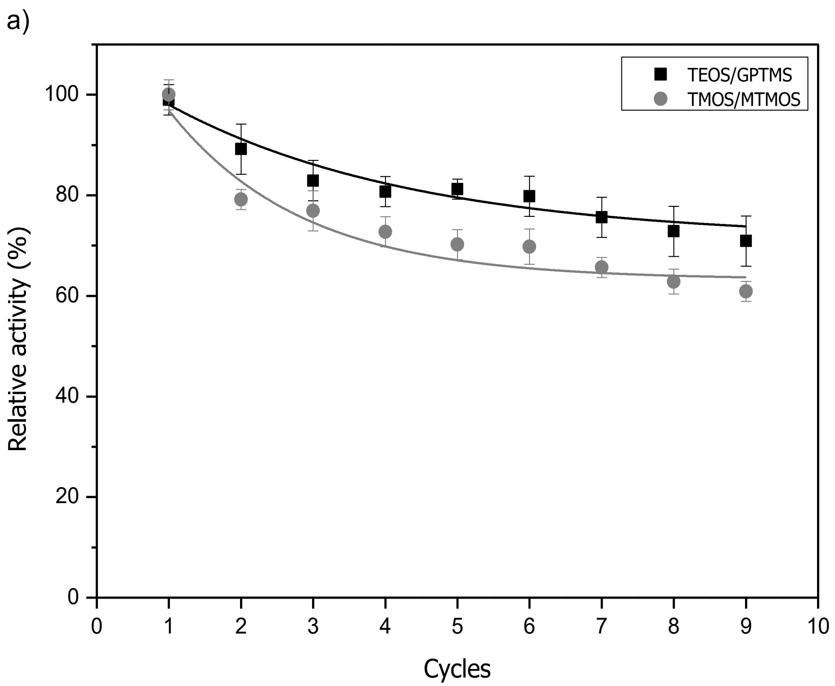
N. Francic, A. Lobnik, E. Efremenko, Bioscience and Technology, 2012)

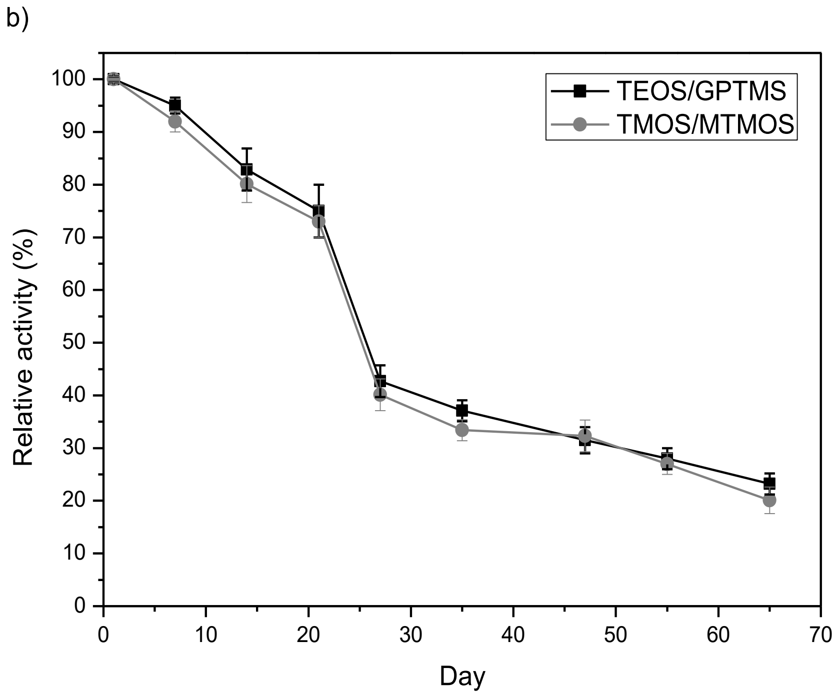
- **His_6-OPH (EC 3.1.8.1.) – organophosphorous hydrolase**
- Enzyme hydrolyzing a broad spectrum of organophosphorous compounds (OPCs) containing P–O, P–F and P–S bonds in the triesters of orthophosphoric acid
- Metalloenzyme: cofactors are Co^{2+} and other bivalent ions
  - Optimal activity :
    - T = 45 - 53 °C (pH 10.5)
    - pH between 10 in 11.5
  - High specific activity: ~ 5000 U/mg

- **hexahistidine (His_6) tag fused to OPH** → improving the catalytic efficiency, especially towards P–S-containing substrates, and the stability under alkaline hydrolysis conditions compared to native OPH

Votchitseva Y. A., Efremenko E.N., Aliev T.K., Volfomeyev S.D., “Properties of Heksahistidine-Tagged Organopgosphate Hydrolase”, Biocemistry (Moscow) 71, 167-172 (2006)

Entraped His_6-OPH within hybrid SiO_2 sol-gel layer



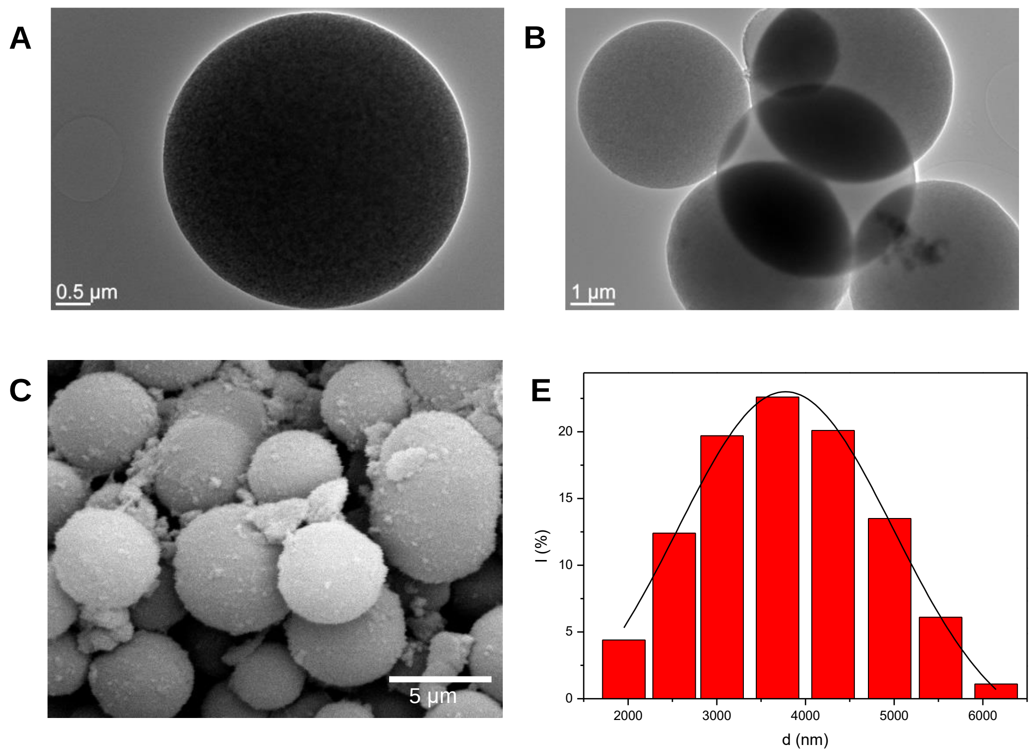


Comparison of two types of biocatalyst films TEOS/GPTMS (R=188, P=5:1) and TMOS/MTMOS (R=148, P=1:2) for a) repeated use in the detoxification of POX. Conditions: 0.675 mM paraoxon, temperature 25 °C, 50-mM Na-carbonate buffer (pH 9.5); and b) stability of SiO_2 thin films with entraped His_6-OPH

Anal Bioanal Chem (2011) 401:2631–2638

J Sol-Gel Sci Technol (2015) 74:387–397

Silica particles with immobilized His_6-OPH for POX determination/detoxification



TEM micrographs of silica particles. (A-B), SEM microgram (C), and particle size distribution (D) of MPS 5 particles.

Silica particles with immobilized His_6-OPH for POX determination/detoxification

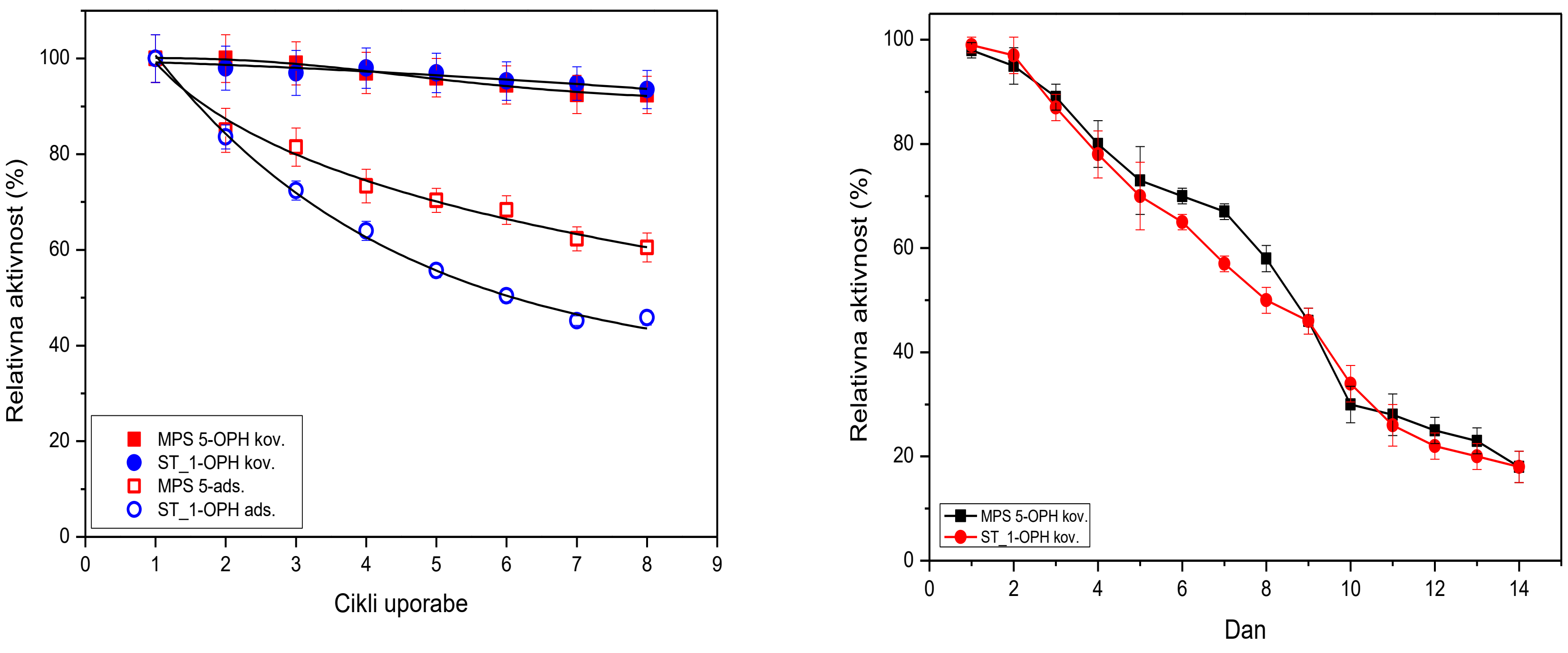
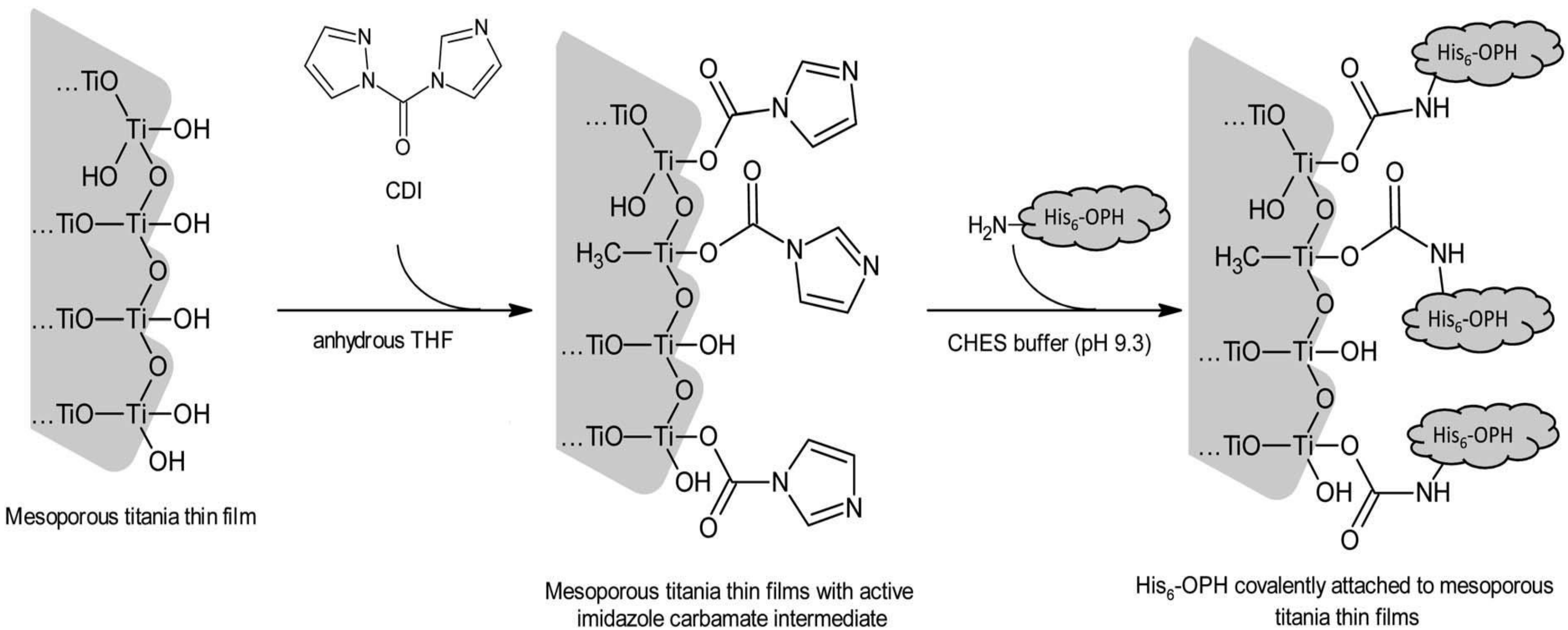


Fig. 4: Cycles of usage (a) and stability (b) of silica particles with immobilized His_6-OPH.

Mesoporous TiO_2 thin films as efficient enzyme carriers for paraoxon determination/detoxification



Schematic representation of the preparation route of His_6-OPH-conjugated mesoporous titania thin films trough CDI mediated reaction.

Analyst, 2014, 139, 3127–3136

Mesoporous TiO_2 thin films as efficient enzyme carriers for paraoxon determination/detoxification

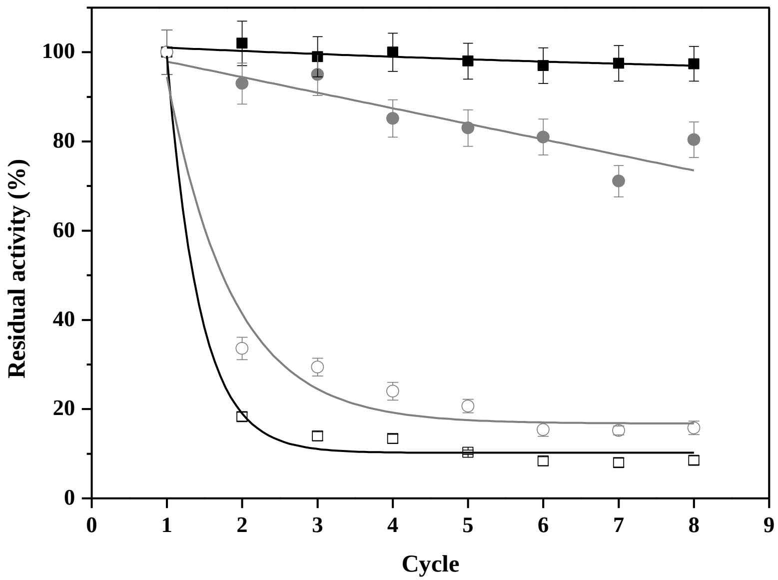


Figure Cycles of usage for covalently attached His_6-OPH, TiF-10 and TiF-bim (black and grey squares), and adsorbed His_6-OPH, TiF-10, TiF-10 and TiF-bim (black and grey circles). Measurements were performed with selected 50 mm^2 bio-functionalized mesoporous titania thin-films with covalently attached His_6-OPH at 20 °C and pH 10.5 (CB, 50 mM). Substrate: 0.3 mM paraoxon.

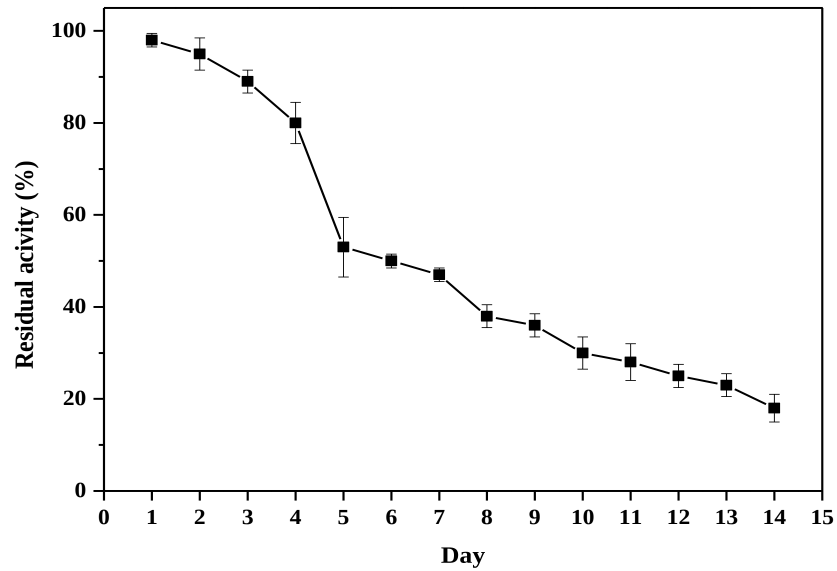


Figure Stability of titania bio-sensing film (TiF-9) with covalently attached enzyme several days after film preparation.

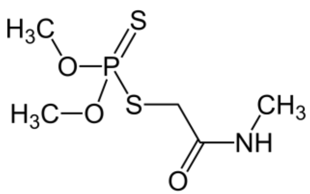
Analyst, 2014, 139, 3127–3136

PESTICIDE FLUORESCENT DETECTION

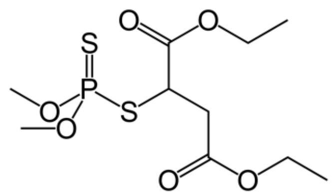
Edoardo DONA, ITN pH.D. Student in FoodTraNet project lead by prof.dr. Nives Ogrinc

ORGANOPHOSPHATES TESTED

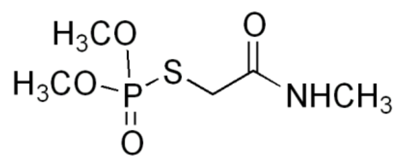
Dimethoate



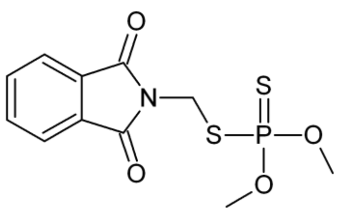
Malathion



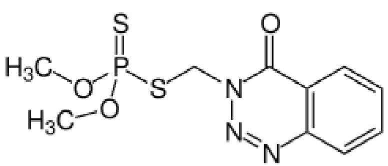
Omethoate



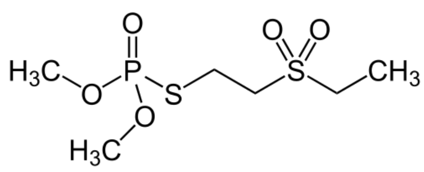
Phosmet



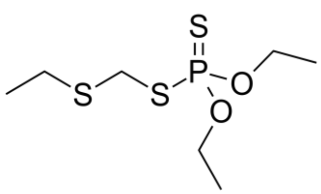
Azinphos methyl



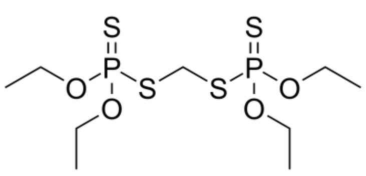
Demeton S



Phorate



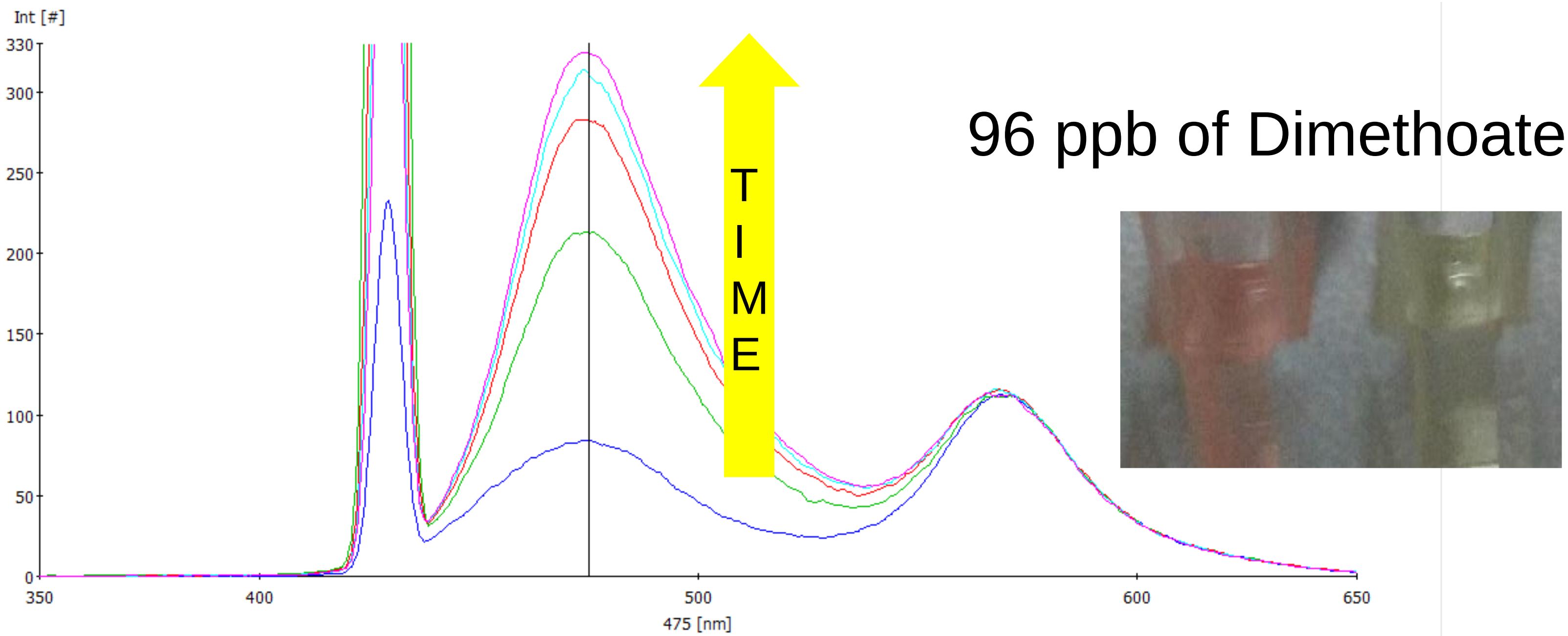
Ethion



REACTION SCHEME

$$\text{PESTICIDE} \xrightarrow[\text{2) HCl(aq) pH}\approx 7]{\text{1) NaOH(aq)}} \text{R-SH} + \text{FLUORESCENT DYE}$$

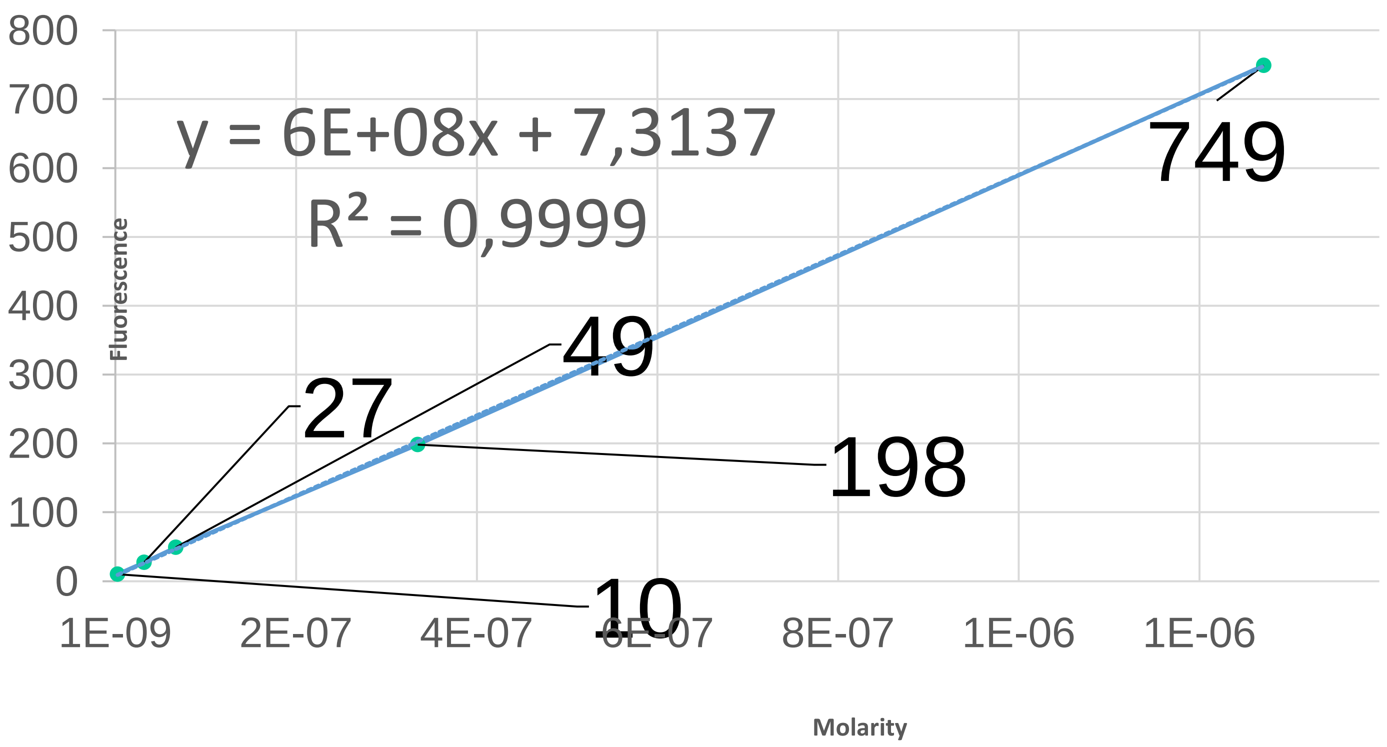
DIMETHOATE FLUORESCENCE



96 ppb of Dimethoate

λ_{exc}= 430nm λ_{em}= 475nm

CALIBRATION CURVE



ppb
1
10
20
96
366

LOD

Pesticide	LOD	Estimate LOD
Dimethoate	1 ppb	No improvements
Omethoate	302 ppb	Very big (10ppb)
Azinphos methyl	3,6 ppb	Little (1ppb)
Phosmet	60 ppb	Little (30 ppb)
Phorate	143 ppb	Medium (50 ppb)
Demeton	422 ppb	Very big (20ppb)
Malathion	336 ppb	Medium (150ppb)

THANK YOU FOR YOUR ATTENTION