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# DETECTION OF NITRATE($NO_2^-$) IONS PRODUCED IN DISPROPORTIONATION OF NITROGEN(II) OXIDE IN AQUEOUS SOLUTION

## DETEKCJA JONÓW AZOTANOWYCH ($NO_2^-$) POWSTAJĄCYCH W REAKCJI DYSPROPORCJONOWANIA TLENKU AZOTU(II) W ROZTWORZE WODNYM

**Abstract:** The nitrate ions ($NO_2^-$), products of disproportionation of NO in aqueous solution, were detected by an Orion Nitrite Electrode 97-46. Calibrations by means of standard $NaNO_2$ solutions within the range 0.001÷100 ppm indicated linear dependence of EMF on ppm within 1÷100 ppm. Measurements justified the usefulness of this detection method of NO in solutions of $OH^-$ concentration lower than $10^{-2}$ mol $dm^{-3}$ since at higher concentrations the EMF values exceeded the measurement range of the electrode. Occurrence of nitrate ions produced in the disproportionation reaction was additionally confirmed in dependence of $OH^-$ concentration by near UV and fluorescence spectra. The calibrated ion-selective nitrate electrode has also been shown, on the basis of Co(II)-dipeptide-$OH^-$ systems, as a useful tool in studying reversible NO uptake by Co(II) chelates in aqueous solution. Such a reaction may be regarded as simulating the harmful binding of NO by hemoglobin, where it substitutes the isoelectronic dioxygen.

**Keywords:** nitrogen(II) oxide, disproportionation of NO, nitrate ($NO_2^-$) ion-selective electrode, UV spectrophotometry, spectrofluorimetry, harmful uptake of NO

Nitric(II) oxide is recently one of the most intensively studied signaling particles in living systems. This small, versatile molecule takes part in a number of activities of the living system, eg blood-vascular system control, nerve signal conductance, organism immune response to pathogens as well as cell partitioning [1, 2]. The studies on biochemistry of NO started in the 70-ties of the last century when it was stated that nitric(II) oxide is an active metabolite of nitrate medicines used in the therapy of cardiac ischemia [3, 4]. Soon, it was also found that nitric(II) oxide is produced endogenously in the living

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system and the molecule was called an *endothelium-derived relaxing factor* (EDRF), responsible for a number of activities mentioned above [5, 6].

Despite the significant physiological and pathological role of NO, the harmful influence of this gas on the natural environment is connected with intensive development of industry and motor transport but also, to a lower degree, with some natural phenomena such as volcanic eruptions, electric discharges and action of bacteria. The essential arduousness of nitrogen oxides (among others NO) is due to their disproportionation reactions yielding nitrogen oxoacids: $HNO_3$ and $HNO_2$ - components of the so called "acid rains".

In the presence of hydroxyl ions the disproportionation reaction of nitrogen(II) oxide to nitrogen(IV) and nitrogen(I) oxides [7] may be written as:

$$2\ NO + OH^- \longrightarrow NO_2^- + \tfrac{1}{2}N_2O + \tfrac{1}{2}H_2O \qquad (1)$$

Hence, the detection of NO may be carried out via measurement of $NO_2^-$ concentration by various methods.

In living systems nitrogen(II) oxide is a harmful factor able to bind hemoglobin where it substitutes the isoelectronic dioxygen yielding a Fe(III)heme-$NO^-$ moiety [8, 9]. Up to now this reaction was also simulated by NO uptake by Co(II) chelates with dipeptides, partially reversible like in reactions with $O_2$, but finally leading to Co(III) products [10, 11]. The aim of the present work was to prove the $NO_2^-$ ion selective electrode in studying the disproportionation reaction under various pH and then to test her usefulness for evaluating the reversible NO uptake by Co(II) chelates with dipeptides in aqueous solution.

## Experimental

### Potentiometric measurements

An ion-selective Orion Nitrite Electrode 97-46 (Thermo Electron Corporation) for measurements of $NO_2^-$ concentration was used. The filling solution was Orion 900046. A calibration solution of concentration 100 ppm was prepared from Orion standard 0.1 M $NO_2^-$ (Orion 954606) according to the instruction manual. Subsequently, solutions of 10, 1.0, 0.1, 0.01 and 0.001 ppm have been obtained by dilution.

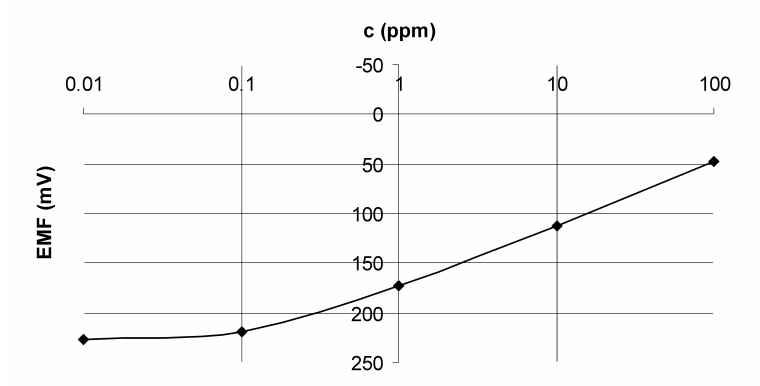


Fig. 1. Nitrate electrode calibration curve

Equal volumes of Nitrite Interference Suppressor Solutions were added to each standard. The electrode potential was measured by a multifunctional CX-731 device (Elmetron) at intensive stirring of solution. Calibration (EMF as a function of concentration, *c*) preceded all the measurements of $NO_2^-$. A linear dependence $E = E_0 - k \log c$, where $E_0$ is the standard potential regarding the reference electrode, was fulfilled within the range 1÷100 ppm (Fig. 1). The mean k coefficient at temperature 25°C amounted to 61 mV.

The NaOH solutions of concentrations 0.01 M and 0.001 M were prepared by dilution of standard 0.100 M NaOH (J.T. Baker) and then poured into the thermostated vessel used in a conventional isobaric laboratory apparatus. The disproportionation reaction was investigated at 25°C both by the $NO_2^-$ ion-selective electrode (after calibration) and a combined glass electrode OSH-10-10 (after calibration with standard buffers: phthalate pH = 4.00 and borax pH = 9.00). The measurement vessel of volume 80 $cm^3$ was filled up either with pure water or an appropriate NaOH solution. During the experiments the liquid was vigorously stirred with a magnetic bar. A stream of argon was used to remove oxygen from the sample during ca 30 minutes. Then the sample was purged with NO during ca 15 minutes. After cutting off the NO inlet the concentration of $NO_2^-$ ions and pH were measured by both electrodes. The values of SEM recorded by the $NO_2^-$ electrode attained minimum after 20÷40 min, by that showing maximum concentration of the ions.

**Spectroscopic measurements**

A UV grade cell (path length 1 cm), of volume ca 3 $cm^3$, filled up with twice distilled water or appropriate aqueous solutions of NaOH and firmly closed with silica stopper, was purged at room temperature with pure argon in order to remove excess of oxygen. Then the sample was saturated with NO during ca 20 min. As it is known, at room temperature the association of gaseous NO to $N_2O_2$ dimers is extremely small [12]. Since then, absorption curves were recorded in time intervals on a Cary 50 Bio (Varian) Spectrophotometer within the range $\lambda$ = 250÷450 nm. Slit width: 1.5 nm. The scan speed was medium (600 nm/min), data interval 1 nm, averaging time 0.1 s.

Fluorescence measurements were carried out on a Hitachi F-4500 Fluorescence Spectrophotometer. A UV grade cell with all the walls transparent was used, path length 1 cm. Samples were prepared similarly as in the UV absorption measurements but the scans were made only once, after ca 30 min. Main parameters: EX Start WL - 200 nm, EX End WL - 500 nm, EX Sampling Interval - 10 nm; EM Start WL - 300 nm, EM End WL - 600 nm, EM Sampling Interval - 10 nm; Scan speed: 12000 nm/min; EX Slit - 5 nm, EM Slit - 2.5 nm.

## Results and discussion

**Potentiometric detection of NO disproportionation**

The pH and EMF values from the glass and $NO_2^-$ electrodes, respectively, are shown in Table 1. As it follows from Table 1 the concentration of $NO_2^-$ ions was very low in pure water and by that undetectable by the Nitric Orion Electrode. The measurable amounts of

these ions could be shown only in the presence of the alkali (in spite of lowered sensitivity), but at high concentrations of NaOH the detection limits were evidently exceeded.

Table 1

Maximum concentrations of $NO_2^-$ ions, $C_{max}$, produced as a result of disproportionation of NO in aqueous solution (measurements carried out in triplicate)

| Solvent | Time [min] | pH (glass electrode) | SEM [mV] ($NO_2^-$ ion selective electrode) | $C_{max}$ [ppm] |
|---|---|---|---|---|
| $H_2O$ | 30 | 4.96 | 247.5 | - [a)] |
| 0.001 M NaOH | 15 | 10.37 | 59.8 | 65 |
| 0.01 M NaOH | 20 | 11.76 | 51.8 | 80 |
| 0.1 M NaOH | 30 | 12.57 | 43.6 | >100 |

a) beyond lowest detection limits

**Near UV measurements**

Absorption spectrum of nitric ion in aqueous solution at 25°C, needed to further comparisons, is shown in Figure 2. The spectrum exhibits an absorption band at $\lambda = 354$ nm ($\varepsilon_{max} = 22.8$; Beer-Lambert's law: $R^2 = 0.9975$) and a weak shoulder near $\lambda = 290$ nm. Both absorption bands are of $n \rightarrow \pi^*$ type [13].

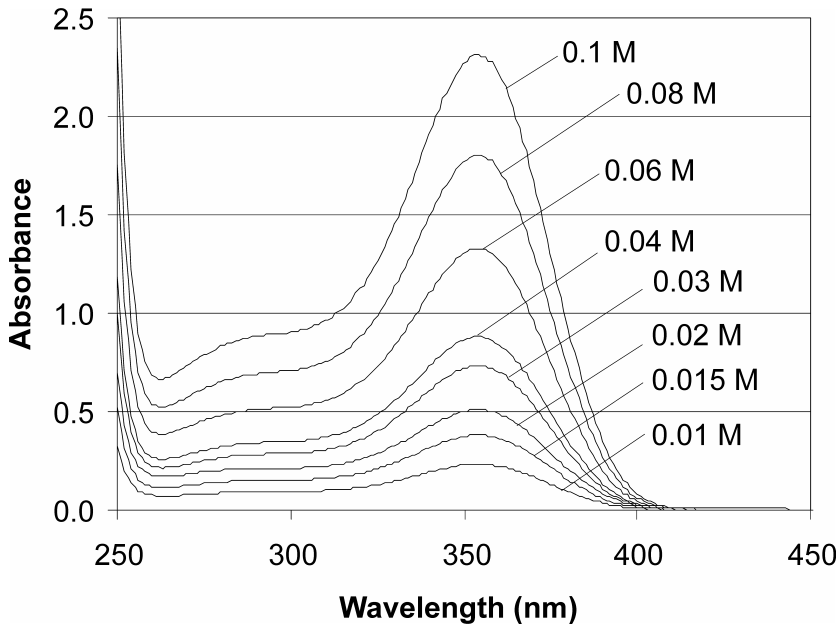


Fig. 2. UV absorption spectra of $NaNO_2$ in aqueous solutions of various concentrations

The UV spectra obtained after saturating the water or aqueous solutions of NaOH with NO are shown in Figure 3. As can be seen the absorption curves were roughly similar to the

ones of nitric ion (as in Fig. 2) due to the proceeding disproportionation reaction. In particular, the similarity of the absorption band at $\lambda$ = 354 nm becomes quite obvious. However, in pure water and to a more and more lower extent in the NaOH solutions along with rise in concentration, electronic spectra revealed the known concomitant vibrational structure of the lower energetic $n \rightarrow \pi^*$ transition of $NO_2^-$ [14, 15]. The absorbance rose in time due to dissolution of consecutive amounts of NO during the purge and but also, after cutting of the gas inlet, due to diffusion of NO volume remaining in the measurement cell, above the surface (ca 1 $cm^3$). The whole process was limited by the solubility and diffusivity of nitric oxide, which in pure water are known as $\alpha_{NO} = 1.94 \cdot 10^{-6}$ mol $cm^{-3}$ $atm^{-1}$ and $D_{NO} = 2.21 \cdot 10^{-5}$ $cm^2$ $s^{-1}$, respectively [16].

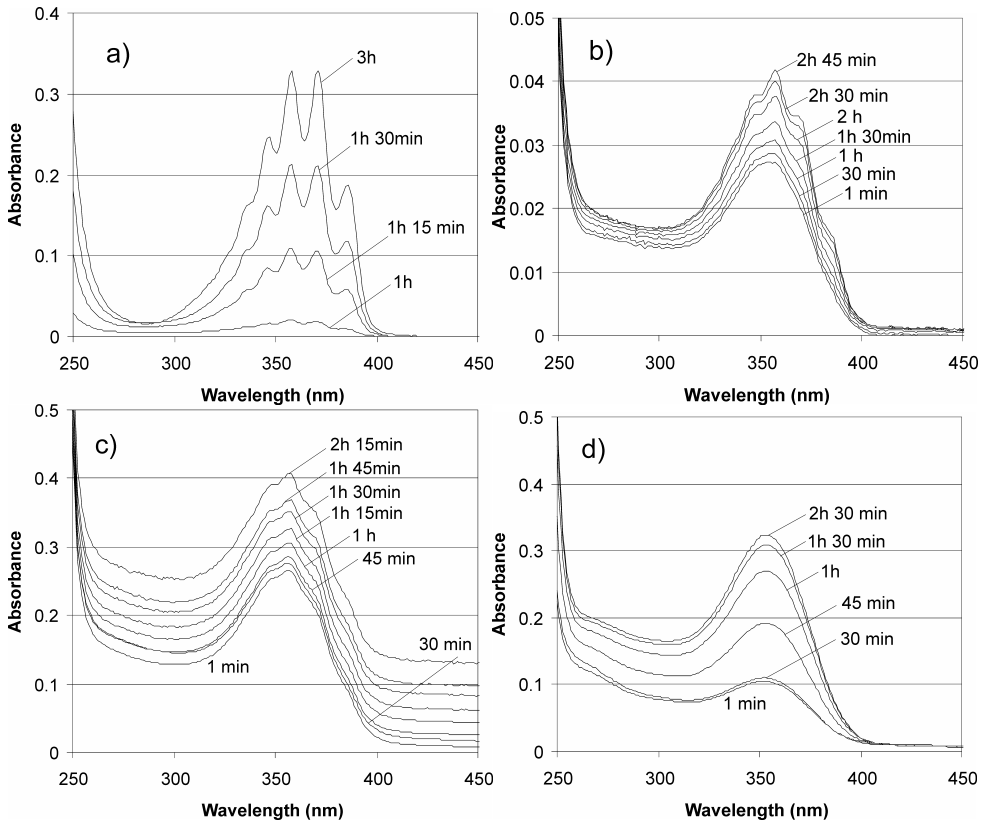


Fig. 3. UV absorption spectra in time intervals after NO-saturating. The cell was filled up with: a) pure water, b) 0.001 M NaOH, c) 0.01 M NaOH, d) 0.1 M NaOH

## Spectrofluorometric measurements

The 2-D fluorescence spectra of the $NO_2^-$ ion, product of NO disproportionation are shown in Figure 4.

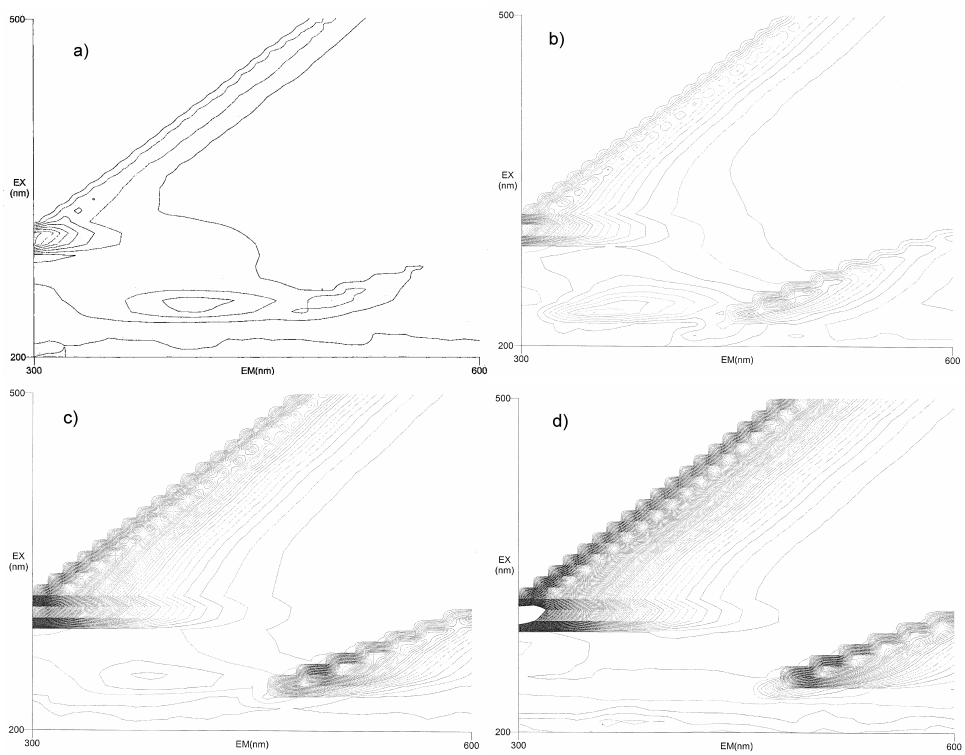


Fig. 4. 2D-fluorescence spectra recorded ca 20 min after NO-saturating. EX - excitation, EM - emission. The cell was filled up with: a) pure water, b) 0.001 M NaOH, c) 0.01 M NaOH, d) 0.1 M NaOH

As can be seen the intensity (displayed by line densities in the 2-D spectra) of the maximum absorption region of $NO_2^-$ and maximum fluorescence range were higher for the solutions of NaOH then for pure water. The effect corresponds to the spectrophotometric results shown in Figure 3 at approximately the same time after cutting of the NO inlet, ie about 20 min. On the other hand, the 2-D fluorescence spectra are more distinct and show vibrational features more clearly in the more basic solutions contrariwise to the absorption spectra. This effect may be explained by the lowering share of pure water molecules of known high distance between the vibration levels and by that more significant fluorescence quenching.

**Reversible NO uptake by Co(II) chelates with dipeptides in aqueous solution**

The Orion Nitrite Electrode has been applied to control the gas atmosphere in vessel during the consecutive steps observed in experiments with the reaction of NO uptake by Co(II) chelates with dipeptides in aqueous solution:

$$CoL_2 + NO + 2OH^- = Co(LH_{-1})_2NO^{2-} + 2H_2O \qquad (2)$$

where product $[Co^{III}(LH_{-1})_2NO^-]^{2-}$ is described formally as a Co(III) adduct with electron density displaced from Co(II) to $\pi^*2p_y(NO)$. This redox rearrangement is partially

reversible. The degree of reversibility is dependent on the kind of dipeptide as auxiliary ligand. The NO uptake may be to some extent drawn back upon flushing with pure argon, which has been already shown by UV/Vis/NIR data [11]. In the present work the changes in solution were monitored, as exemplary shown in Table 2, by the nitrate electrode.

Table 2

Changes in aqueous solution of 1 mmol of PheGly, 0.5 mmole of $Co(NO_3)_2$ observed as a result of the reaction with NO. Total sample volume 75 $cm^3$

| **Calibration** | | **pH** | **EMF [mV] (nitrate electrode)** | **Color** |
|---|---|---|---|---|
| [ppm] | [mV] | 5.233 (under argon) | 277.9 | Pink |
| 0.01 | 176.7 | 5.109 (NO 20 min) | 206.8 | Pink |
| 0.1 | 161.1 | 10.033 (+ 1.4 $cm^3$ NaOH in portions) | 45.1 | Dark violet |
| 1 | 157.7 | 9.987÷10.176 (+ argon 0÷130 min) | 48.5÷111.2 (+ argon 0÷130 min) | Violet |
| 10 | 86.7 | | | |
| 100 | 18.0 | | | |

At first (Table 1) the nitrate( $NO_2^-$ ) electrode has a high potential by that indicating a very low concentration of the nitrate( $NO_2^-$ ) ion in aqueous solution of NO below lowest detection limits (cf. Table 1, first line). The pink color corresponds to the Co(II) at relatively low concentration (ca $7 \cdot 10^{-3}$ M). Then, the concentration of nitrate( $NO_2^-$ ) ions increases dramatically due to alkalization - effect of disproprtionation. At the same time the dark violet color shows the formation of the Co(II)-nitrosyl adduct with PheGly as auxiliary ligand. By turns, purging the solution with pure argon removes NO and leads to a decrease of nitrate( $NO_2^-$ )-ion concentration (below 10 ppm). Moreover, as a result of irreversible autooxidation of Co(II) to Co(III) and then decay of the nitrosyl complex solution becomes violet due to the remaining Co(III) species.

## Conclusions

The Orion Nitrite Electrode proved to be a useful tool in studying the disproportionation reaction under various pH and by that enabled evaluating the partially reversible NO uptake by Co(II) chelates with dipeptides in aqueous solution. This reaction may be regarded as to some extent simulating the harmful binding of NO by hemoglobin where it substitutes the isoelectronic dioxygen and yields a Fe(III)heme-$NO^-$ moiety. Although the predicted pH range for this electrode is 4 to 8, the some higher basicity of the solution (even up to pH 10), necessary to attain the NO uptake by the Co(II) chelate, does not exclude the observation of the phenomena occurring during the main reaction and during the evolution of NO in the inert atmosphere. The nitrate ions produced in the disproportionation reaction were additionally observed by near UV and fluorescence spectra along with the vibrational states in dependence of $OH^-$ concentration.

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## DETEKCJA JONÓW AZOTANOWYCH ( $NO_2^-$ ) POWSTAJĄCYCH W REAKCJI DYSPROPORCJONOWANIA TLENKU AZOTU(II) W ROZTWORZE WODNYM


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**Abstrakt:** Jony azotanowe ($NO_2^-$), produkty dysproporcjonowania NO w roztworze wodnym, oznaczano za pomocą jonoselektywnej elektrody Orion Nitride Electrode 97-46. Kalibracje za pomocą standardowych roztworów $NaNO_2$ w zakresie 0.001÷100 ppm wskazywały liniową zależność SEM od ppm w zakresie 1÷100 ppm. Pomiary uzasadniły użyteczność tej metody wykrywania NO w roztworach o stężeniu $OH^-$ mniejszym niż $10^{-2}$ mol $dm^{-3}$, ponieważ przy większych stężeniach wartości SEM przekraczały zakres pomiarowy elektrody. Występowanie jonów azotanowych tworzonych w reakcji dysproporcjonowania było dodatkowo potwierdzane, w zależności od stężenia $OH^-$, za pomocą widm w bliskim UV i widm fluorescencyjnych. Wykazano także, na przykładzie układów Co(II)-dipeptyd-$OH^-$, że kalibrowana jonoselektywna elektroda na jony azotanowe ($NO_2^-$) może być użytecznym narzędziem w badaniu odwracalnego wiązania NO przez chelaty Co(II) w roztworze wodnym, reakcji symulującej szkodliwe działanie tlenku azotu(II) na hemoglobinę.



**Słowa kluczowe:** tlenek azotu(II), dysproporcjonowanie NO, elektroda jonoselektywna $NO_2^-$, spektrofotometria UV, spektrofluorymetria, szkodliwe wiązanie NO