BFÚ

Biofyzikální ústav

Akademie věd České republiky, v.v.i.

DFRP
Patofyziologie volných radikálů

- Informace o oddělení
- Zaměstnanci
- Výzkum
- Výsledky výzkumu
- Publikace
- Metody
- Výuka
- Mezinárodní projekty
- Národní projekty

Vedoucí oddělení:
Antonín Lojek

Oblasti zájmu:

- **Modulace mechanismů vedoucích ke tvorbě reaktivních metabolitů kyslíku a dusíku fagocyty**
- **Vliv nenasycených mastných kyselin a produktů jejich peroxidace na metabolickou aktivitu fagocytů**
- **Vzájemné interakce fagocytů s ostatními buněčnými typy a složkami mezibuněčné hmoty.**
- **Antioxidační vlastnosti tělních tekutin, léčiv a přírodních látek**
- **Role myeloperoxidázy v regulaci cévní fyziologie**
- **Redoxní regulace intracelulárního signálování**
- **Role NADPH oxidáz ve fyziologii nefagocytujících buněk**

Zaměstnanci oddělení Patofyziologie volných radikálů

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transplantation (heart, liver, kidney) or regular hemodialysis treatment.

Samples are analyzed using the following methods:

Parameter	Method
cell numbers	coulter-counter, microscopy
cell viability	spectrophotometry, flow cytometry, microscopy
cell morphology	microscopy
cell cycle	flow cytometry
metabolic activity as a measure of the number of viable cells	spectrophotometry, luminometry
oxidative burst of neutrophils	luminescence, flow cytometry
MPO activity	luminescence, spectrophotometry
NO synthesis	luminescence, flow cytometry, spectrophotometry
total radical-trapping antioxidant parameter	luminescence
individual antioxidants	spectrophotometry
lipid peroxidation	spectrophotometry
surface molecule expression	flow cytometry
activity of specific enzymes	luminometry, spectrophotometry

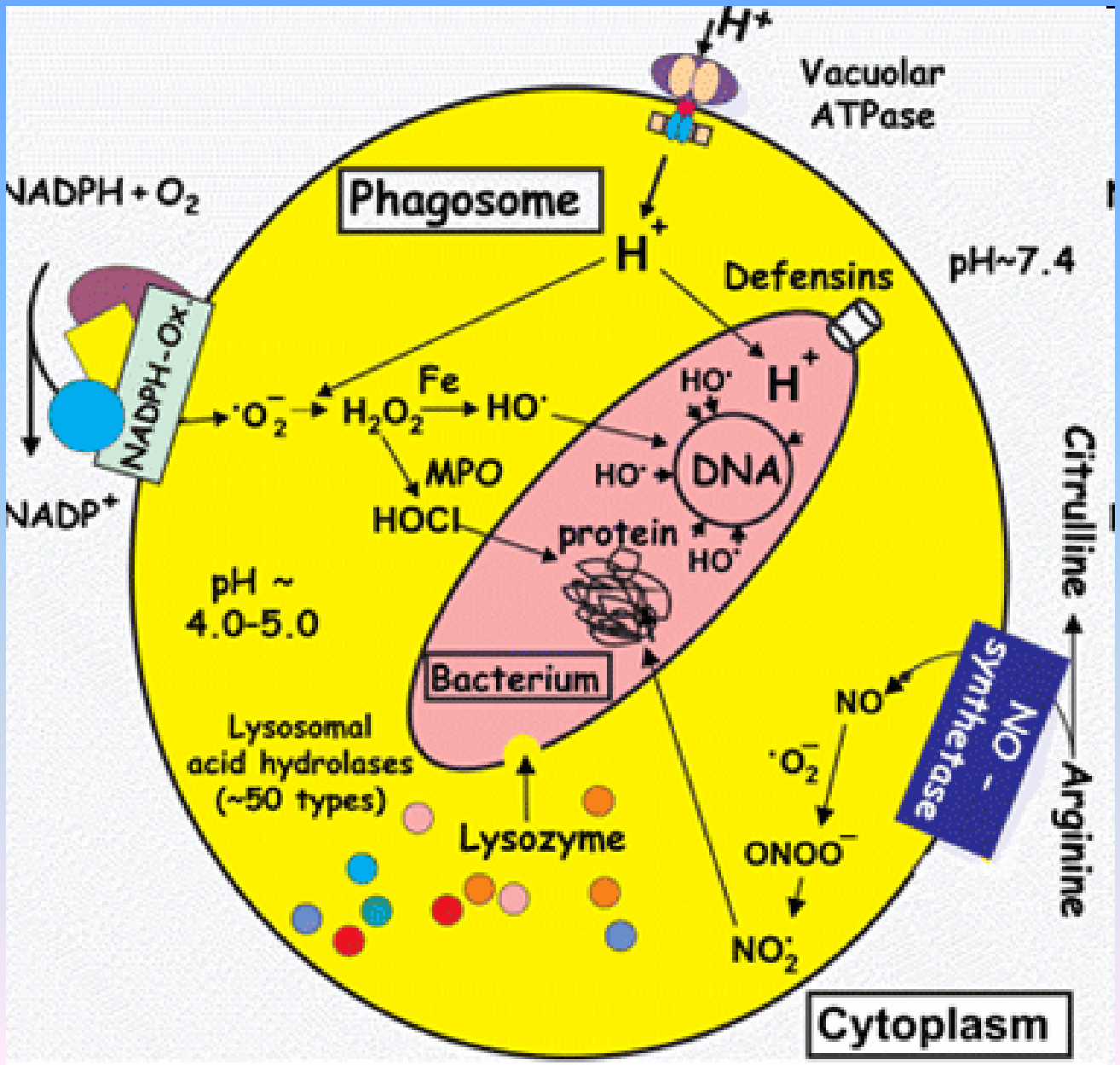
Chemiluminescence methods

Laboratory is equipped with cuvette (BioOrbit) and microtitre plate (Immunotech) luminometers.

- Luminol- or lucigenin-enhanced chemiluminescence is used for the measurement of ROS

Luminometry, fluorimetry and electrochemistry in the analysis of phagocyte-derived reactive oxygen and nitrogen species and antioxidative capacity of biological samples

Phagocyte-derived reactive oxygen and nitrogen species



Assays used to measure ROS production

- **NBT test**
- **Cytochrom c reduction assay**
- **Oxygen uptake (Clark type electrodes)**
- **Fluorescence**
- **Chemiluminescence**

Luminometric methods enable:

1. continual analysis of the oxidative burst

2. to differentiate between the intra- and extracellularly generated reactive oxygen species.

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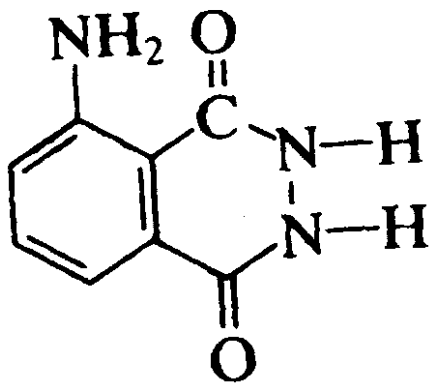
DETECTION SYSTEMS

Orion II Microplate Luminometer

Chemiluminescent Indicators

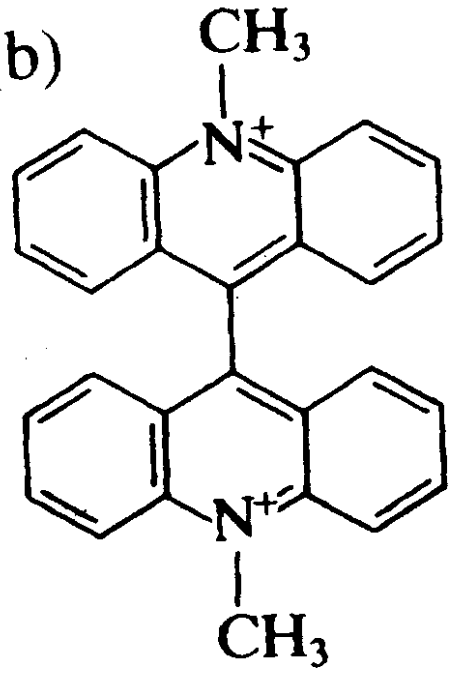
- **Lucigenin**
- **Luminol**
- **Isoluminol**
- **Pholasin**

(a)



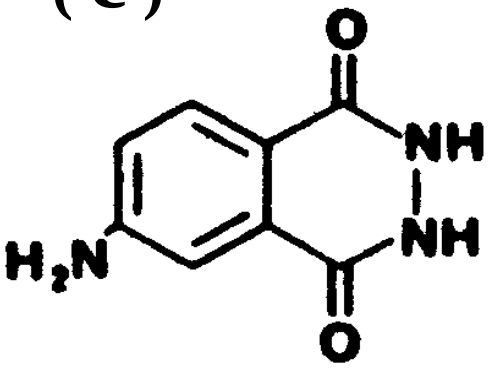
luminol

(b)



lucigenin

(C)



isoluminol

LUMINOL

Luminol-enhanced CL reflects primarily myeloperoxidase activity

Luminol-enhanced CL reflects reflects also production of other oxidants in physiological pH:

.> Peroxynitrite

> Hydrogen peroxide together with hypochlorite

> Hydrogen peroxide together with heme iron (MPO, HRP)

At alkaline pH, luminol is easily oxidised by week oxidants (hydrogen peroxide alone)

ISOLUMINOL

Differs from luminol only with respect to the position of the amino group in the phthalate ring of the molecule.

It does not produce luminescence as efficiently as luminol.

As published by several authors (e.g. Lundquist and Dahlgreen, 1996), isoluminol does not cross the plasma membrane.

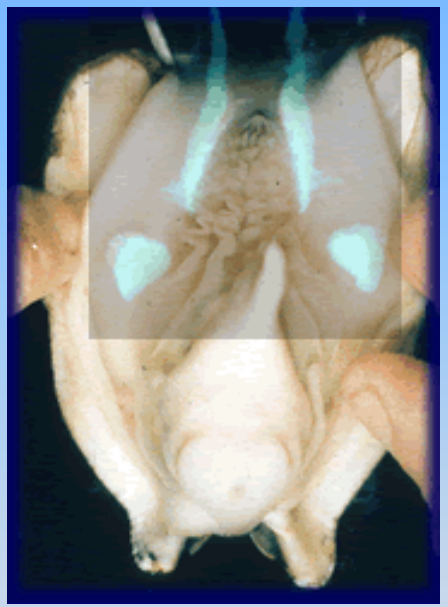
LUCIGENIN

Lucigenin-dependent CL is myeloperoxidase independent and measures NADPH oxidase associated oxygenation, essentially superoxide.

Criticism of superoxide-lucigenin method:
in higher concentrations lucigenin itself can generate superoxide and confound the results.

PHOLASIN

Photoprotein of the bioluminescent mollusc (*Pholas dactylus)*



Emits light in the presence of free radicals and oxidants.

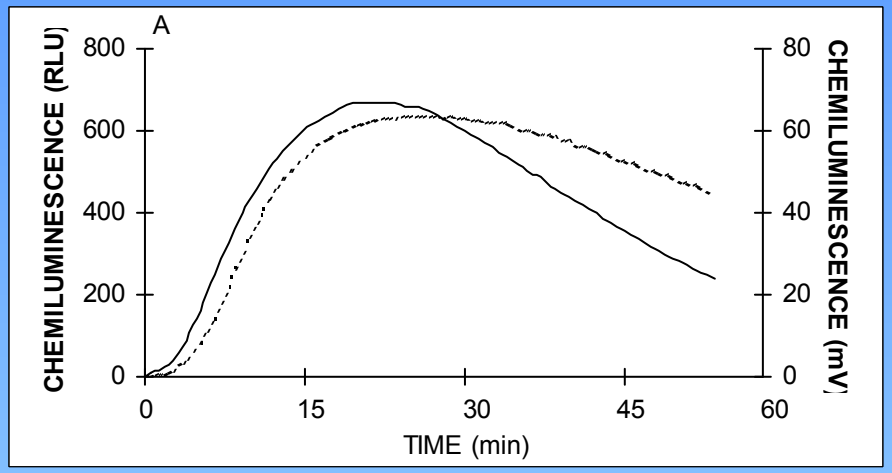
Pholasin is a 34 kDa protein that is impermeable to cells (Arnhold et al., 2002)

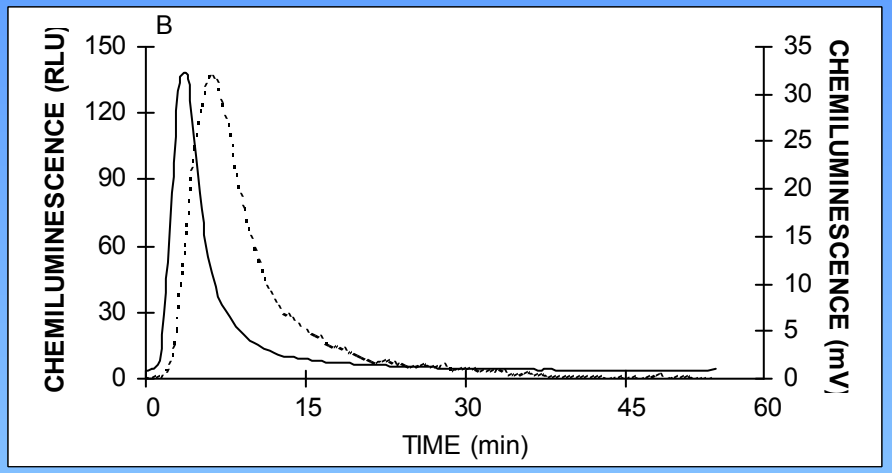
Peak of CL (RLU)

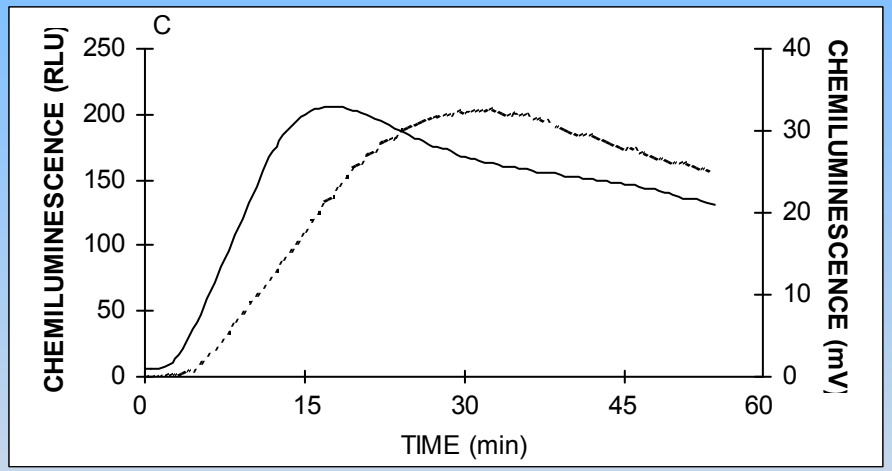
	Final C (stock 10 mM in water)			
	1mM	0,1mM	0,01mM	1microM
Luminol	163,33	116,67	26,17	27,67
izoluminol	111,33	43,50	13,33	12,17
lucigenin	30,17	30,67	20,33	12,50

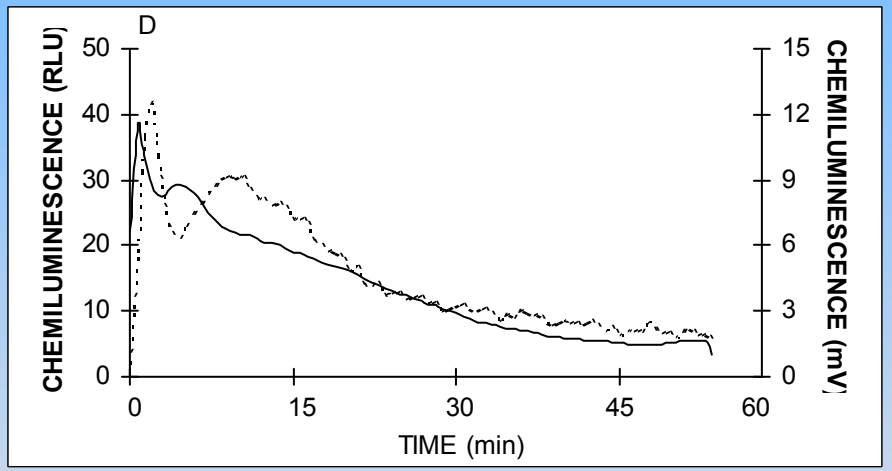
	Final C (stock 10 mM in DMSO)			
	1mM	0,1mM	0,01mM	1microM
Luminol	44,17	185,83	39,17	14,17
izoluminol	83,67	49,17	15,33	11,17
lucigenin	27,00	35,17	19,17	12,00

	Final C (stock 10 mM in borate buffer)			
	1mM	0,1mM	0,01mM	1microM
Luminol	415,83	193,33	43,50	14,00
izoluminol	271,00	48,17	14,33	11,17
lucigenin	51,83	24,17	14,17	12,33

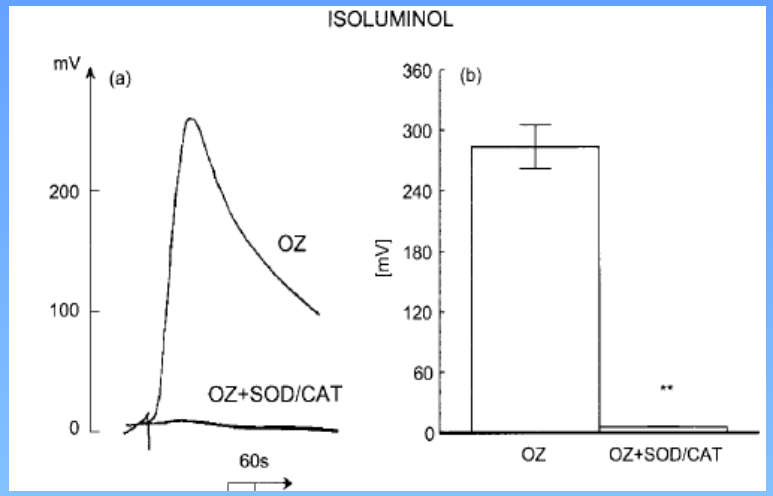


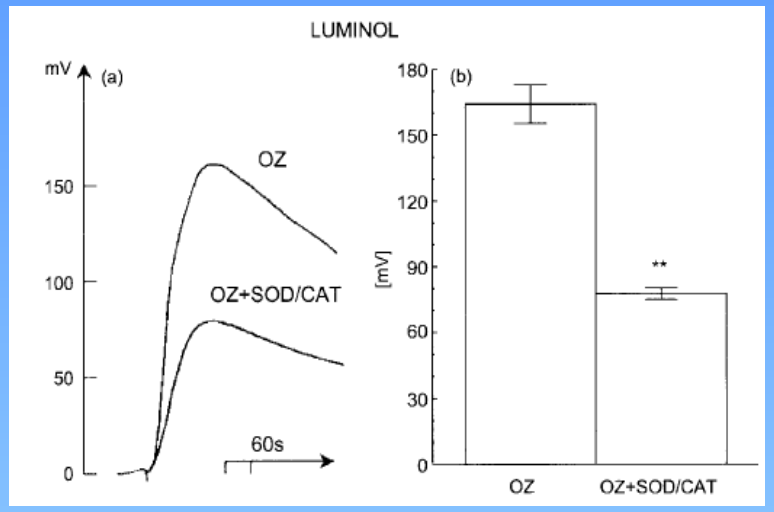


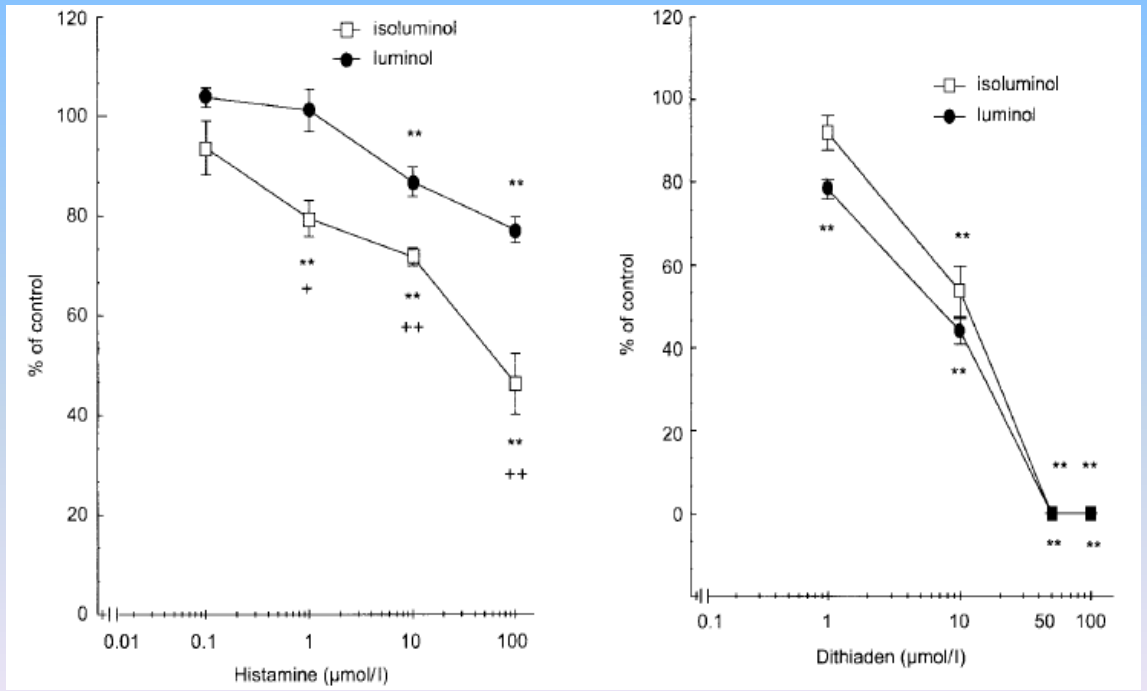




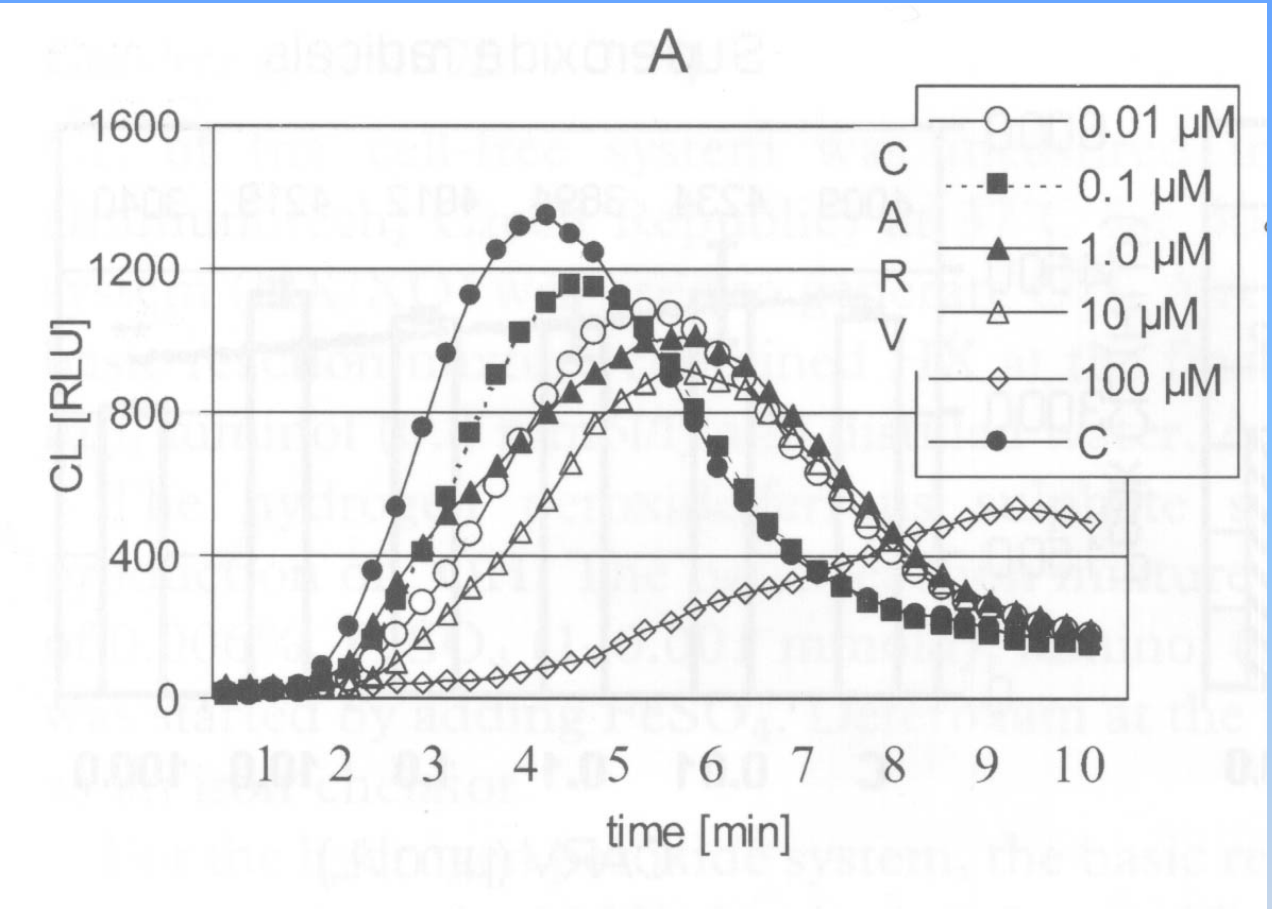
Lojek A., Kubala L., Čížová H., Číž M. (2002): A comparison of neutrophil chemiluminescence in cuvettes and microtittre plates. Luminescence, 17, 1-4.







Drábiková K., Nosáľ R., Jančinová V., Číž M., Lojek A (2002).: Reactive oxygen metabolites production is inhibited by histamine and H1-antagonist dithiaden in human PMN-leukocyte. Free Rad. Res. 36(9), 975-980



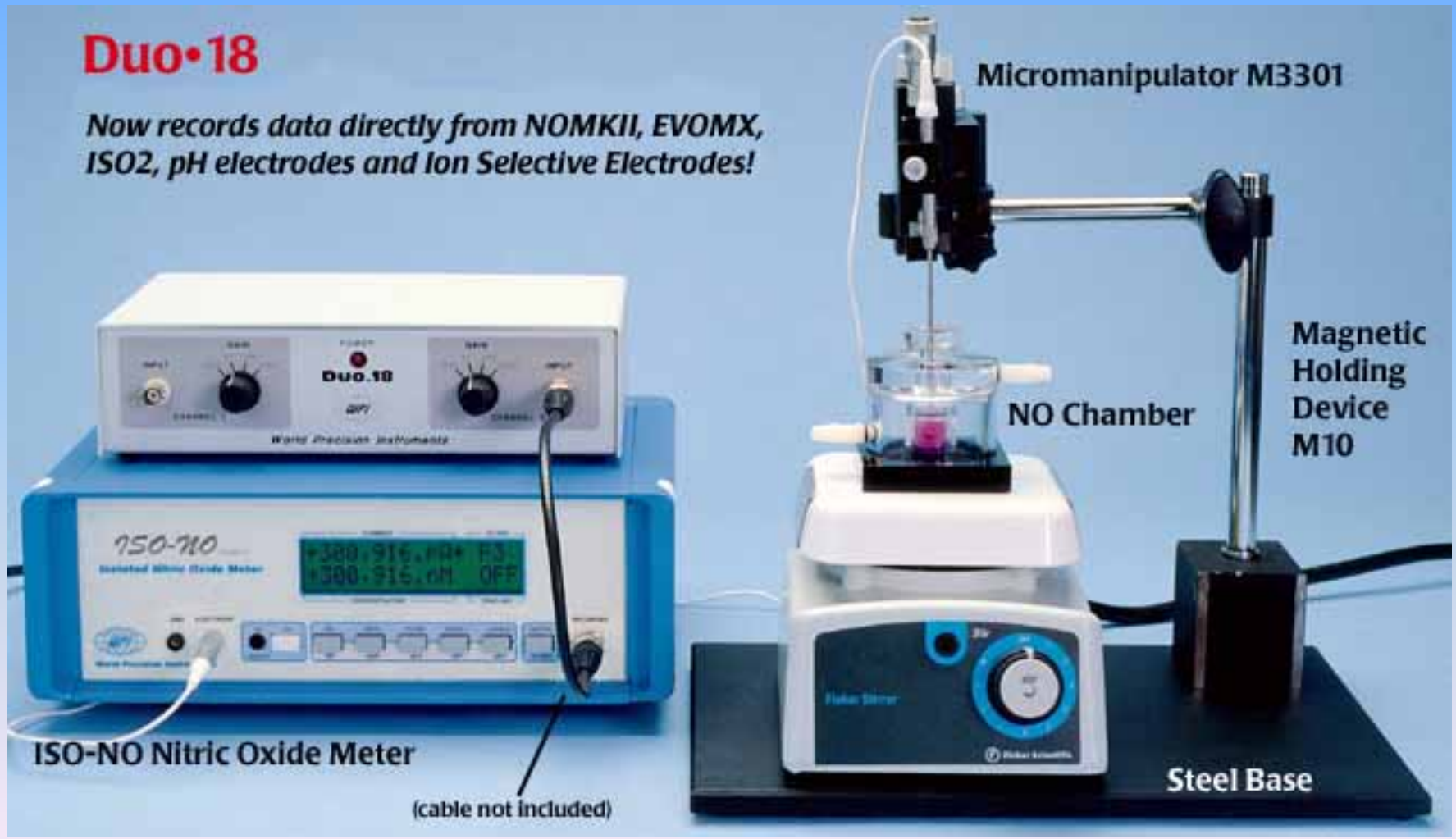
Nosal R., Jancinova V., Ciz M., Drabikova K., Lojek A., Fabryova V.(2005): Inhibition of chemiluminescence by carvedilol in the cell-free system, whole human blood and blood cells. Scand J Clin Lab Invest 65, 55-64

Phagocyte-derived nitric oxide production

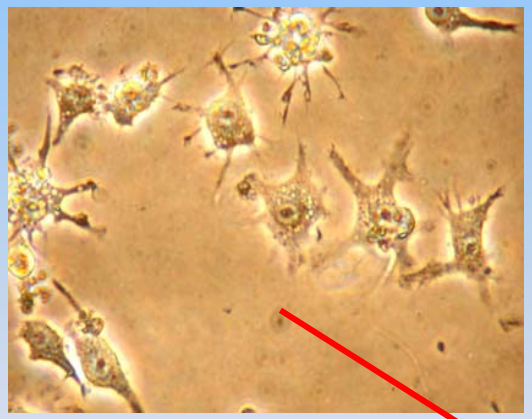
Assays used to measure NO production

- cell-permeable fluorescent indicators (4,5-diaminofluorescein diacetate (DAF-2 DA)
- total nitrate/nitrite concentration
- NO donor compounds, NO scavengers
- NOS activity in cell homogenates by measuring the enzymatic conversion of arginine to citrulline during NO formation
- NOS inhibitors
- antibodies to NOS isoforms by immunocytochemistry or by immunoblotting
- electrochemical method for direct measurement of NO concentration

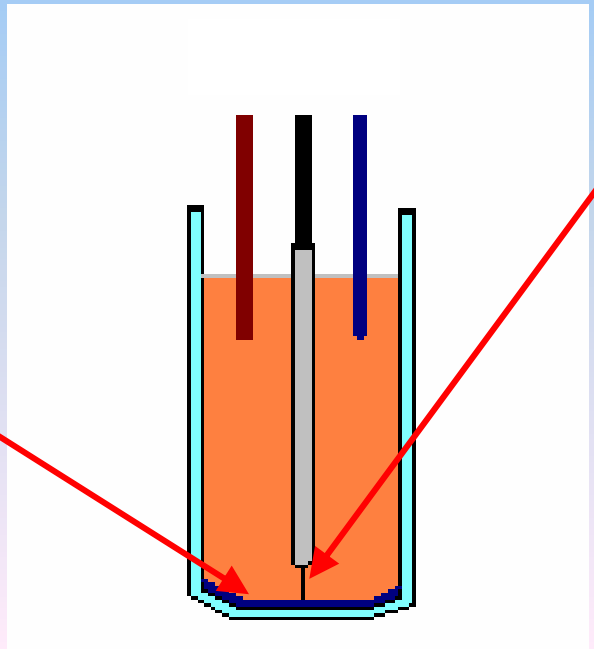
Electrochemical method



Carbon fibre electrode



RAW 264.7 ($1x10^6$)



Hrbac J, Gregor C, Machova M, Kralova J, Bystron T, Ciz M, Lojek A. (2007) Nitric oxide sensor based on carbon fiber covered with nickel porphyrin layer deposited using optimized electropolymerization procedure. Bioelectrochemistry. Sep 27; [Epub ahead of print]

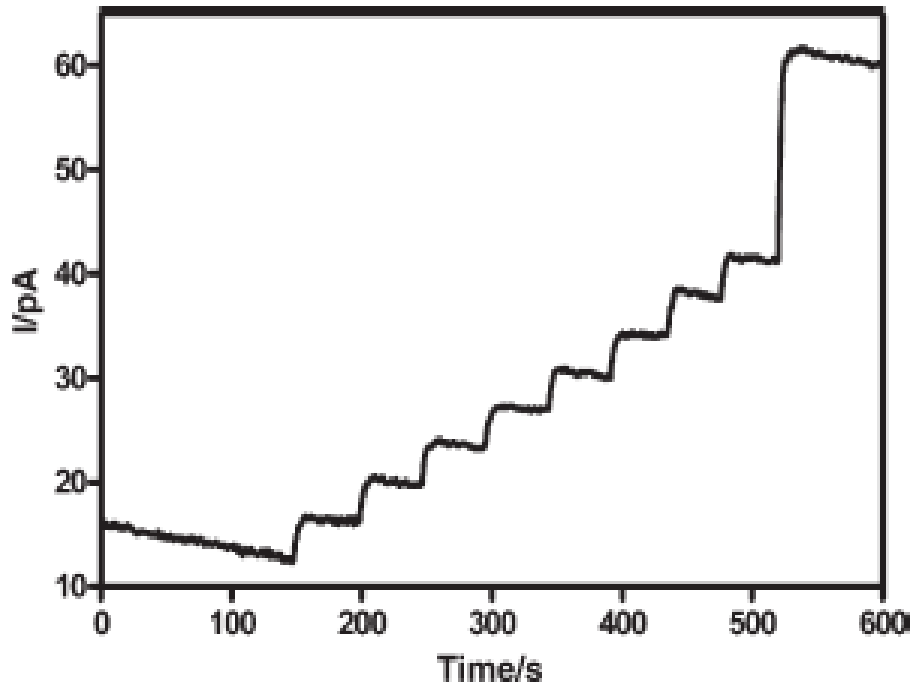
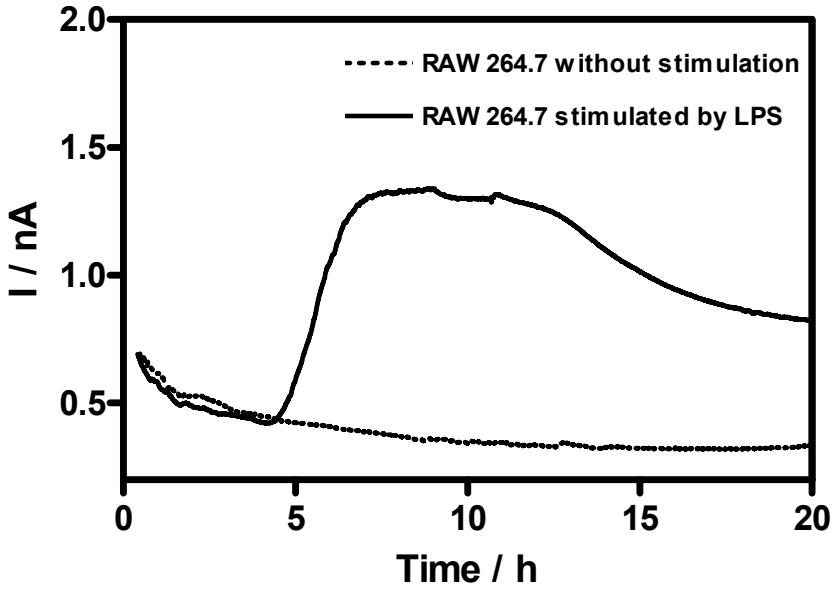
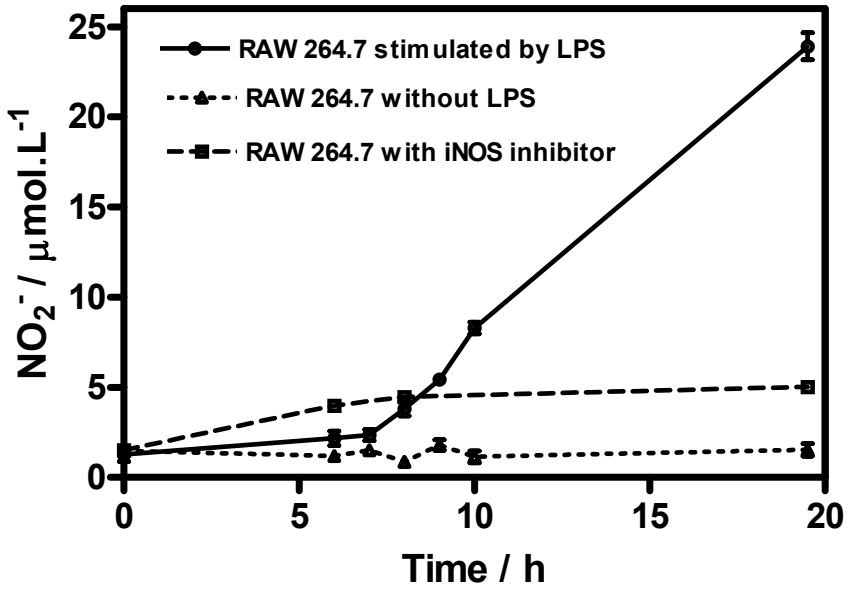


Fig. 2. Carbon fiber sensor's performance at nanomolar NO concentrations (CPA at 830 mV (vs. Ag/AgCl)). Eight additions of NO into aerated PBS, each resulting in 4 nM NO concentration, followed by response for 20 nM NO are shown. After pretreatment the electrode was coated with poly-NiTMHPP, electropolymerized from 0.4 mM NiTMHPP by 100 cycles from 0 to 1200 mV (vs. Ag/AgCl), scan rate 100 mV/s).



Time course of NO production by RAW 264.7 cell culture after stimulation (priming) by LPS



Time course of nitrite accumulation in the supernatant collected from RAW 264.7 cell culture

Fluorimetric and Luminometric

determination of

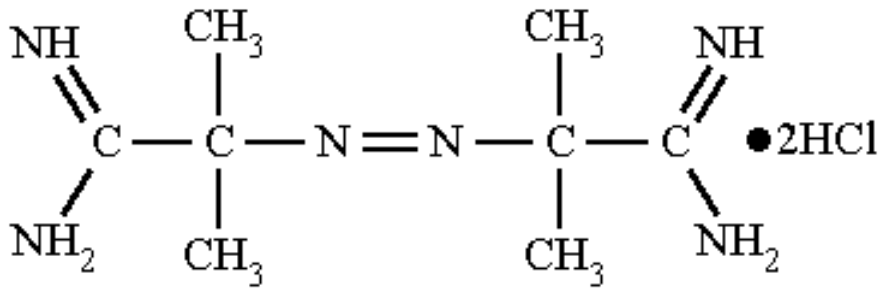
antioxidative activity

Luminometric determination of antioxidative activity

All individual antioxidants can be analysed at the same time using the measurement of

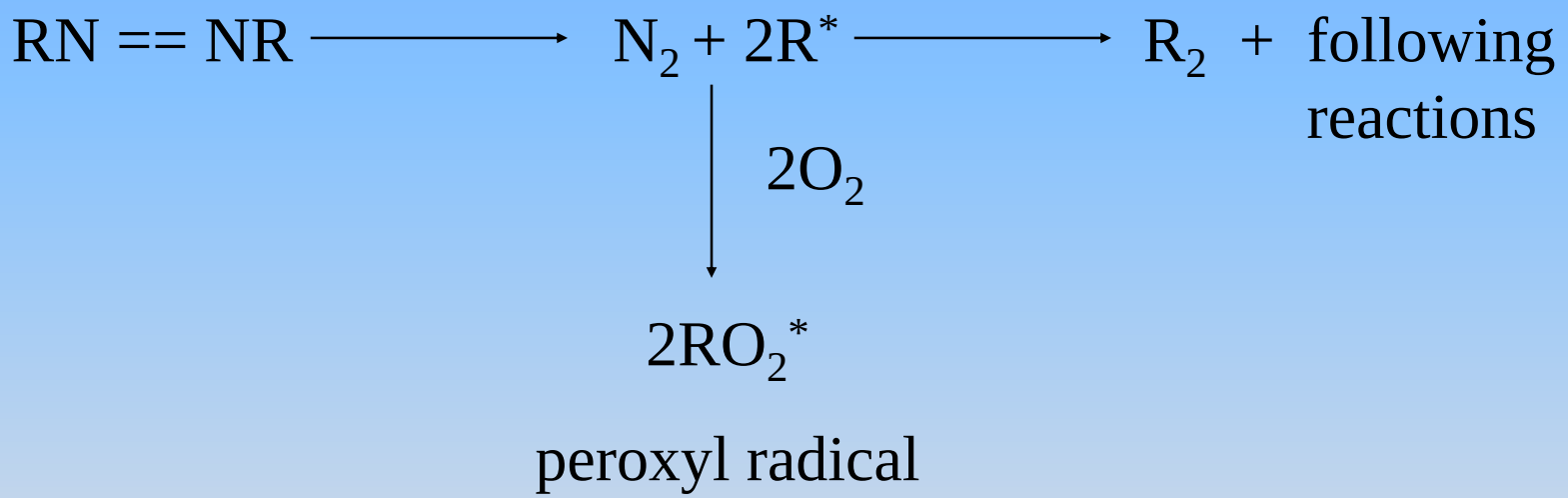
Total Radical-trapping Antioxidative Potential

TRAP – basic principle

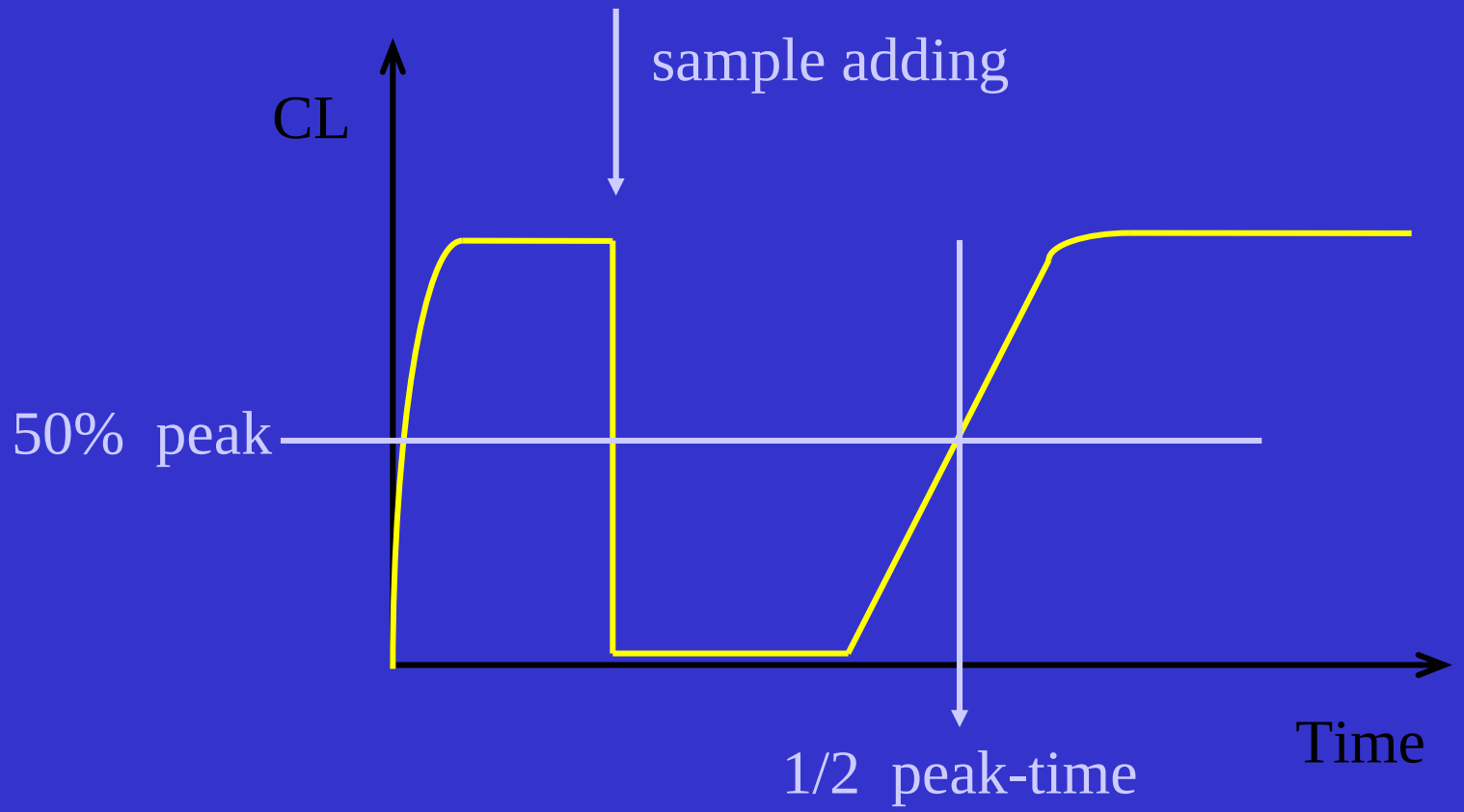


- 2,2‘-azobis(2-amidinopropane) dihydrochloride
- 2,2‘-azobis(2-methylpropionamide) dihydrochloride

TRAP – basic priciple



TRAP – basic principle



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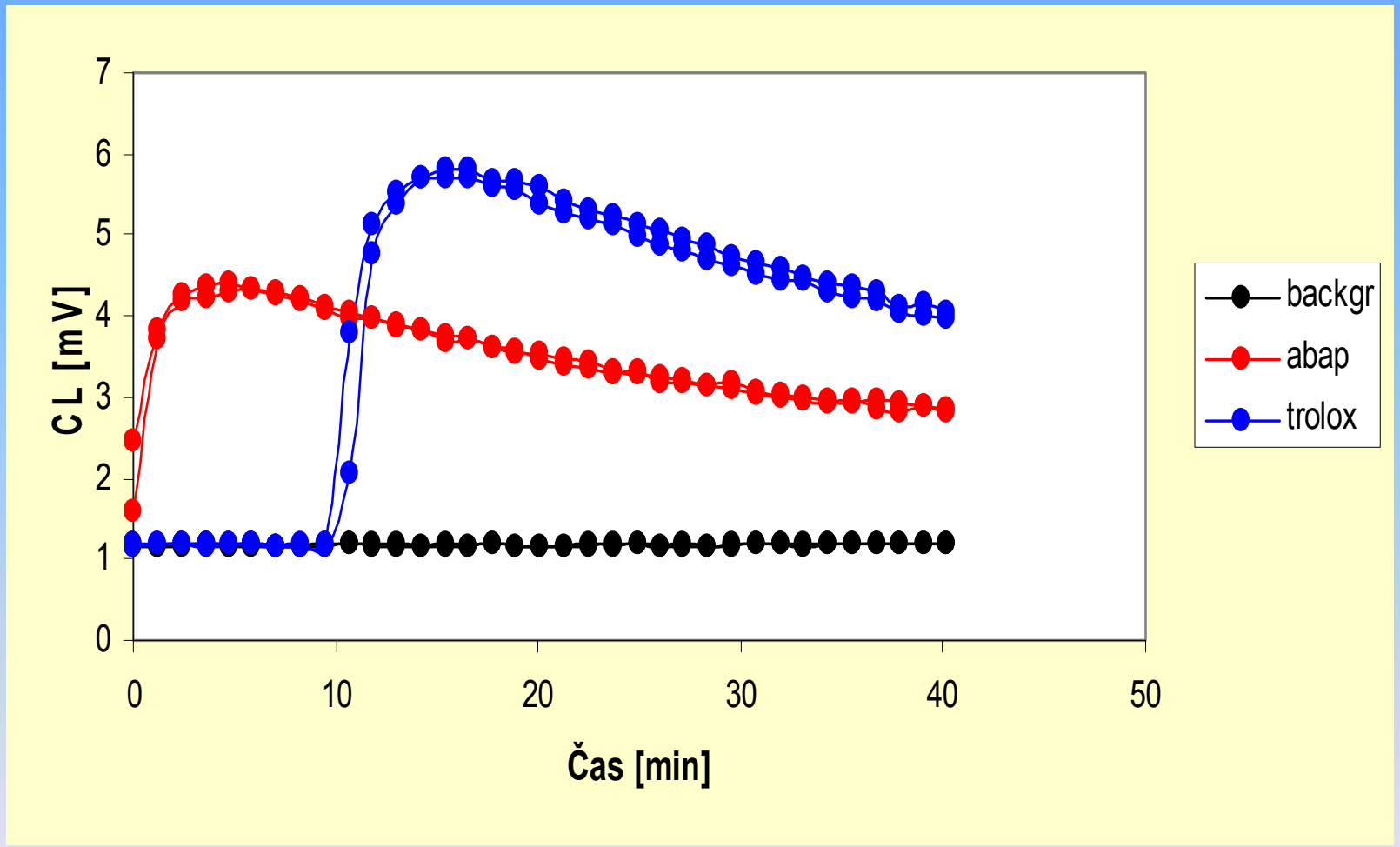
DETECTION SYSTEMS

Orion II Microplate Luminometer


CL [mV]
0
1
2
3
4
5
6
7
0
10
20
30
40
50
Čas [min]
backgr
abap
trolox

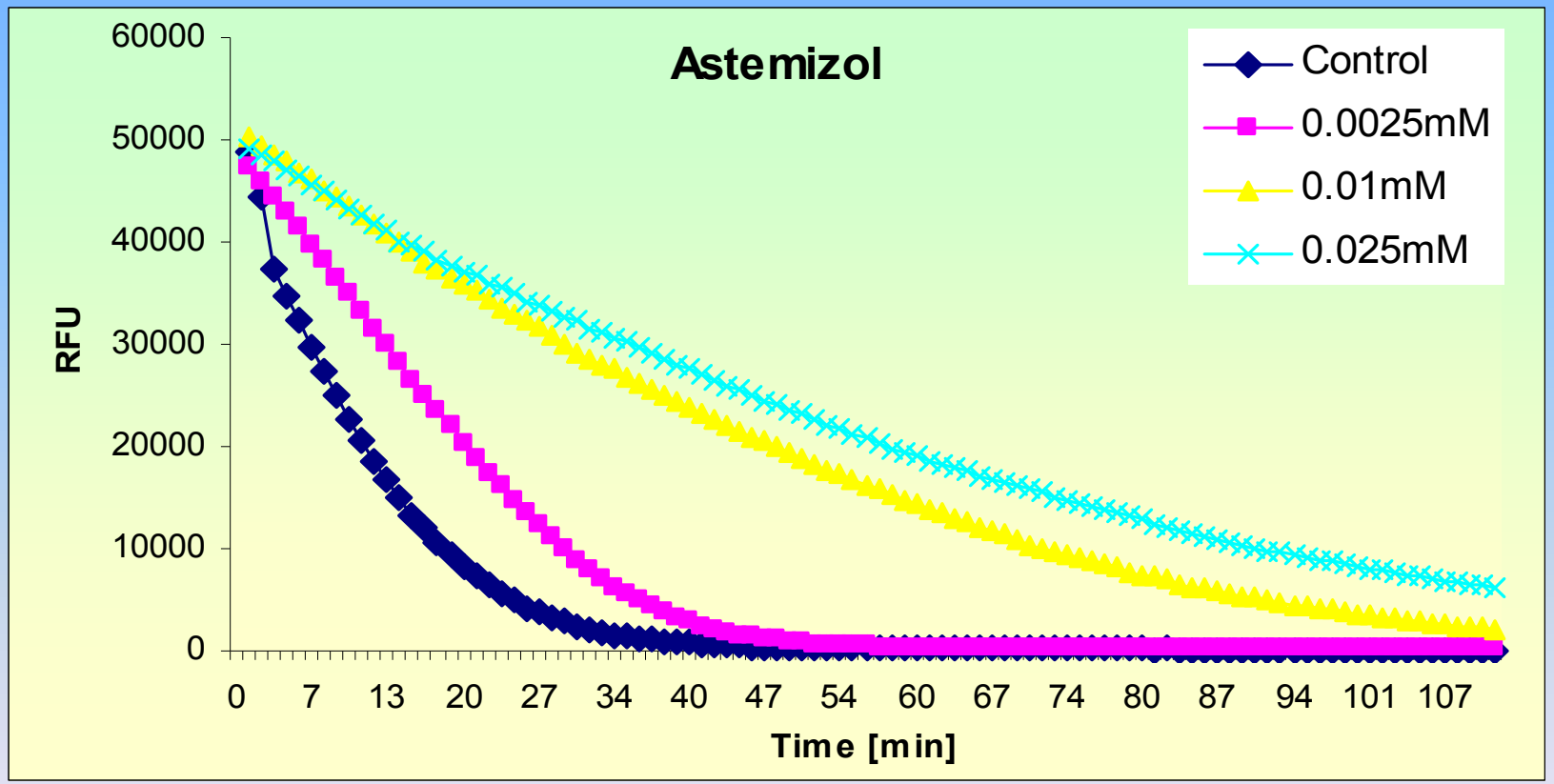
Infinite M200

Fluorimetrical assay [Prior and Cao, 1999].

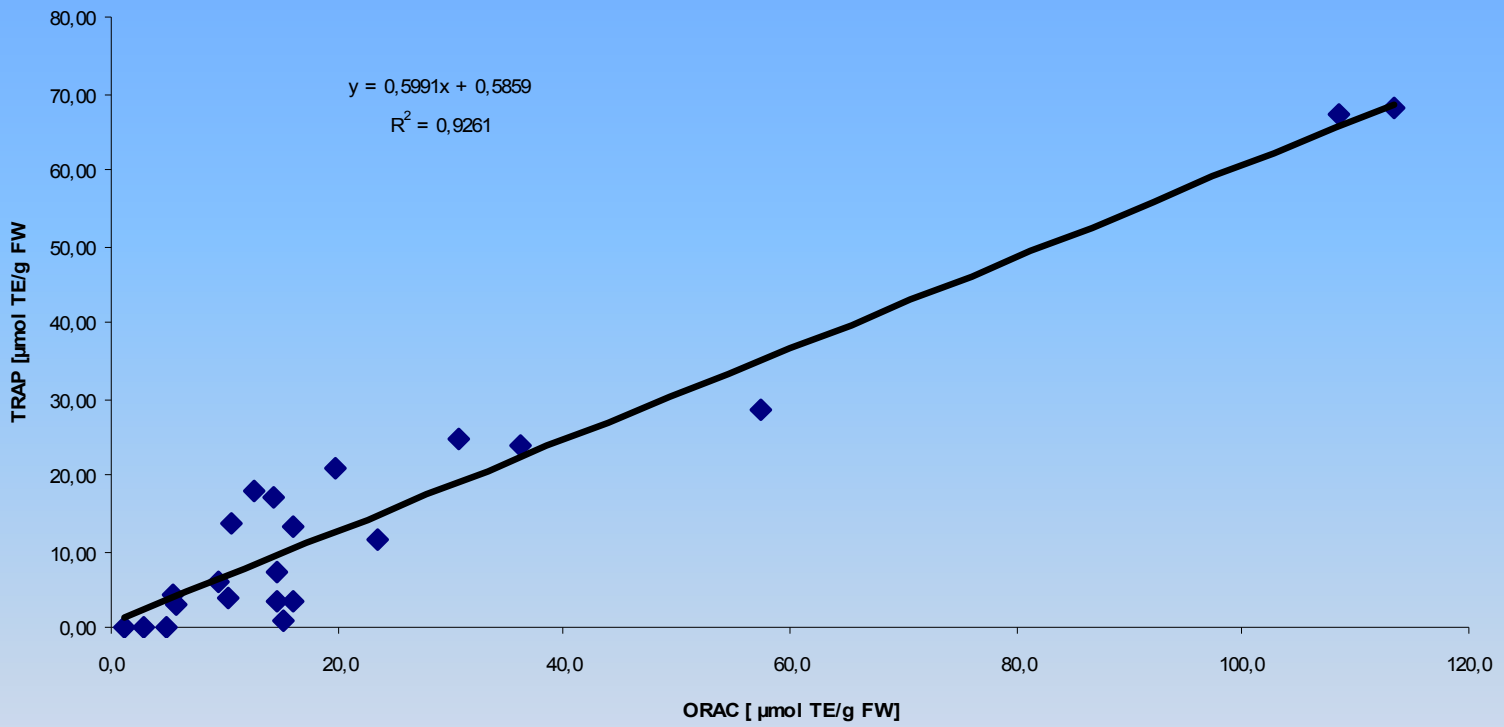
OXYGEN RADICAL ABSORBANCE CAPACITY

- Antioxidant scavenging activity against peroxyl radical induced by 2,2-azobis (2-amidinopropane)hydrochloride (ABAP) at 37˚C
- Fluorescein (FL) - fluorescent probe
- The lost of fluorescence of FL is an indication of the extent of damage from its reaction with the peroxyl radical
- The antioxidative effects of a drugs are measured by assesing the area under the fluorescence decay curve (AUC)

Example:
ORAC of antihistamine drug Astemizol



		ORAC		TRAP
		µmol TE/g FW		µmol TE/g FW
1	celery - leaves	113,5 ± 6,1	celery - leaves	68,14 ± 4,79
2	parsley	108,6 ± 13,1	parsley	67,15 ± 8,92
3	lovage	57,3 ± 5,0	lovage	28,41 ± 1,30
4	chilli pepper	36,1± 6,5	goathorn pepper	24,8 ± 1,18
5	goathorn pepper	30,6 ±1,4	chilli pepper	23,72 ± 3,06
6	radish	23,6 ± 1,7	capsicum	20,91 ± 1,15
7	capsicum	19,9 ± 1,4	red beet	17,85 ± 0,25
8	eggplant	16,2 ± 2,0	green bean	16,88 ± 0,45
9	broccoli	16,1± 1,2	dill	13,66 ± 1,50
10	celery - root	15,3 ± 1,2	eggplant	13,40 ± 1,47
11	green onion	14,7± 1,5	radish	11,39 ± 2,24
12	gumbo	14,6 ± 0,8	gumbo	7,42 ± 0,77
13	green bean	14,5 ± 1,2	red pepper	5,93 ± 0,14
14	red beet	12,6 ± 1,6	tomato	4,35 ± 0,47
15	dill	10,5 ± 1,1	potato	3,78 ± 0,10
16	potato	10,3 ± 1,3	green onion	3,48 ± 0,52
17	red pepper	9,3 ± 0,9	broccoli	3,39 ± 0,12
18	green pepper	5,6 ± 0,3	green pepper	2,81 ± 1,88
19	tomato	5,4 ± 0,3	celery - root	1,00 ± 0,43
20	carrot	4,8 ± 1,1	carrot	0,00
21	vegetable marrow	2,9 ± 0,3	vegetable marrow	0,00
22	cucumber	1,2 ± 0,2	cucumber	0,00


y = 0,5991x + 0,5859
R2 = 0,9261
TRAP [µmol TE/g FW
ORAC [µmol TE/g FW]
80,00
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
0,0
20,0
40,0
60,0
80,0
100,0
120,0

Institute of Biophysics of the AS CR

Department of Free Radical Pathophysiology