Ultra-sensitive detection of nitric oxide isotopologues

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Abstract
This paper reviews recent technical advances of resonance enhanced multiphoton ionization (REMPI) spectroscopy and laser-induced fluorescence (LIF) spectroscopy, both extremely sensitive techniques for the determination of low nitric oxide (NO) concentrations. Because NO is involved in a multitude of biological functions, labeling and isotope-specific detection are important tools for elucidating chemical pathways. Mass selective REMPI discovers spectroscopic windows for monitoring the most abundant isotopologues such as $^{14}\text{N}^{16}\text{O}$, $^{15}\text{N}^{16}\text{O}$ and $^{14}\text{N}^{18}\text{O}$ without these species interfering spectroscopically with each other. This has been realized by using the $A^2\Sigma^+(v' = 0) \leftarrow X^2\Pi_2(v'' = 0)$ transition (NO $\gamma$-band) in the ultraviolet region around 226 nm. Selected rovibrational transitions are employed for the ultra-sensitive and isotope-specific detection of exhaled NO by LIF. The detection limit for $^{14}\text{N}^{18}\text{O}$ has been improved to 0.8 parts per trillion.

Furthermore, preliminary results from a study of the $A^2\Sigma^+(v' = 1) \leftarrow X^2\Pi_2(v'' = 0)$ transition interfering with the $B^2\Pi(v' = 1) \leftarrow X^2\Pi_2(v'' = 0)$ transition are presented as an alternative approach for isotopologue selective measurements of $^{14}\text{N}^{18}\text{O}$. From experimental data the rotational constant $B = 1.10310 \text{cm}^{-1}$ and the spin–orbit coupling constant $A = 33.170 \text{cm}^{-1}$ of $^{14}\text{N}^{16}\text{O}$ ($B^2\Pi(v' = 1)$) have been calculated.

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1. Introduction
The Nobel Prize for medicine and physiology in 1998 was awarded to Furchgott [1]. Ignarro and Murad, who identified nitric oxide (NO) as a key player in mammalian blood pressure regulation [2]. As is known today, NO is involved in a multitude of physiological processes such as immune defense and brain memory [3]. The average lifetime of NO is very low under physiological conditions. Due to the role of NO in the immunological defense of the human body, the molecule is an important marker for monitoring inflammatory diseases [4] of the airways, e.g. asthma [5]. Common clinical devices achieve detection limits of about 1 part per billion (ppb), which is sufficient for exhaled NO concentrations in the range of 10–100 ppb. The main disadvantages of such devices are the relatively low temporal resolution of a few seconds and the incapability of differentiation between several NO isotopologues, which are both important aspects in medical studies concerning the metabolism behavior and reactive pathways of NO inside the body. Both aspects are significantly improved by the application of laser-induced fluorescence (LIF) [6, 7] spectroscopy as shown later on. Besides airway-originated NO a small amount is emitted by the skin, which has also been studied extensively [8]. During the last years it has turned out that NO is also involved in plant signaling [9], e.g. as a transmitter during biological and abiological stress.

The global impact of NO as part of the NOx cycle is of significant importance. NO is involved in ozone depletion in the stratosphere as well as ozone formation in the lower atmosphere of urban regions. Main sources of NO production [10] are fossil fuel combustion, combustion of biomass and emission from soils and plants. The overall output of NO is estimated to be around 40 MT a$^{-1}$. All mentioned fields of research imply extremely low local NO concentrations. Therefore, a need for very sensitive and isotopologue selective detection methods rises. The most abundant isotopic compounds of NO are $^{14}\text{N}^{16}\text{O}$, $^{15}\text{N}^{16}\text{O}$ and $^{14}\text{N}^{18}\text{O}$.

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14N18O with fractions due to the natural isotopic distribution of 99.394, 0.363 and 0.204%, respectively [11].

Many methods have been recently employed for the detection of NO. In chemiluminescence measurements NO is converted to excited NO2, e.g. by ozone [12, 13]. The quantity of fluorescence from NO2 can be detected and related to a NO calibration procedure. This technique is mostly used in commercially distributed devices and is selective for NO with detection limits about 10 ppb to 1 ppb according to the average time. The method is not isotopologue specific. Cavity ring down spectroscopy (CRDS) is a classical absorption technique that refers to the Lambert–Beers law [14, 15]. This fast method is essentially free of calibration procedures and can be used for many different molecules. CRDS for the detection of NO is mostly done by application of infrared light which can be a problem concerning interference with other trace gases, e.g. water in exhaled air analysis. The detection limit is around 10 ppb. In laser magnetic resonance (LMR) experiments a magnetic field is applied to the sample [16, 17]. Different isotopologues can be detected with a detection limit in the ppb region. Furthermore, photoacoustic spectroscopy [18], electron paramagnetic resonance (EPR) [19] and different mass spectroscopic methods [20, 21] can be used for NO detection. We applied a combination of resonance enhanced multi photon ionization (REMPI) spectroscopy with a time-of-flight (TOF) mass spectrometer (MS) and LIF spectroscopy for the selective detection of different NO isotopologues down to concentrations in the sub-ppt region with a maximum time resolution of 20 ms.

Numerous studies have been carried out on the spectroscopy of NO, especially of the γ-band system [22–26]. Nevertheless, spectroscopic constants for the excited A2Σ+(v′′ = 2) state of the 14N18O isotopologue can hardly be found in the literature. The selective detection of NO via the A2Σ+(v′′ = 1) state is very important and will be discussed later. This state is interfered by the B2Π(v′′ = 1) state, which is not well studied due to its interference with A2Σ+ (v′′ = 1) [27–29]. Application of LIF spectroscopy and REMPI can help for a better understanding of these transitions. Comparison and fitting of calculated spectra to the measured ones can provide exact calculations of spectroscopic constants. Hence, these techniques can enrich both the highly sensitive isotopologue selective detection of NO in various biological environments as well as classical spectroscopic studies.

2. Detection procedure

The REMPI setup consists of a XeCl Excimer (Lambda Physik LPX 600) pumped dye laser (Lambda Physik FL 2002, operated with Coumarin 47) with a β-barium-borate crystal for second harmonic generation. Separation of the second harmonic is achieved by a setup of four Pellin-Broca prisms which finally generates 600 µJ pulses around 226.6 nm at 10 Hz. A schematic overview is given in figure 1. For the (0+1) REMPI measurement we excite the A2Σ+(v′ = 0) → X2Π1 (v″ = 0) around 226.6 nm as a resonant intermediate and then add another 226.6 nm photon for ionization of NO. Due to a frequency change of the dye laser NO isotopologues can be ionized selectively. The ionization takes place in a homebuilt stainless-steel cube with an integrated single-field TOF spectrometer. The TOF device has a total length of 0.57 m with a ratio of the acceleration region to the drift region of 1 : 2. The ionized NO+ is accelerated in an electric field and detected with a multi-channel plate (MCP) in chevron arrangement. The combination of a 40 m3 h−1 oil diffusion pump and a 150 L s−1 turbo molecular pump evacuates the spectrometer to a base pressure of 10−6 mbar. A detailed description of the setup is given elsewhere [30].

Due to different flight-times of the three main isotopologues of NO (15N218O, 16N18O and 14N18O with the masses 30, 31 and 32 u) a differentiation in the MCP signal is possible. The MCP signal is gated and processed with three boxcars, each on a single NO isotopologue. The output signals are recorded by a LabViewTM program. This setup has been applied to reveal spectroscopic windows for the detection of single NO isotopologues. As soon as these windows could be found, an LIF device has been used for a further observation because of its higher flexibility for the application of dynamic samples as well as a higher sensitivity for NO detection.

For the isotopologue selective detection of NO, we apply the well-known γ-band system. NO is excited by A2Σ+(v′ = 0) → X2Π1 (v″ = 0) around 226.6 nm in the ultraviolet (UV), and subsequent detection of fluorescence from A2Σ+(v′ = 0) → X2Π1 (v″ ≥ 2) is recorded. Alternatively, the A2Σ+(v′ = 1) → X2Π1 (v″ = 0) transition around 215.0 nm interfering with the B2Π(v′ = 1) ← X2Π1 (v″ = 0) transition is investigated for isotopologue selective detection of NO. Due to the higher vibrational state of 2Σ+(v′ = 1), the band origins of the different NO isotopologues are more shifted against each other and therefore more suitable for selective observations.

The applied laser system for LIF experiments is identical to the previously described one for REMPI measurements. A schematic overview is given in figure 1. The output beam is directed through CaF windows in Brewster angle arrangement to the measuring cell and furthermore through a comparable reference cell. Both cells benefit from a Woods horn on the entrance and exit side as well as from two pinholes for better adjustment and reduction of scattered light. Fluorescence is collected from the excitation region by a 1/f lens system perpendicular to the excitation beam. Furthermore, an Ag-covered spherical mirror with focus inside the fluorescence region reflects fluorescence to the lens system. The parallelized fluorescence is spectrally filtered by a bandpass filter (Andover Corporation 248FS10–50, transmission of 0.166 at central wavelength 250.964 nm, full-width at half-maximum (FWHM) is 10.97 nm) to essentially collect fluorescence from A2Σ+(v′ = 0) → X2Π1 (v″ = 0 → 3) and amplified by a solar blind side-on photomultiplier (PM) (Hamamatsu R7154). Alternatively, a longpass filter (Glen Spectra, 238AELP) with a cut-on wavelength of 237.5 nm and a maximum transmission of about 0.9 has been applied. The PM signal with an input resistance of 50 Ω is gated by a standard boxcar arrangement (SR 250) controlled by a LabViewTM (National Instruments Corporation) program. The boxcar as well as the excimer laser is triggered by a delay generator (Quantum Composers 9514).
The mass flux system of the measuring cell is optimized for the application of variable gas flows and application of the standard addition method (SAM) for calculation of unknown NO concentrations. The mass flux controller 1 (MFC 1) (MKS Instruments 1179A) can be operated with a 2.5 ppm mixture of NO and nitrogen between 0.1 and 10 standard cubic centimeters per minute (sccm min\(^{-1}\)). MFC 2 ranges from 50 to 5000 sccm min\(^{-1}\) for variable samples such as breath measurements. The vacuum system consists of a piston pump (Pfeiffer Vakuum XtraDry) in combination with a digitally controlled (MKS Instruments 250E) automatic valve (MKS Instruments 248A). For higher mass fluxes a bypass has been installed. The optimum cell pressure for fluorescence measurements is around 10 mbar. A more detailed description of the experimental setup can be found elsewhere [31].

For the determination of absolute NO concentrations two methods are applied. In principal, the amount of detectable fluorescence is proportional to the NO concentration of the unknown sample. The fluorescence signal can be evaluated by the more or less conventional application of calibration curves or the standard addition method (SAM). The main problem with calibration curves is the effect of quenching due to ambient air and trace gases [31]. This disadvantage is avoided by the SAM but large amounts of the unknown sample are required. During the SAM the signal from the unknown sample is observed continuously while different NO concentrations of a known sample are added. From this procedure it is possible to calculate very accurately the NO concentration of the unknown sample.

3. Results and discussion

The mass selective REMPI measurements have been performed to find spectroscopic windows for the interference-free detection of the most abundant NO isotopologues. We were mainly interested in \(^{14}\)N\(^{16}\)O because no spectroscopic constants could be found in the literature for the excited A-state. Accordingly, an accurate calculation of line positions for this isotopologue is not possible. The MCP signal has been gated with three boxcars each on a single mass of the main isotopologues \(^{14}\)N\(^{16}\)O, \(^{15}\)N\(^{16}\)O and \(^{14}\)N\(^{18}\)O with 30, 31 and 32 u, respectively. Due to extremely strong signals from \(^{14}\)N\(^{16}\)O (because of its high concentration in comparison to the minor isotopologues), the signal also appears on the mass-31-boxcar and the mass-32-boxcar signal. \(^{15}\)N\(^{16}\)O and \(^{14}\)N\(^{18}\)O can only be observed without strong \(^{14}\)N\(^{16}\)O transitions. For the \(A^3\Sigma^+(v' = 0) \rightarrow X^2\Pi_\Omega(v'' = 0)\) transition the region from 224 to 229 nm has been recorded by wavelength variation of the dye laser. A section of these measurements is displayed in figure 2. It could be shown that in certain areas of the spectra \(^{15}\)N\(^{16}\)O as well as \(^{14}\)N\(^{18}\)O can be observed without interference with the main compound \(^{14}\)N\(^{16}\)O [30]. Simulations for all three isotopologues have been performed with the program pgopher [32]. The results of fitting procedures are in excellent agreement with measured spectra.

By application of the LIF method selected interference-free sections of the REMPI spectra have been observed in more detail. The spectral resolution of the dye laser output has been determined by a Fabry–Perot interferometer to be 7 GHz. Cross sensitivities during the
Figure 2. REMPI spectrum between 226.45 and 226.50 nm from \(A^2\Sigma^+(v' = 0) \leftarrow X^2\Pi_O(v'' = 0)\) of NO (solid line = \(^{15}\)N\(^{18}\)O; dotted line = \(^{18}\)N\(^{16}\)O; dashed line = \(^{15}\)N\(^{17}\)O).

Figure 3. Dilution series for \(^{15}\)N\(^{18}\)O with Ar. The straight line is a fit taking into account the errors of both coordinates. The dotted lines are the upper and lower 95\% confidence levels for the fit.

Further studies evaluated the suitability of the \(A^2\Sigma^+(v' = 1) \leftarrow X^2\Pi_O(v'' = 0)\) transition around 215.0 nm for the application on isotopologue selective detection of NO. Again, REMPI measurements provided spectroscopic windows for interference-free detection of the minor isotopologues. We introduce here the red end of the \(AX(v' = 1)\) transition. In this region between 215.42 and 215.51 nm, \(^{15}\)N\(^{18}\)O can be observed without interference. Therefore, this spectral region is extremely suitable for an isotopologue selective detection. The previously described LIF setup has been used for these experiments with Coumarin 120 as dye. The measurements have been performed with 2.5 ppm NO in \(N_2\) at cell pressures of 10 mbar. Figure 4 shows a measured spectrum of this region (positive values). The upside down solid black line is a simulation for \(^{14}\)N\(^{18}\)O \(AX(v' = 1)\) of the \(^6\)P\(_{1/2}(J'')\) branch. This region is interfered by the extremely weak measurement can be excluded as discussed in [31]. A dilution series has been recorded to estimate the detection limit for NO detection. A mixture of 2.5 ppm NO in \(N_2\) has been diluted stepwise with Ar until the detected fluorescence approached the noise level of pure Ar (value for 0 ppt). Constant excitation of the \(^P\)Q\(_{12}(2.5) + ^P\)P\(_{22}(2.5) + ^P\)Q\(_{12}(3.5) + ^P\)P\(_{22}(3.5)\) transitions of \(^{14}\)N\(^{18}\)O and subsequent collection of radiated fluorescence resulted in the averaged values of figure 3. The straight line is a fit of the recorded data. Each point has been averaged over 400 laser shots. The fit has been performed with a Matlab program for straight line data with errors in both coordinates. The main causes for uncertainties of the concentration are the accuracy of the applied MFCs as well as the uncertainty of the 2.5 ppm NO mixture. Calculation of \(^{15}\)N\(^{18}\)O concentrations has been carried out under the assumption of an isotopologue distribution from the 2.5 ppm mixture due to the natural isotopic distribution. This assumption has been supported by strength comparisons of the same transitions from different isotopologues. The dotted lines are the upper and lower 95\% confidence levels for the fit. Corresponding to a signal-to-noise ratio of 2, the detection limit is at about 0.8 ppt. Due to the application of boxcars, a single dilution series can just cover a certain range of concentrations. In principle, the behavior of data points is linear to about 100 ppb. For higher concentrations self-quenching of NO has to be taken into account. Furthermore, the \(^{14}\)N\(^{18}\)O transition has been used to monitor the output of the isotopologue from exhaled air in an online measurement [30]. Comparable procedures have been performed for \(^{15}\)N\(^{16}\)O [7, 31, 33]. Hence, application of LIF is an excellent tool for highly sensitive isotopologue selective detection of NO. \(^{15}\)N-labeled drugs or amino acids, e.g. l-arginine which is metabolized to \(^{15}\)N\(^{16}\)O, can be used to reveal reaction pathways and temporal conversion in biological systems [6]. This application has been enriched by providing a tool for the selective observation of \(^{14}\)N\(^{18}\)O as a metabolite from \(^{18}\)O-labeled agents.
$B^2\Pi(v' = 1) \leftrightarrow X^2\Pi_O(v'' = 0)$ transition whose simulation is displayed upside down as dotted lines. Very precise fittings concerning the estimation of spectroscopic constants for the different isotopologues can be performed in this region for the $AX(1, 0)$ of $^{14}\text{N}^{18}\text{O}$ [30] and the $BX(1, 0)$ of $^{14}\text{N}^{16}\text{O}$. The fitting procedure provided for the excited $B^2\Pi(v' = 1)$ state of $^{14}\text{N}^{16}\text{O}$ a rotational constant $B = 1.10310 \text{ cm}^{-1}$ and a spin–orbit coupling constant $A = 33.170 \text{ cm}^{-1}$. Concerning an isotopologue-specific detection of $^{14}\text{N}^{18}\text{O}$, the $^{16}\text{O}$ and $^{18}\text{O}$ transitions are most suitable.

4. Conclusions and perspectives

(1+1) REMPI has been applied to reveal spectroscopic windows from $A^2\Sigma^+(v' = 0) \leftrightarrow X^2\Pi_O(v'' = 0)$ for the isotopologue selective detection of NO. These regions have been studied in more detail by the application of an LIF experiment. As shown, it is possible to detect $^{14}\text{N}^{16}\text{O}$, $^{15}\text{N}^{16}\text{O}$ and $^{14}\text{N}^{18}\text{O}$ separately without interference with each other down to concentrations in the sub-ppt region. The detection limit for $^{14}\text{N}^{18}\text{O}$ has been improved to 0.8 ppt. This technique can be used for label studies with drugs or amino acids metabolizing NO to reveal reactive pathways in biological systems as well as its temporal evolution. An application is the non-invasive detection of NO in exhaled air.

It has been shown that specific detection of NO is also possible in the 215 nm excitation region in addition to the conventional 226 nm region by application of the $A^2\Sigma^+(v' = 1) \leftrightarrow X^2\Pi_O(v'' = 0)$ transition for LIF. The higher vibrational state gives rise to a better separation of the isotopologue band heads, which results in a bigger shift of the spectra at the red end of the spectrum compared to $AX(0, 0)$. Furthermore, precise LIF measurements can provide exact spectroscopic constants for the calculation of rovibronic states from $^{14}\text{N}^{16}\text{O}$, $^{15}\text{N}^{16}\text{O}$ and $^{14}\text{N}^{18}\text{O}$. The 215 nm region is interfered by the weak $B^2\Pi(v' = 1) \leftrightarrow X^2\Pi_O(v'' = 0)$ transition of $^{14}\text{N}^{16}\text{O}$. Calculations from measured transitions provided a rotational constant $B = 1.10310 \text{ cm}^{-1}$ and a spin–orbit coupling constant $A = 33.170 \text{ cm}^{-1}$ for the excited $B^2\Pi(v' = 1)$ state of $^{14}\text{N}^{16}\text{O}$. In the future we will apply the REMPI setup in combination with a molecular beam to observe cold NO with the aim of separated $AX(1, 0)$ and $BX(1, 0)$ states for characterization of the latter and calculation of the band head.

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