Physico-Chemistry of Dinitrosyl Iron Complexes with Thiol-Containing Ligands as a Determinant of Their Biological Activity

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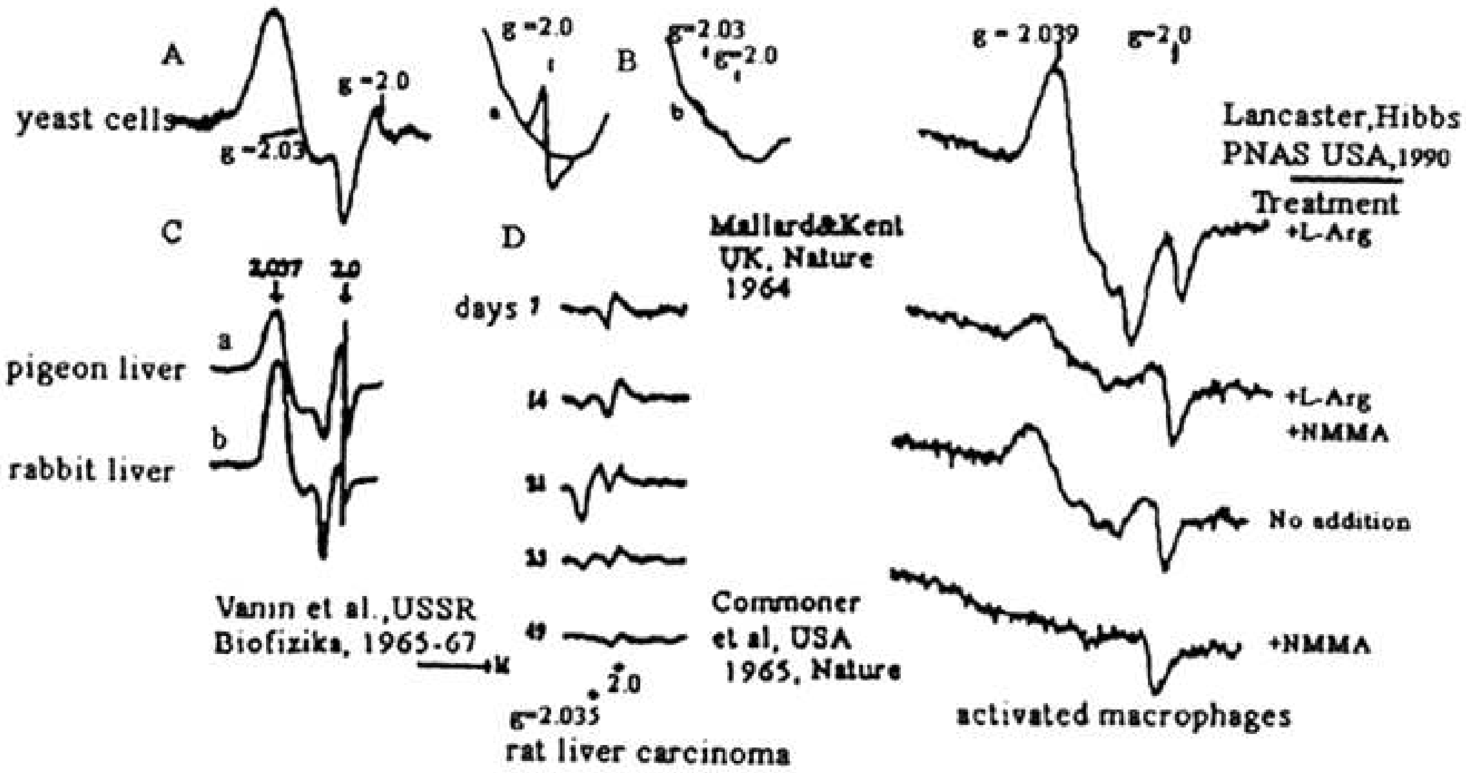
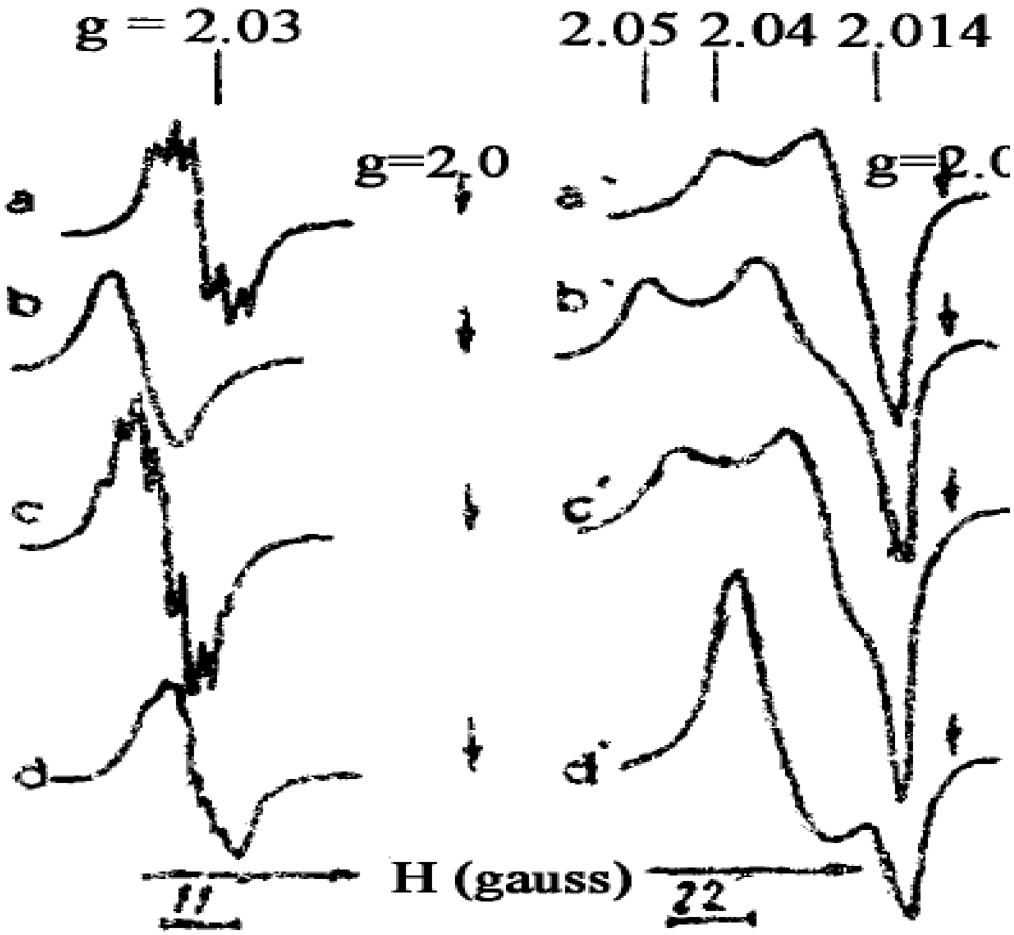
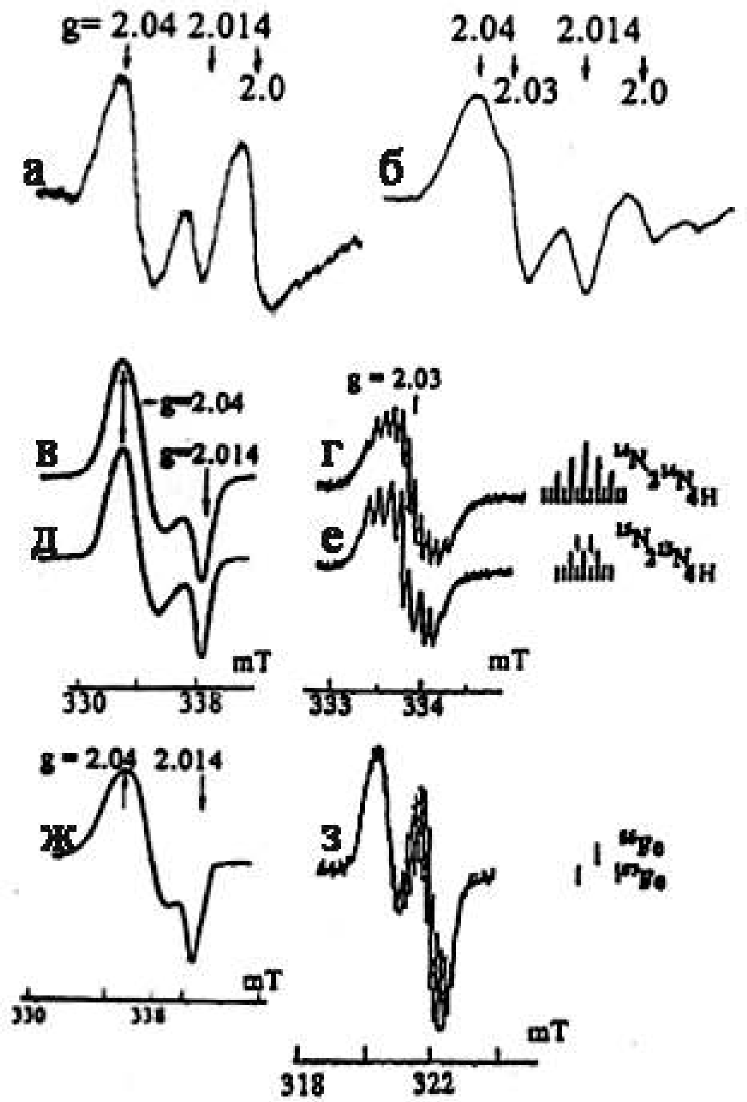
First observations of 2.03 EPR signal in living organisms

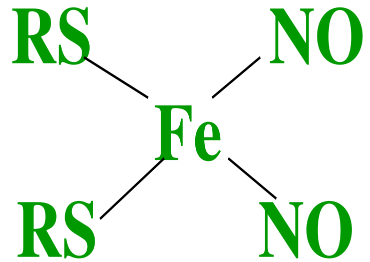


EPR signals of M-DNIC with hydroxyl ions (a), citrate (b), phosphate (c) or cysteine (d). Recordings were made at room temperature (left side) or at 77K (right side) *(Vanin, 1967)*

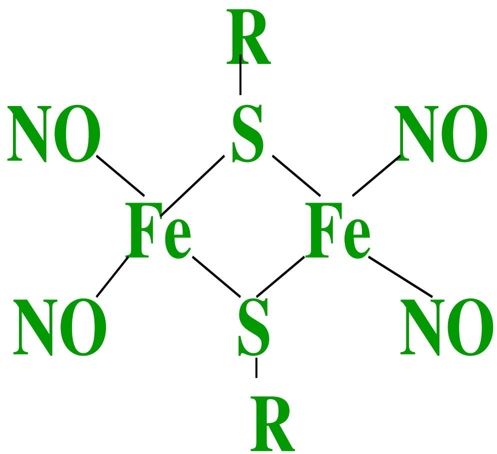


EPR spectra of 2.03 complexes in rabbit liver or in yeast cells (а,б) and DNIC with thiol-containing ligands (в-з)





M-DNIC



B-DNIC

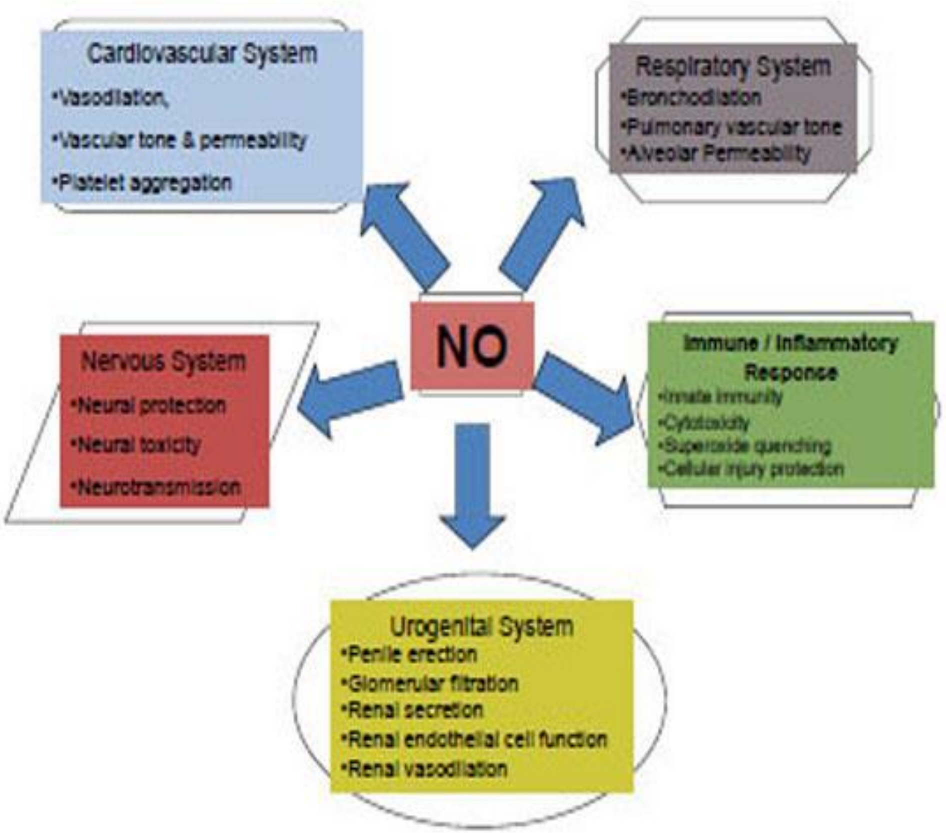
$-RS^-$

М-ДНКЖ ⇔ Б-ДНКЖ

Ferid Murad Louis Ignarro Robert Furchgott

The Nobel Prize in Physiology or Medicine 1998 was awarded jointly to Robert F. Furchgott, Louis J. Ignarro and Ferid Murad "for their discoveries concerning nitric oxide as a signalling molecule in the cardiovascular system"

Biological functions of NO system



DNIC with thiol-containing ligands as a «working form» of nitric oxide in living organisms

1.Identical biological activity of DNIC and endogenous NO system

2. Including the most part of endogenous NO into M- and B-DNIC

3. DNICs can function as a NO and NO^{+} donors

[illegible]

[illegible]

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Dinitrosyl Iron Complexes as a "Working Form" of Nitric Oxide in Living Organisms

Anatoly F. Vanin

Dinitrosyl Iron Complexes as a "Working Form" of Nitric Oxide in Living Organisms

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One-electronic oxidation/reduction of diamagnetic (EPR-silent)$(RS^-)_2Fe^{2+}(NO)_2$ with d^8 iron electron configuration leading to its transformation of paramagnetic (EPR-active) M-DNIC with d^7 or d^9 iron electronic configuration

Oxidation $(RS^-)_2Fe^{2+}(NO)_2 \rightarrow (RS^-)_2Fe^{2+}(NO,NO^+)_2$

d^8 d^7

Reduction $(RS^-)_2Fe^{2+}(NO)_2 \rightarrow (RS^-)_2Fe^{2+}(NO,NO^-)_2$

d^8 d^9

Disproportionation reaction of NO molecules in gaseous phase

$$2NO \rightarrow NO^{+} + NO^{-}$$

$$3NO \rightarrow NO_2 + N_2O$$

$$4\,NO + 4H_2O \leftrightarrow (\underbrace{2NO^{+} + 2OH^{-}}_{2HNO_2} + 2H^{+}) + (\underbrace{2NO^{-} + 2H^{+}}_{2HNO} + 2OH^{-})$$

$$4\,NO + 4H_2O \leftrightarrow (H_2O + NO + NO_2) + 2H^{+} + (H_2O + N_2O) + 2OH^{-}$$

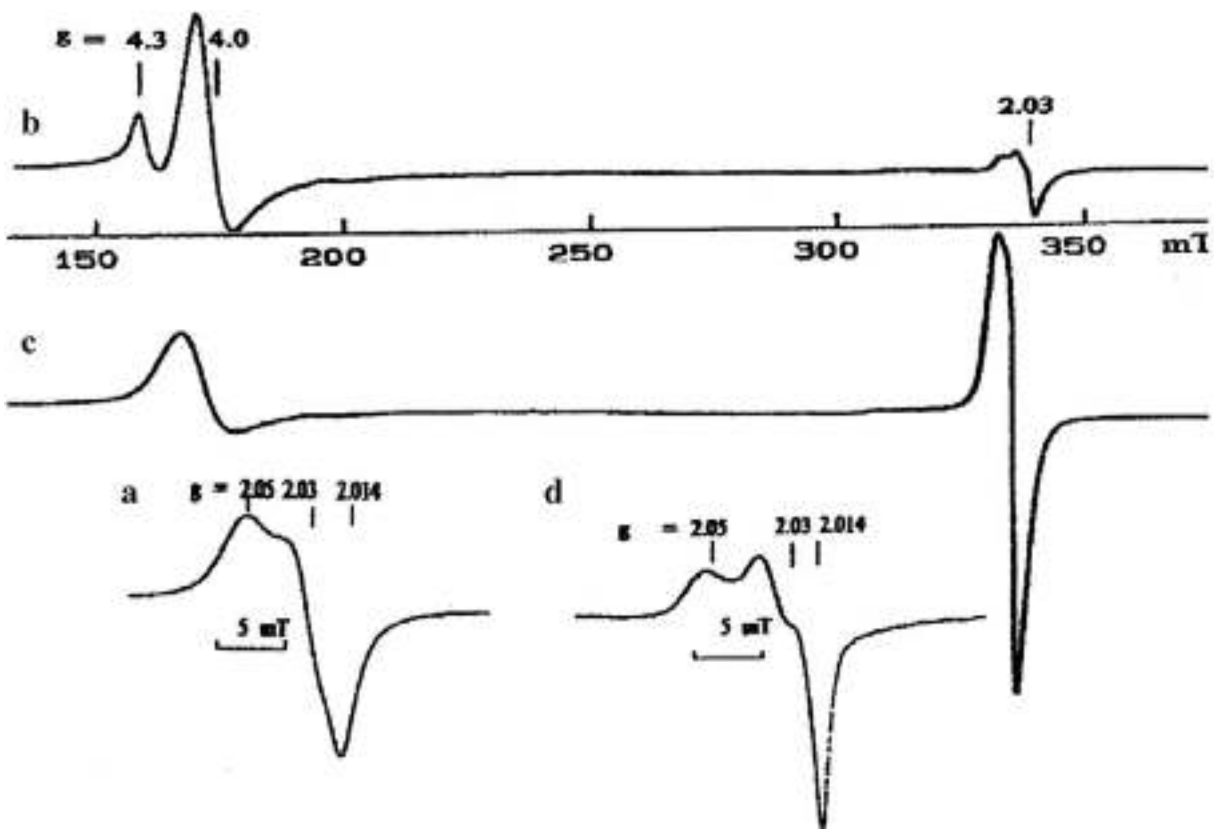
Oxidative mechanism of DNIC formation in the reaction of $NO + Fe^{2+} + L^-$

$$[(L^-)_2Fe^{2+}(NO)_2] \rightarrow [(L^-)_2Fe^{2+}(NO^+)(NO^-)] + NO + H_2O \rightarrow$$
$$\rightarrow [(L^-)_2Fe^{2+}(NO^+)(NO)] + HNO + OH^-$$

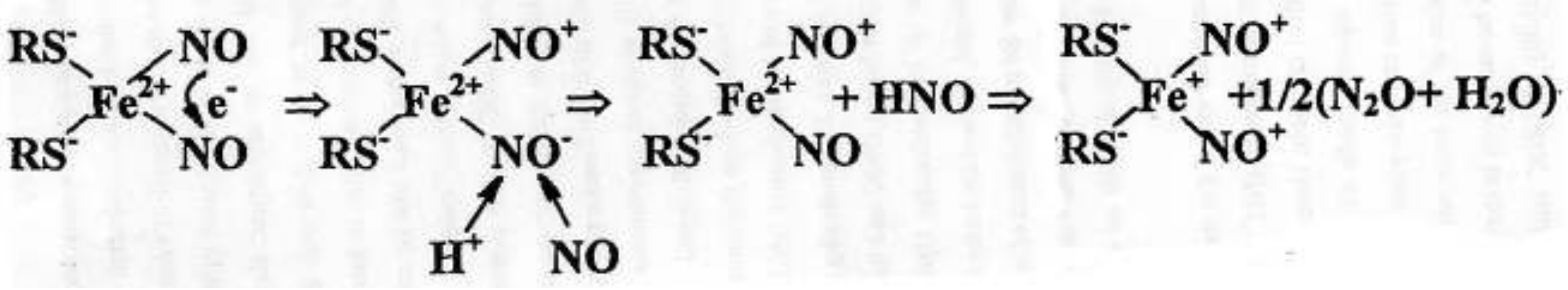
Nitrosonium cation hydrolysis leading to DNIC transformation into MNIC :

$$[(L^-)_2Fe^{2+}(NO^+)(NO)] + OH^- \rightarrow [(L^-)_2Fe^{2+}(NO)] + H^+ + NO_2^-$$

Summary EPR spectra of MNIC (S=3/2) and DNIC (S=1/2) with ATP and phosphate (b and c) and EPR specta of DNIC with ATP and phosphate (a,d) alone. Recordings were made at 77K.

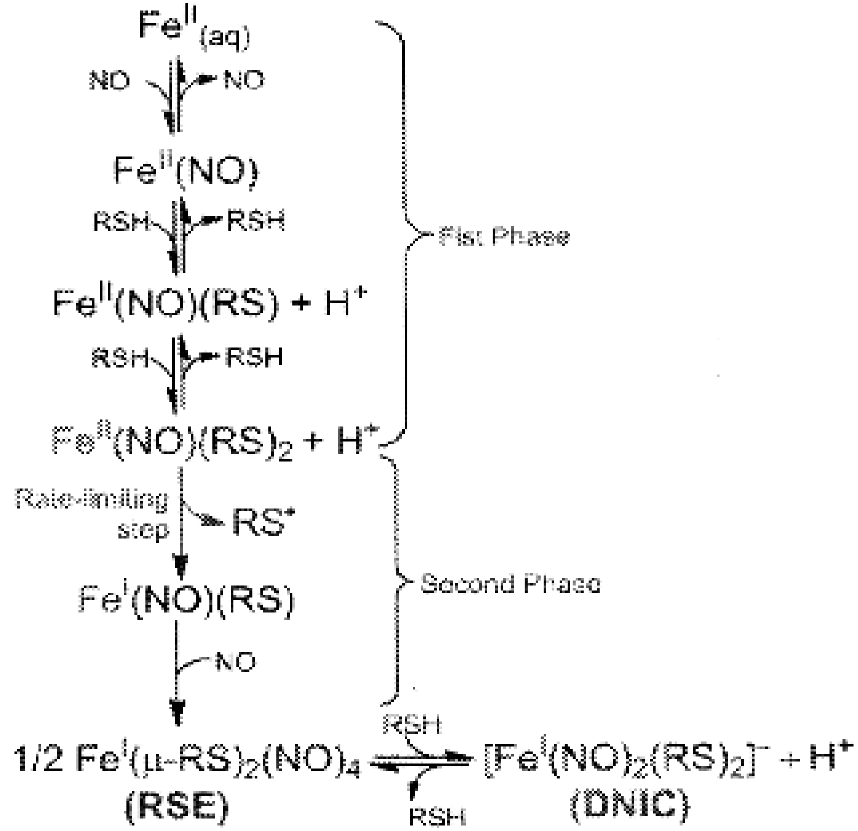


Oxidative mechanism of the formation of paramagnetic M-DNIC in the solutions containing thiol-containing compounds

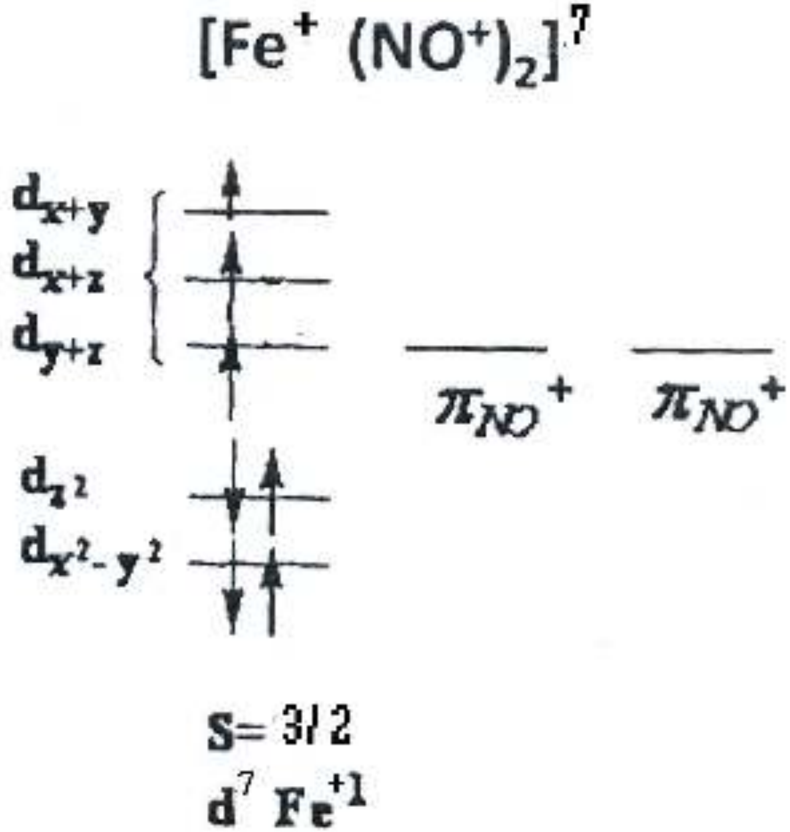


Reductive mechanism of DNIC formation (d^9 iron electron configuration) *(Ford et al. 2018)*

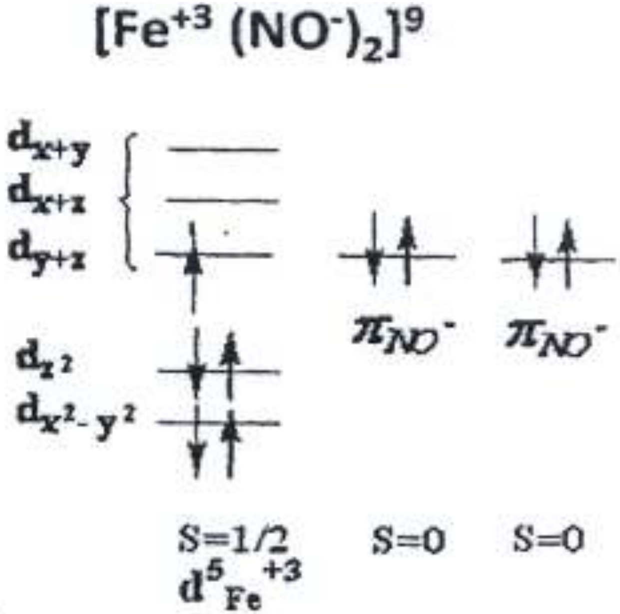
Scheme 1. Proposed General Mechanism for LMW-DNIC Formation in Aqueous Media

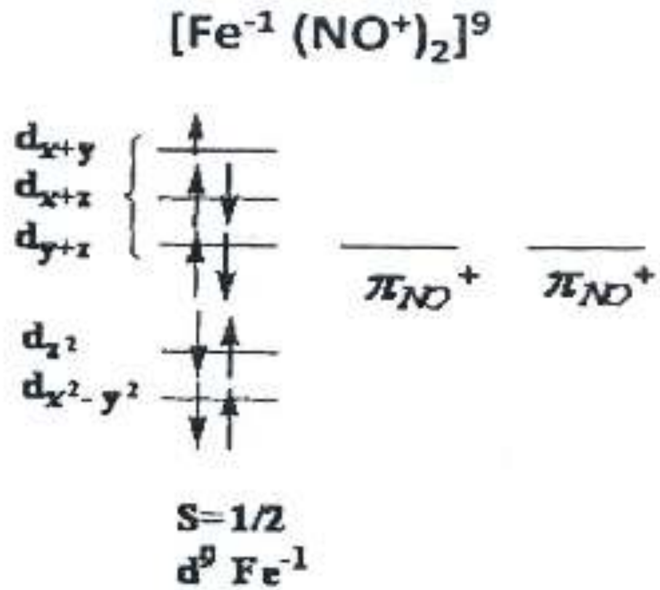


High-spin (S=3/2) of $Fe(NO)_2$ fragment of DNICwith the d^7 electronic configuration of iron and tetrahedral structure

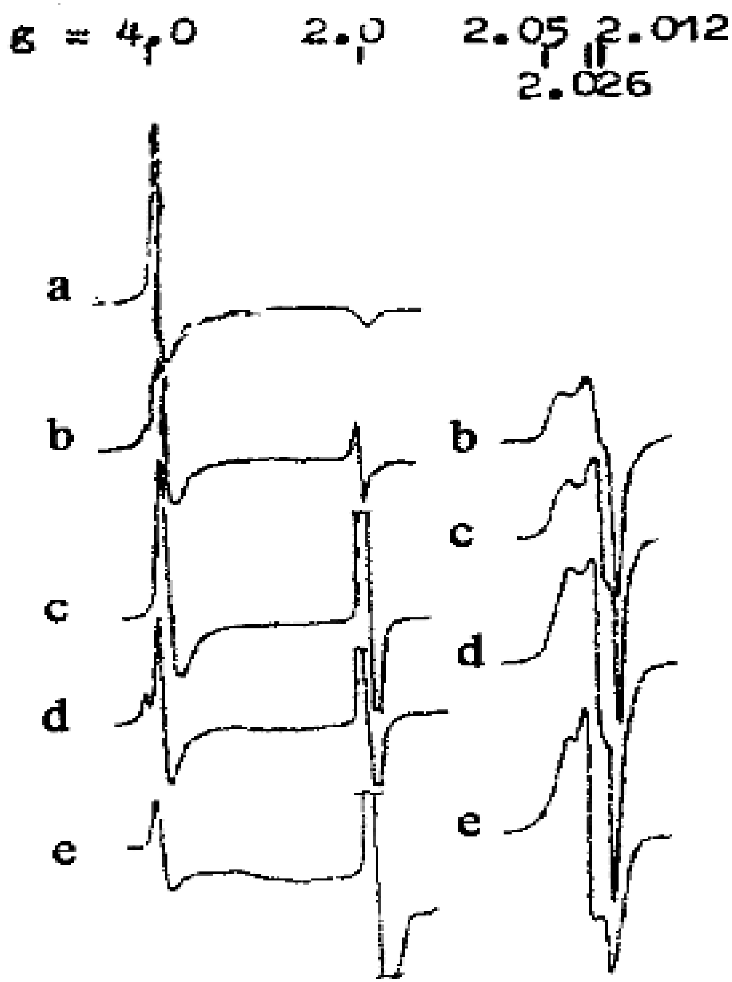


The low-spin (S=1/2) state of $Fe(NO)_2$ fragment with d^5 and d^9 electronic configuration of iron and tetrahedral spatial structure





EPR spectra of low spin (S=1/2) DNIC (right side, b-e, respectively) and high spin (S=3/2) MNIC with non-thiol ligands (EDTA, phosphate, ascorbate, citrate or water) (left side, a-e, respectively)

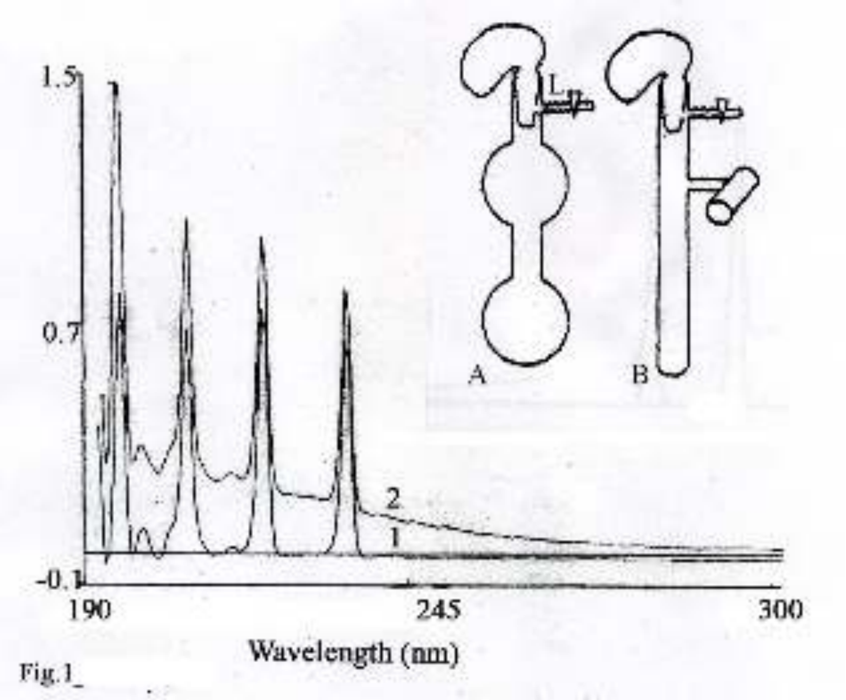


Chemical equilibrium between M-DNIC and its constituents

$$(RS^-)_2\,Fe^+(NO^+)_2 \Longleftrightarrow Fe^{2+} + NO + \underbrace{RS^- + NO^+}_{RS\text{-}NO} + RS^-$$

$$(RS^-)_2Fe^{-1}(NO^+)_2 \Leftrightarrow Fe^{2+} + NO + NO^- + 2RS^-$$

Left side — Optical absorption spectra of gaseous NO (specrum 1, four equidistant bands) and gaseous NO_2 (spectrum 2, structureless optical absorption). Right side — Modified Thunberg apparatus



Panel A: the optical absorption of gaseous NO released from the solution of B-DNIC-GSH (12 mM, 17 mL, pH 1.0) heated at 80°C for 3, 10 or 60 min (curves 1-3, respectively).
Panel B: the absorption spectrum of a standard sample of gaseous NO (200μmol in 100 mL of the free volume of the Thunberg apparatus).

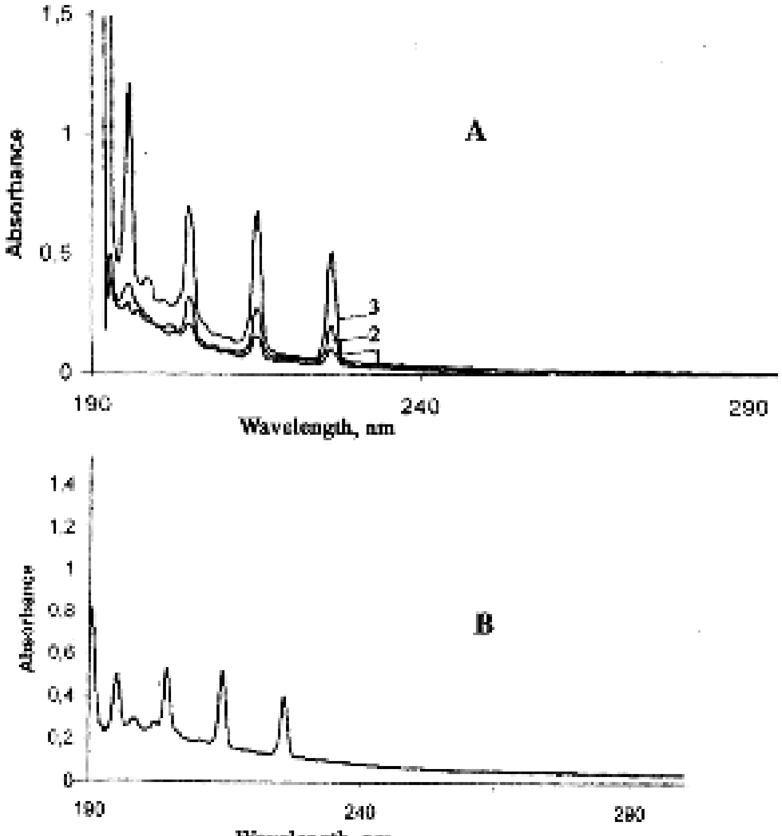
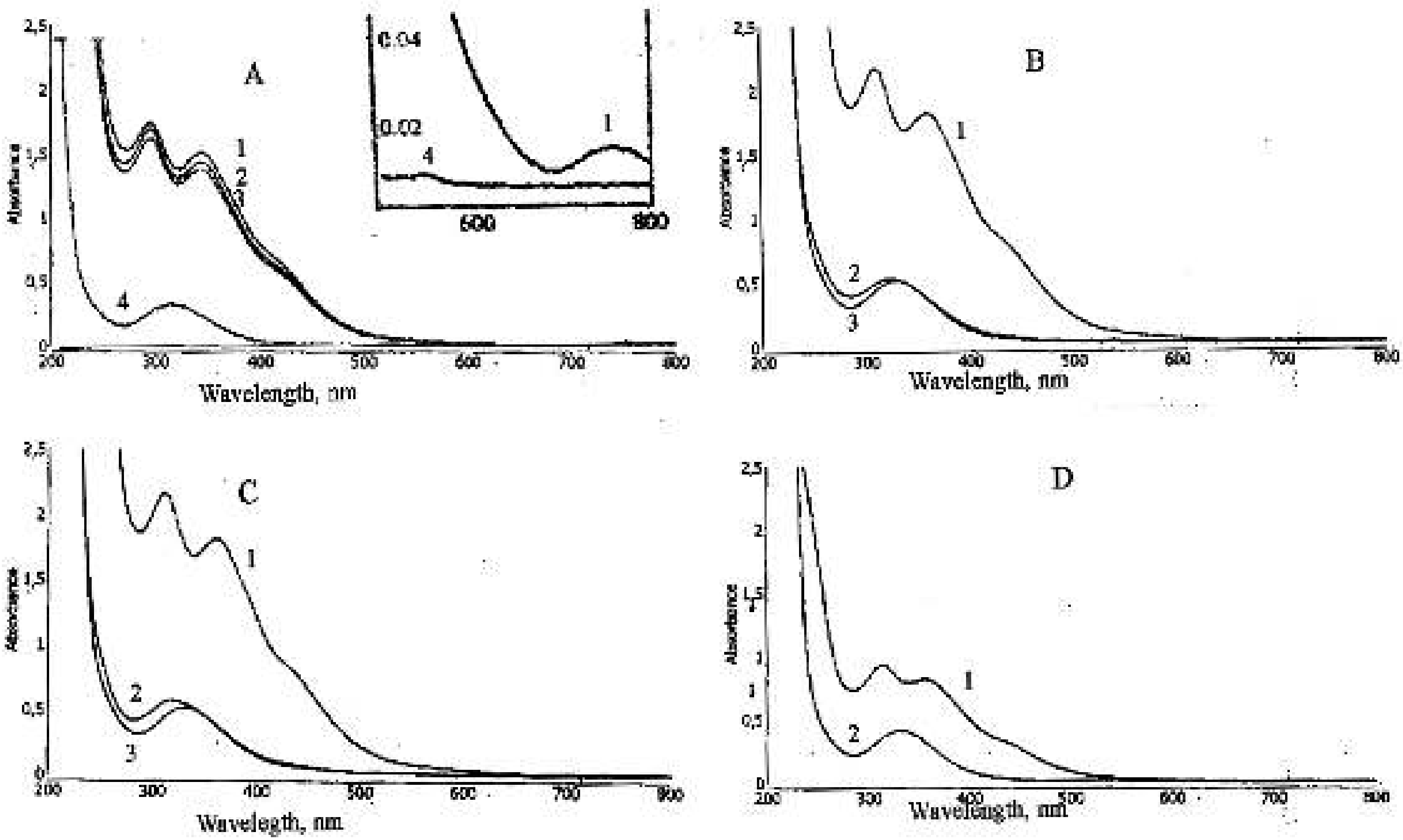


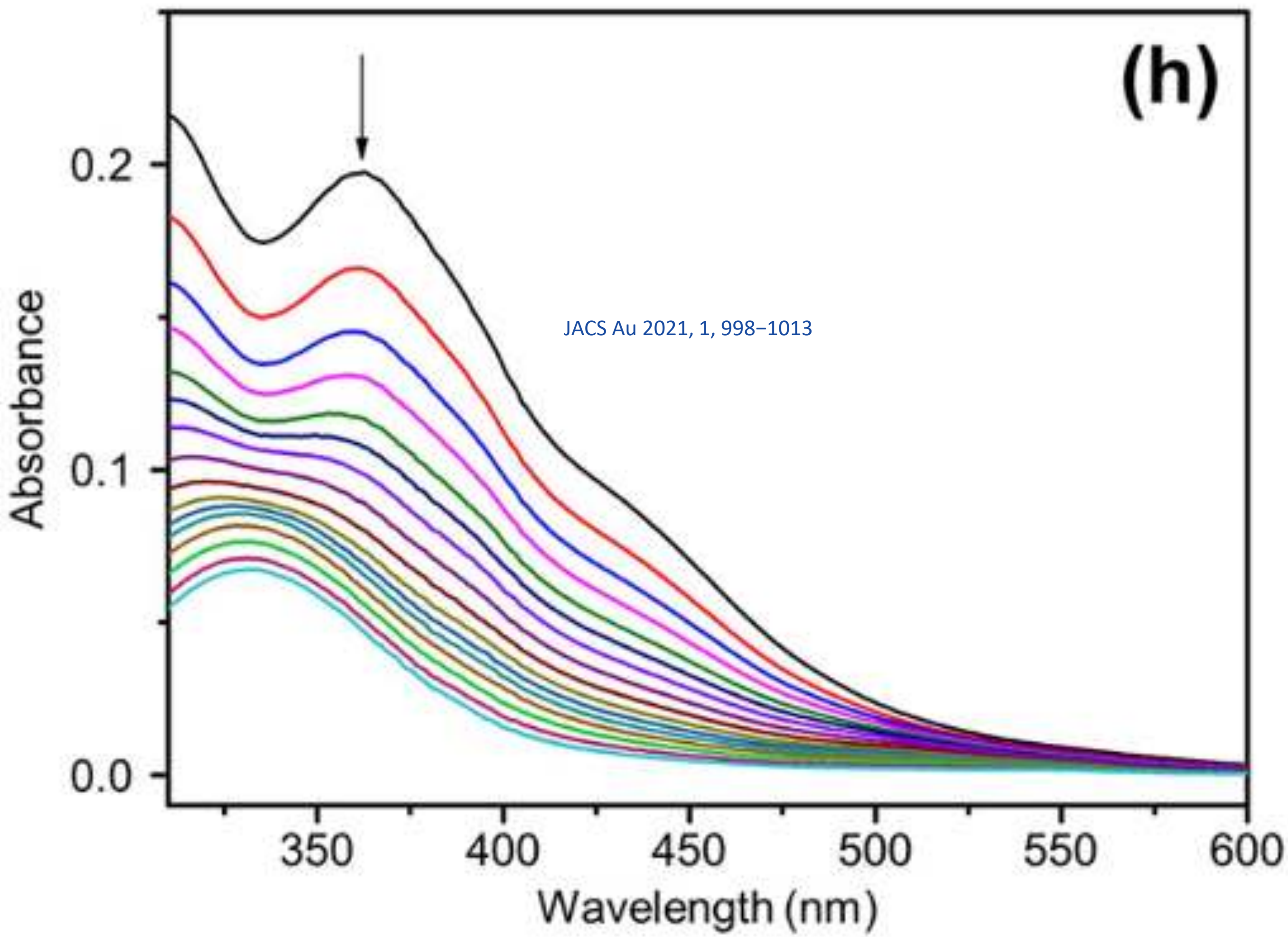
Fig.14

The decomposition of B-DNIC-GSH and their conversion into GS-NO induced by heating of acidified solutions (data from optical absorption measurements).

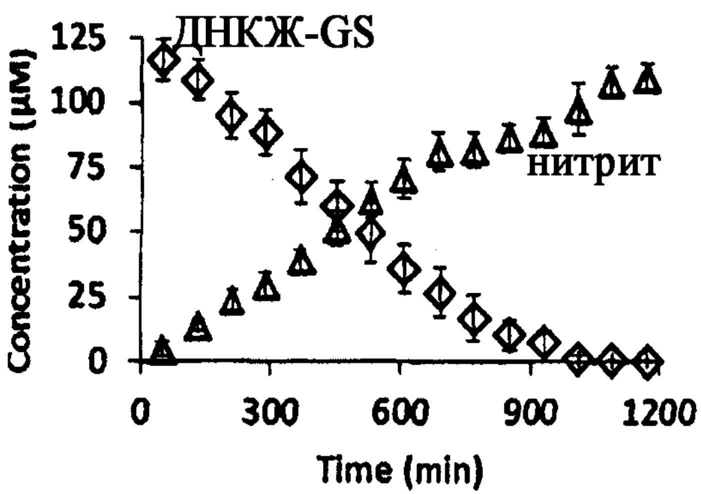
Panel A: The optical absorption spectra recorded 2 and 30 min after acidification of the initial 0.4 mM solution of B-DNIC-GSH (pH 7.4) (curve 1) to pH 1.0 (curves 2 and 3, respectively) and a subsequent 30-s increase in temperature from ambient to 80C (curve 4). Inset: the weak absorption bands of GS-NO and B-DNIC recorded at 543 and 768 nm (curves 4 and 1, respectively). **Panels B and C**: The optical absorption spectra recorded after acidification of a 0.5 mM solution of B-DNIC-GSH to pH 1.0 (curve 1) and 30-s incubation at 80C (**Panel B**) or 25-min incubation at 40C (**Panel C**) in the presence and in the absence of air (curves 2 and 3, respectively). **Panel D**: The absorption spectrum of a 9 mM B-DNIC-GSH acidified to pH 1.0 recorded after a 40-fold dilution with distilled water (curve 1) or a 15-min air incubation at 80C followed by a 20-fold dilution of the final solution with distilled water (curve 2)

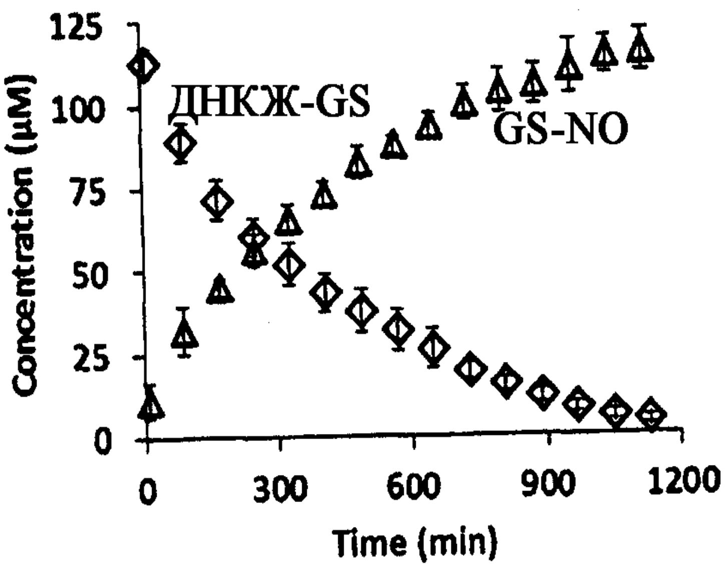


Time-dependentchange of UV−vis spectra for B-DNIC-mercaptoethanol in simulated gastric fluid without pepsin (pH 1.2).
(JACS Au 2021, 1, 998−1013 T.-T. Lu et al)

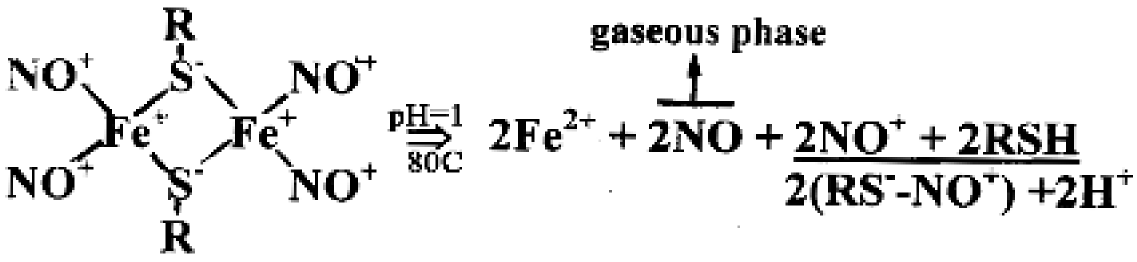


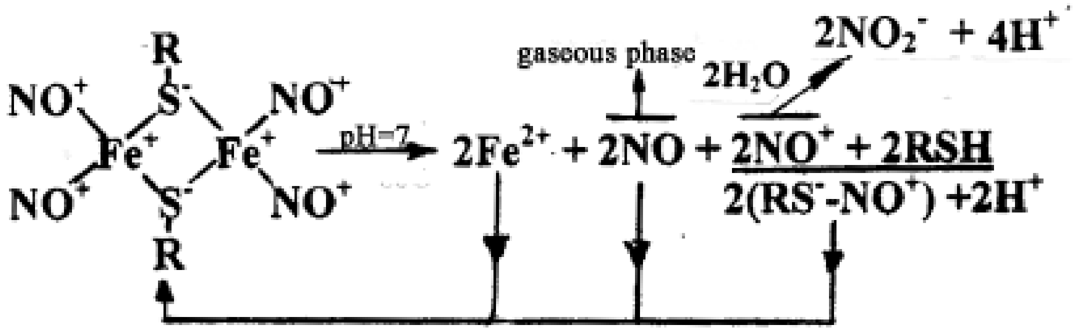
Accumulation of nitrite or S-nitrosoglutathione (GS-NO) during 100 micromole DNIC-GSH decomposition in air in aqueous solutioins at pH 7.0 or 1.0 (Hogg et al. 2017)



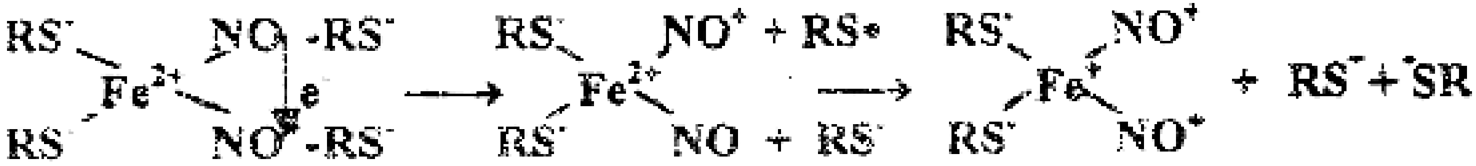


B-DNIC decomposition in a result of thiol group protonation in the solutions at acid condition or in a result of these groups oxidation in the solutions at neutral pH values.



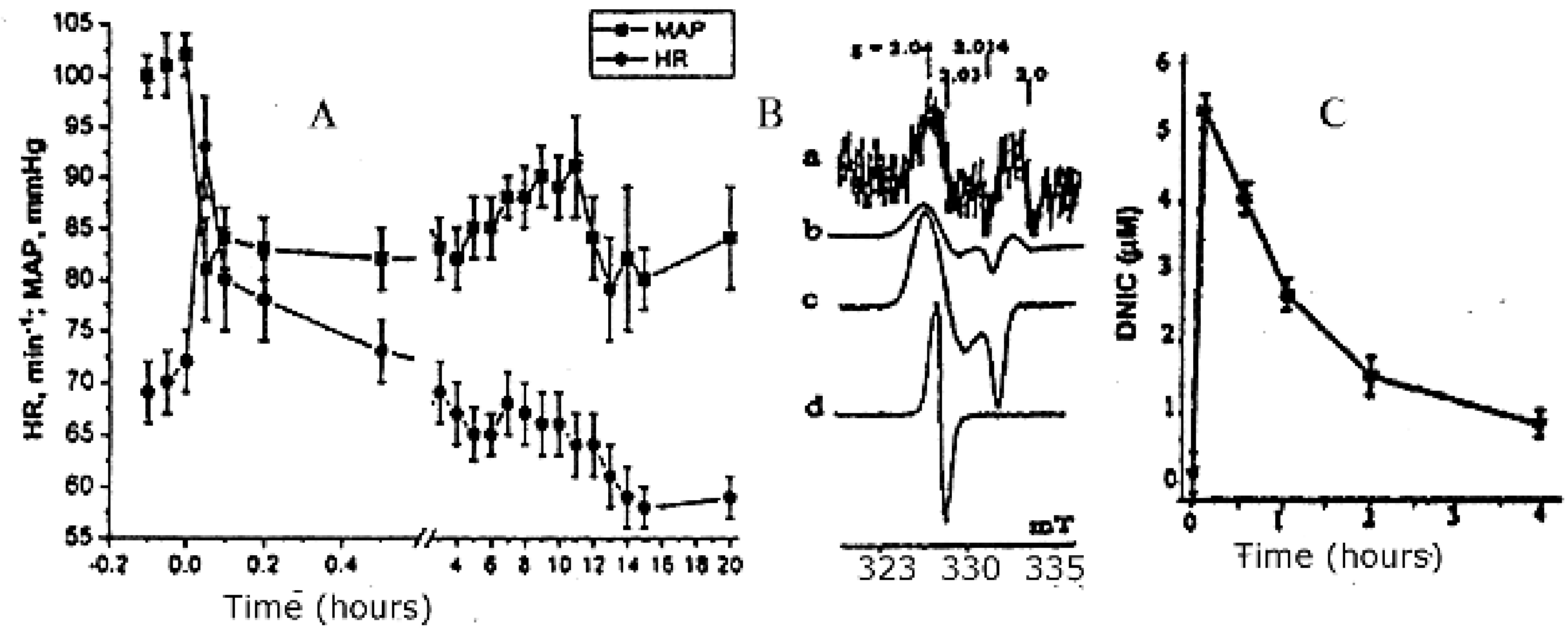


M-DNIC synthesis from S-nitrosiothiol, iron (II) and thiols

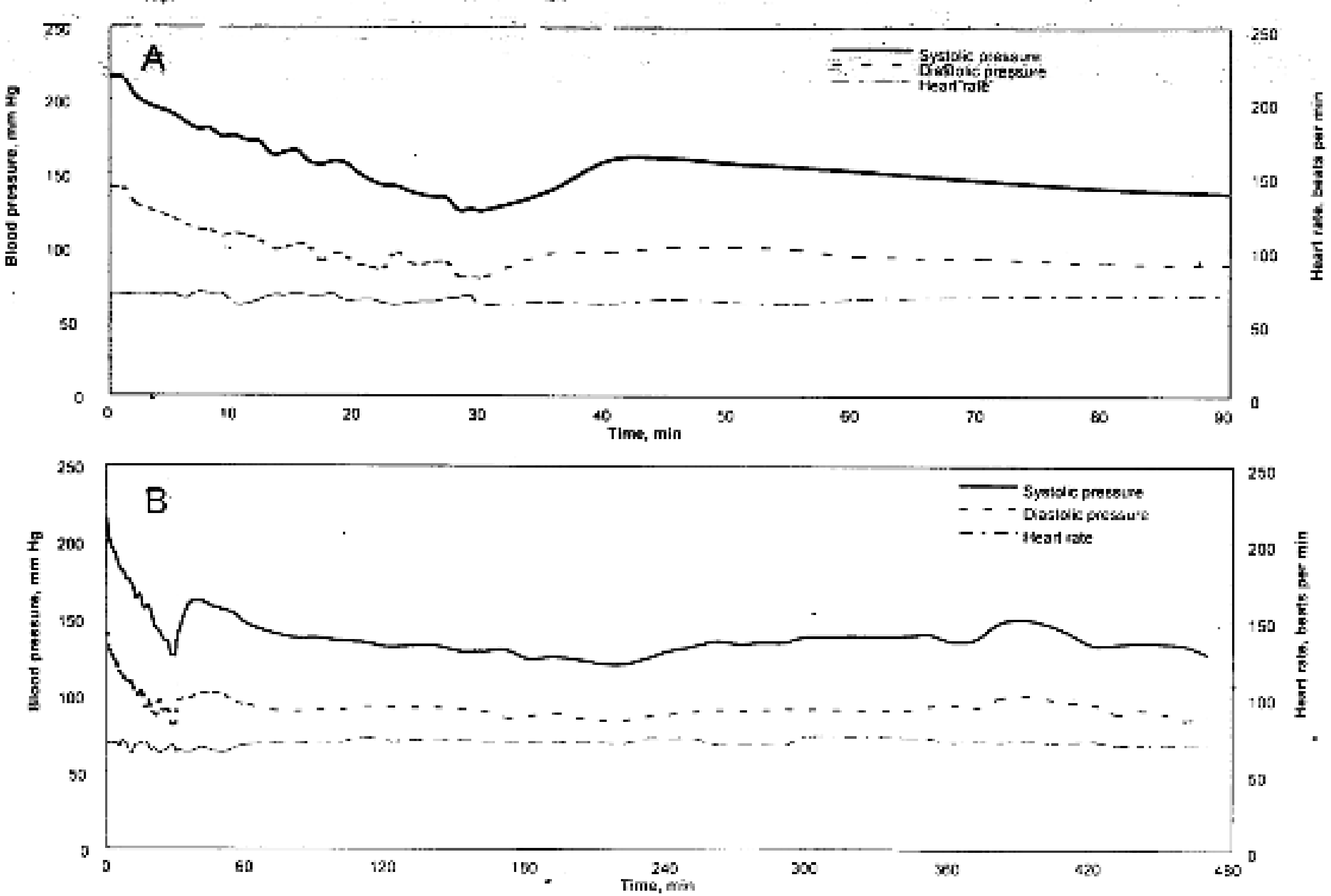


BIOMEDICINE OF DNIC

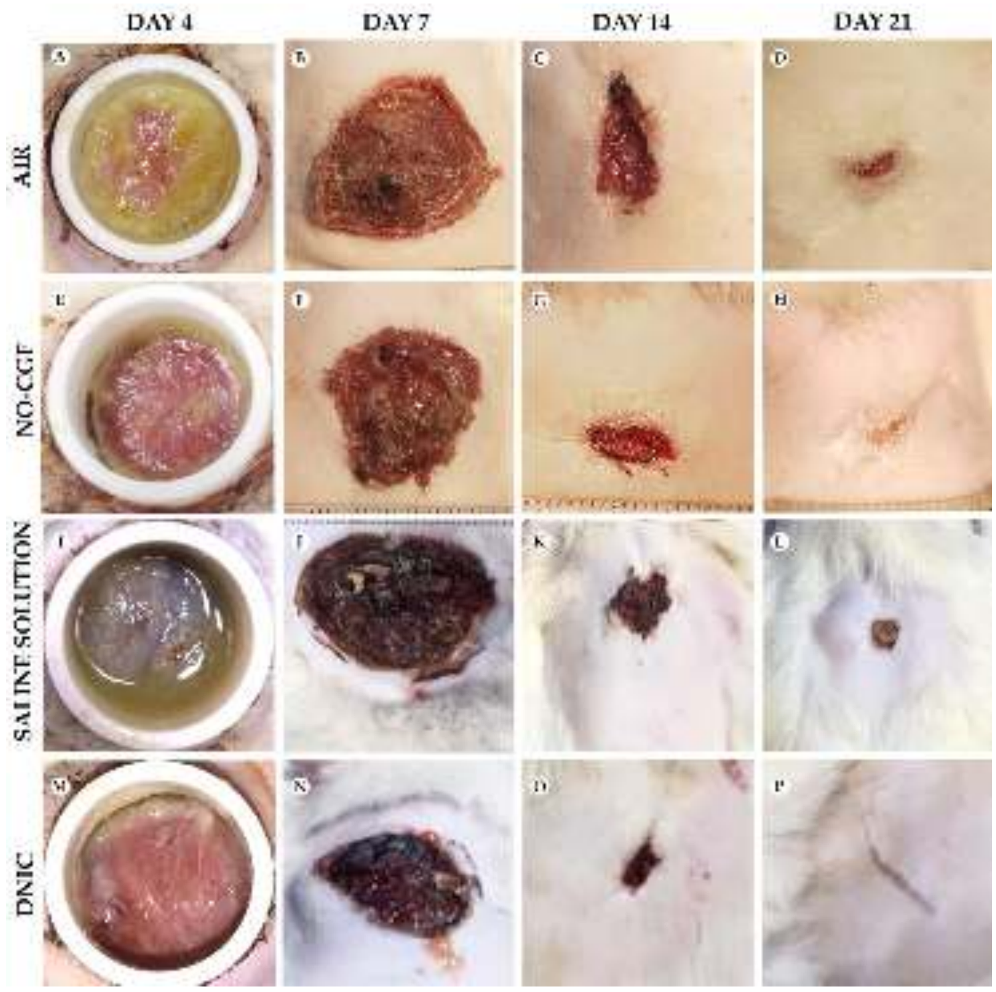
Hypotensive effect of Oxacom (DNIC-GSH) on healthy volunteers (E.Chazov et al. 2012)



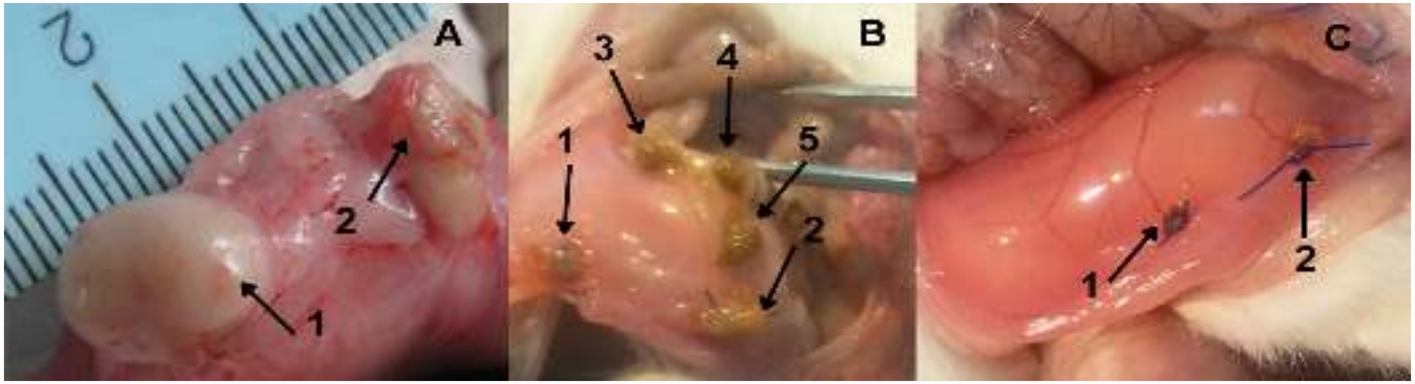
A 59-year-male male patient had a long history of hypertension with maximal blood pressure levels of 240/140 mmHg.The patient was put in intensive care unit and was administrated with 1,5 mg/kg of Oxacom (0.3 micromole/rg of DNIC-GS). After administration of the drug the decrease in blood pressure to mimnimal levels of 125/80 mmHg. Hypotension persisted for 8 hrs.



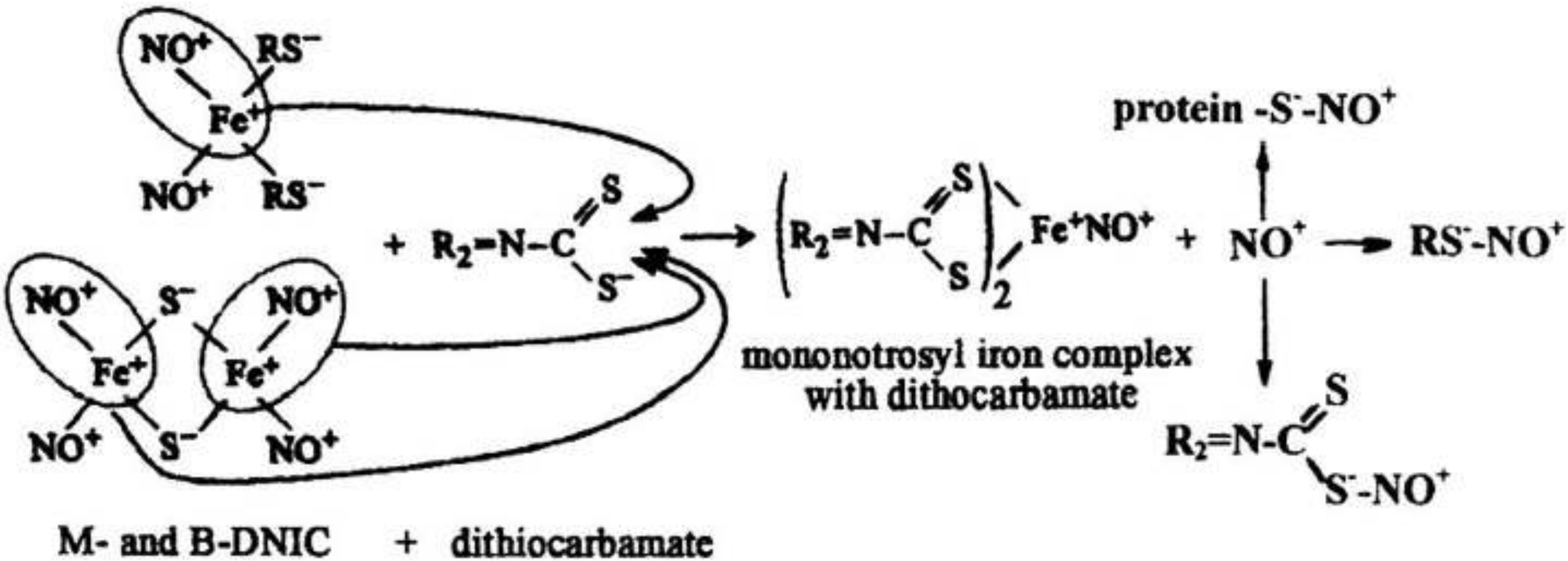
Gross examination of wounds in animals on post-operative days 4 (a, e, i, m), 7 (b, f, j, n), 14 (c, g, k, o), 21 (d, h, l, p) (Igrunkova et al, 2022).



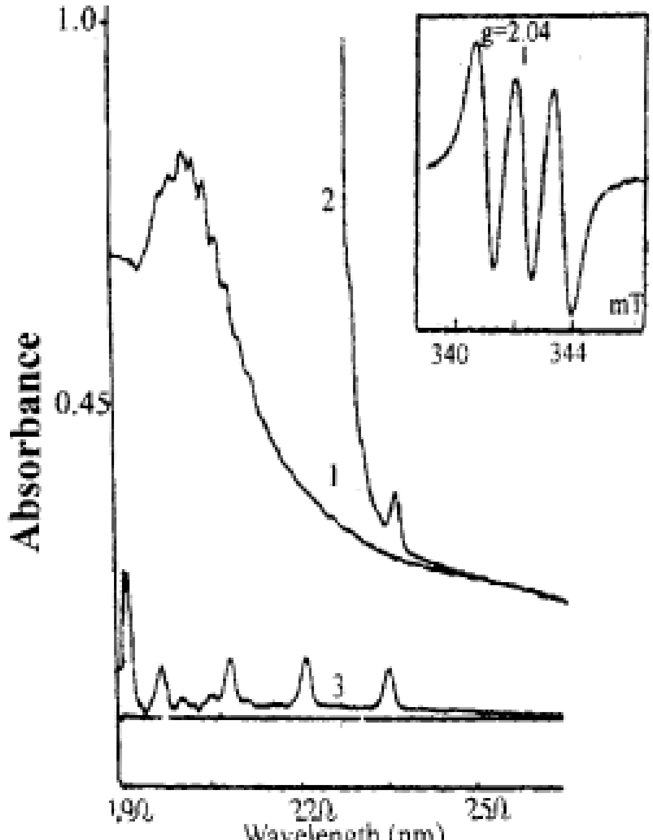
Blocking action of DNIC-GSH on endometrioidal tumor proliferation in rat organisms with experimental endometriosis (Burgova et al.,2018)



NO+ rerleasing from M- and B-DNIC under action of dithiocarbamate derivatives

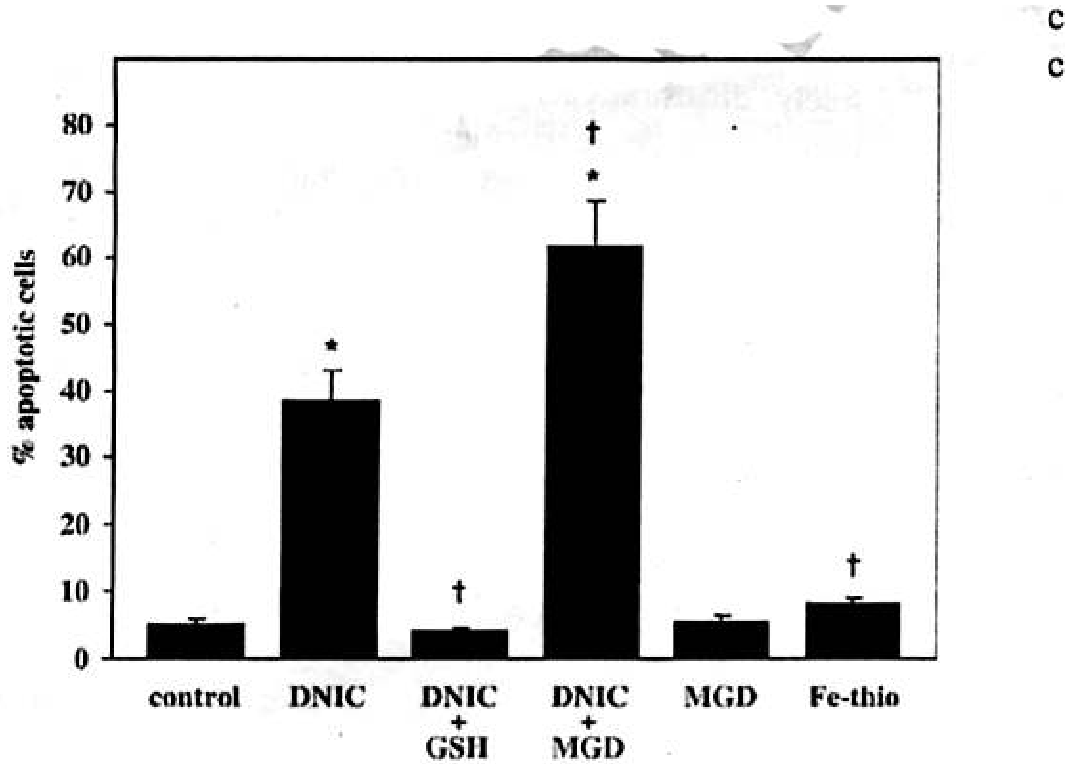


Nitrosonium cation release from B-DNIC-GSH under action of water soluble dithiocarbamate derivative - N-methyl-D-glucamine dithiocarbamate (MGD)

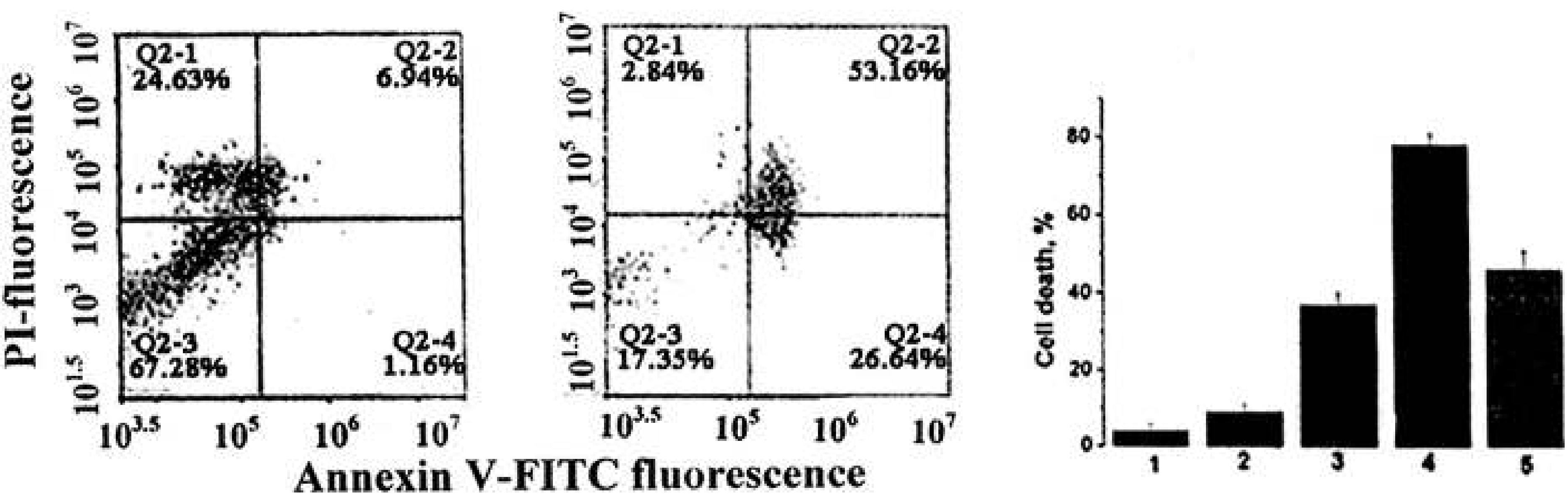


The amount of Jurkat cells in apoptotic state (%), treated with 100 micromole of DNIC witht tiosulphate followed with addition of GSH (2 mM, DNIC+GSH)), MGD (200 micromole, DNIC+MGD) , only MGD or Fe+thiosulphate

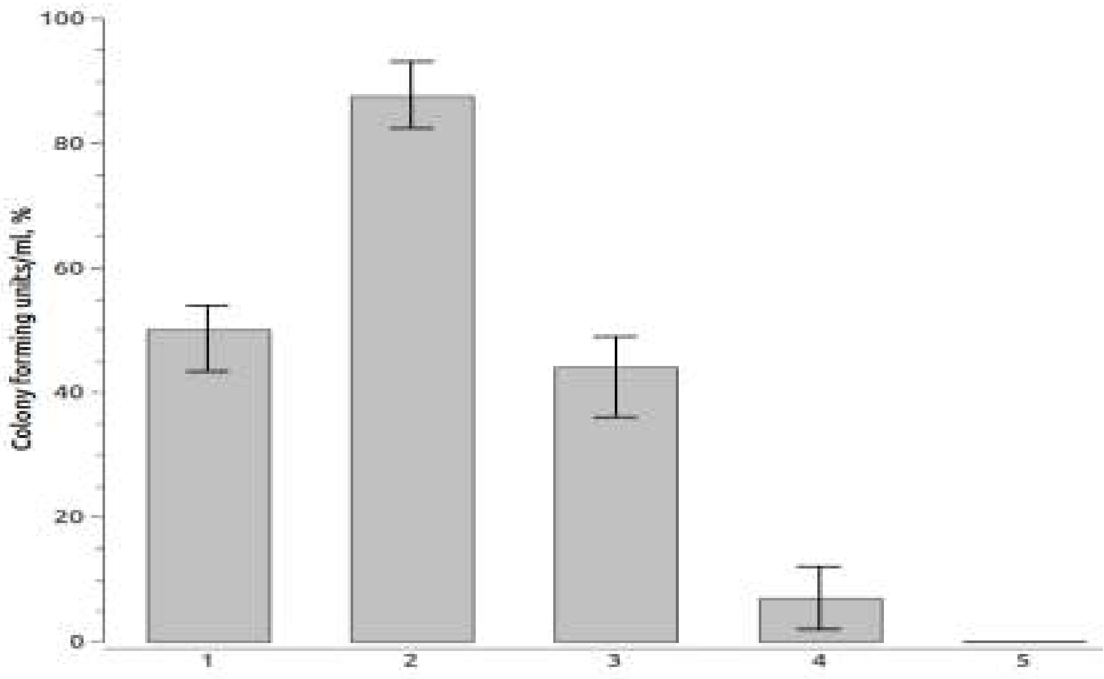
(Kleschyov et al., 2006)



MCF7 cell death in response to the treatment with B-DNIC-MS (0,5 mM) and MGD (1 mM) assessed with Annexin-PI staining. (0,5 mM) and MGD (1mM) assessed with Annexin-PI staining. Left panel -. 2D plots obtained using flow cytometry. Cells were incubated with B-DNIC-MS alone (left plot) and with a mix of B-DNIC-MS and MGD (right plot). Right panel - Mean fraction (%) of dead cells in a suspension. 1 - control, 2 –B-DNIC-MS, 3 – MGD , 4- B-DNIC-MS+MGD (combined action), 5 - sum of the effects in 2+3. Results are presented as mean values from three independent experiments ± SE (Vanin et al. 2020)



Colony forming activity of E. coli TN530 cell (CFU/mL): 1 – DETC (2.5 mM), 2 – B-DNIC-GSH (0,5 mM), 3 — Sum of the effects 1 + 2, 4 – B-DNIC-GSH + DETC (added simultaneously), 5 - B-DNIC-GSH + DETC (DETC added 40 min later, after B-DNIC-GSH).



ПРОТИВОВИРУСНАЯ АКТИВНОСТЬ КАТИОНОВ НИТРОЗОНИЯ В ОТНОШЕНИИ SARS-CoV-2 НА МОДЕЛИ СИРИЙСКОГО ХОМЯЧКА

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АБСТРАКТ

В экспериментах на модели сирийского хомячка продемонстрирована противовирусное действие в отношении вируса SARS-CoV-2 биядерных динитрозильных комплексов железа (Б-ДНКЖ) с глутатионом и диэтилдитиокарбамата натрия (ДЭТК) при последовательной (Б-ДНКЖ+ДЭТК) аэрозольной обработке заражённых животных растворами этих соединений. Методом электронного парамагнитного резонанса (ЭПР) в аналогичных экспериментах на здоровых хомячках установлена преимущественная локализация Б-ДНКЖ и ДЭТК в ткани лёгких. У мышей при таких же измерениях эти агенты равно эффективно локализовались как в лёгких, так и в печени. Предполагается, что при контакте Б-ДНКЖ и ДЭТК в организме животных происходит высвобождение из Б-ДНКЖ катионов нитрозония, вызывающих S-нитрозирование протеаз хозяина и вируса, что приводит к подавлению ковидной инфекции.

Вирусная нагрузка в тканях носовой полости и тканях лёгких хомячков, интраназально инфицированных вирусом SARS-CoV-2 с последующим аэрозольным лечением препаратами Б-ДНКЖ-GSH и Б-ДНКЖ-GSH + ДЭТК.

По данным метода ОТ-ПЦР(Ct) Б-ДНКЖ сам по себе снижал вирусную нагрузку в тканях носовой полости ((по уровню РНК) в 16 раз по сравнению с плацебо. По инфекционному титру (lg $TCID_{50}$/ml) достоверного снижения вирусной нагрузки ни в носу ни в лёгких не обнаружено.

При введении Б-ДНКЖ+ДЭТК по данным ОТ-ПЦР(Ct) отмечено снижение уровня РНК в носу — в 16 раз, в лёгких — в 21 раз. Методом титорования в тканях носовой полости опроеделено ещё большее снижение вирусной нагрузки — в 200 раз. Для тканей лёгких при такой оценке эта величина была снижена в 20 раз

*** Более низкие пороговые значения числа циклов (Ct) указывают на более высокие вирусные нагрузки.**

Соединение	Вирусная нагрузка SARS-CoV-2			
	В тканях носовой полости		В тканях легких	
	lg $TCID_{50}$/ml	Ct*	lg $TCID_{50}$/ml	Ct*
Б-ДНКЖ	4,1±0,5	23,83±1,27	4,4±0,8	20,67±2,13
Б-ДНКЖ+ДЭТК	2,7±0,4	27,28±3,44	2,4±0,4	30,33±1,18
Плацебо	5,0±0,5	19,80±2,19	3,7±0,4	25,96±1,60

Thank you for your attention

Спасибо за внимание!

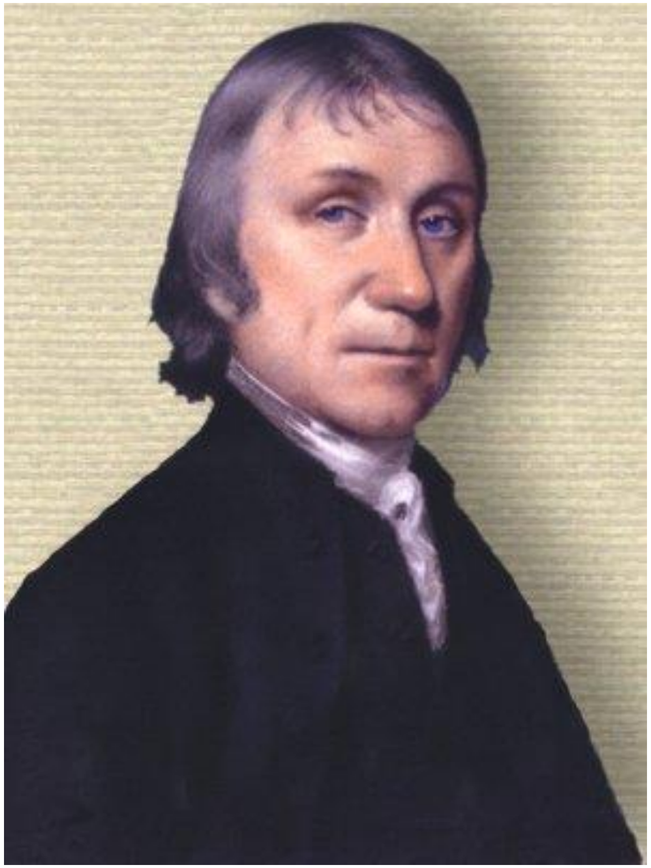
EPR parameters of low spin (S=1/2) M-DNIC with thiolate ligands

$g_{\perp}=2.04$, $g_{||}=2.014$
($g_{\perp}>g_{||}>2.0023$)

$A_{\perp}(^{57}Fe) = -1.7mT$, $A_{||}(^{57}Fe) = -0.25mT$, $A_{iso}=-1.22mT$
$|A_{\perp}(^{57}Fe)| > |A_{||}(^{57}Fe)|$

The values are consistent with unpaired electron localization on MO(d_{z^2}) (low spin d^7 configuration) and square planar spatial structure
(McGarvey, 1973; Vanin&Burbaev, 2011)

Joseph Priestliey (1733-1804)

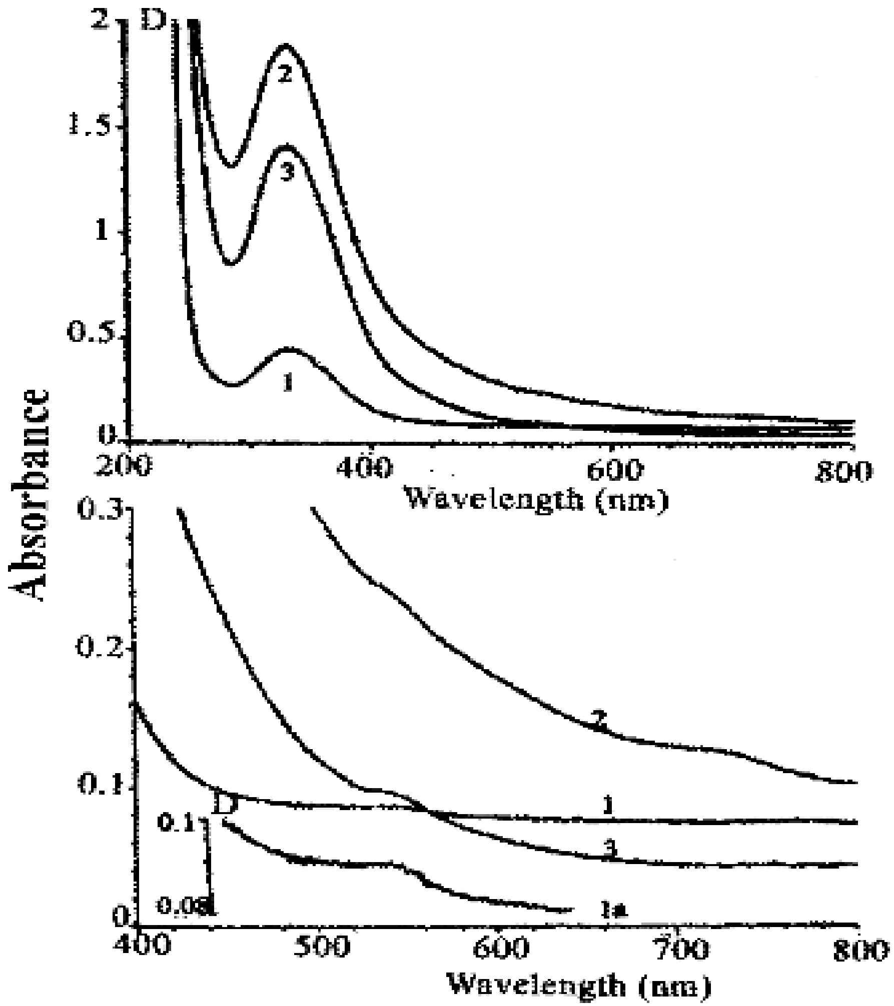


François-Zacharie Roussin (1827-1894)

Evgeny Konstantinovich Zavoisky

1907-1976

Nitrosonium cation release from M-DNIC in aqueous solutions



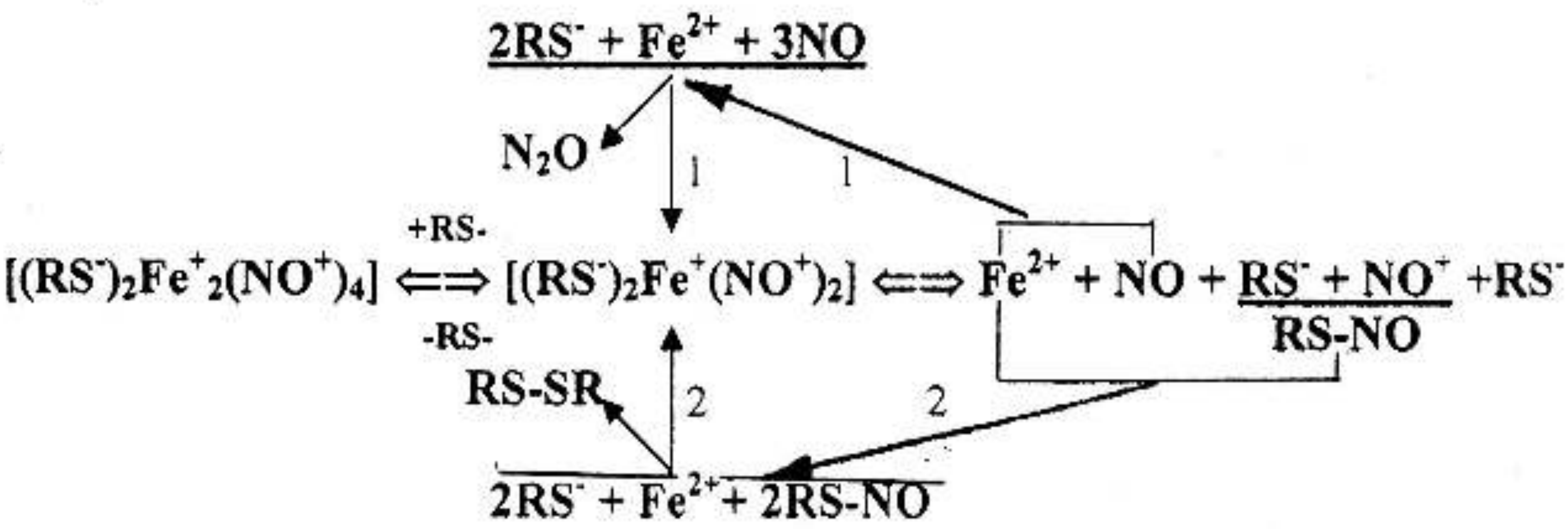
Peroxynitrite and its transformations

$$NO + O_2^- \rightarrow NOOO^-$$

$$NOOO^- + H^+ \rightarrow HNOOO$$

$$HNOO \rightarrow OH^{\cdot} + NO_2$$

Mechanism of formation, the relationships between components and the ability of M- and B-DNIC with thiol-containing ligands to donate NO and NO^+ cations



<u>Left panel</u> - The photo image of a ring-like structure obtained 0.1 sec after application of a spherical drop (0.01 ml) of an aqueous solution of Fe^{2+} (10 microM) + glutathione (0.5 M) onto a 0.3-mm layer of a 0.5 mM solution of GS-NO. The maximum diameter of the concentric rings was nearly 10 mm/

<u>Right panel-</u> Ring-like structures wich appeared after applying of a 0.01 mL drop of nitrite solution onto the same GS-NO solution.