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Sensitive optical NO sensor based on bis [(2,9-dimethyl-1,10-phenanthroline)] copper(II) complex

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Abstract

The reaction of $Cu(dmp)_2{}^{2+}$ with NO has been investigated for potential development into an optical fibre chemical sensor. In aqueous solutions, a linear calibration ($r = 0.9950$) was obtained between 0 and 2.5 μM NO with a detection limit of 0.32 μM (∼0.0096 ppm) (s/n = 3). The complex electrostatically immobilised in nafion produced a highly linear calibration ($r = 0.999$) between 0 and 3 ppm in NO gas phase with a detection limit of 0.072 ppm (∼2.38 μM in solution). The response was shown to be fully reversible by immersing the films in hydroxylamine hydrochloride solutions and a steady state response was achieved within 100 s exposure time. The nafion films were also shown to be more selective to NO over O_2 (183:1), NO_2 (16:1) and CO (795:1). In solution nitrite, H_2O_2 and peroxynitrite anion did not interfere.

Keywords: Nitric oxide detection; Absorbance; Nafion; Nitric oxide sensor

1. Introduction

Since the identification of nitric oxide as a biological signalling molecule, the realisation that NO is involved in the regulation of blood vessels, communication in the brain and in immunological defence against invading organisms has emerged [1].

NO is synthesised from L-arginine and this reaction is catalysed by nitric oxide synthase (NOS) [2,3]. Three distinct isoforms of NO exist, two of which are classed as constitutive NOS (cNOS), including endothelial NOS (eNOS or NOS-III) and neuronal NOS (nNOS or NOS-I). A third NOS species is called inducible NOS (iNOS or NOS-II) [4–6]. The most important distinction is that cNOS produces small amounts of NO for short periods of time and iNOS catalyses higher concentrations of NO for longer.

The realisation of the importance of the diverse roles endogenous NO can play led to the development of a number of techniques to monitor its concentration. This paper is concerned with the development of an optical chemical sensor for monitoring NO concentration that can be applied to biological monitoring. Optical fibre chemical sensors have several advantages over other conventional analytical sensors such as electrodes [7,8]. They are not subject to electrical interference and are safer than electrochemical sensors, no reference electrode is required and they can be miniaturised for

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Abbreviations: ABTS, 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid); Au, absorbance units; CCD, charge coupled detector; cNOS, constitutive nitric oxide synthase; DAF, diaminofluorescein; DAN, diaminonaphthalene; DAR, diaminorhodamine; DEANO, diethylamine NONOate; Dmp, 2,9-dimethyl-1,10-phenanthroline; DMSO, dimethyl sulfoxide; ECR, eriochrome cyanine R; EDTA, ethylenediaminetetraacetic acid; eNOS, endothelial nitric oxide synthases; EPR, electron paramagnetic resonance spectroscopy; H_2O_2, hydrogen peroxide; HHCL, hydroxylamine hydrochloride; iNOS, inducible nitric oxide synthase; KO_2, potassium superoxide; $OH^\bullet$, hydroxyl radical; MLCT, metal to ligand charge transfer; MnO_2, manganese (IV) oxide; NEDD, *N*-(1-naphthyl) ethylenediamine hydrochloride; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NO^-, nitroxyl anion; NOS, nitric oxide synthase; $O_2{}^-$, superoxide anion; $ONOO^-$, peroxynitrite anion; R.S.D., relative standard deviation; S.D., standard deviation; sGC, soluble guanylate cyclase; SOD, superoxide dismutase; SULF, sulfanilamide; TMD-ABODIPY, 1,3,5,7-tetramethyl-8-(3′,4′-diaminophenyl)-difluoroboradiaza-*s*-indacene

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in vivo use. Other analytical techniques used for detection of NO include electrochemistry [7,8], chromatography [9] and electron paramagnetic resonance spectroscopy (EPR) [10]. Electrochemistry is prone to interference from other biological components. The major drawback with chromatography is the expense and size associated with chromatographic instrumentation. EPR is a sensitive and specific technique for NO detection from a purely analytical point of view but many of the required reagents are expensive or not commercially available, and bulky apparatus is required.

In the development of an optical chemical sensor, a reagent phase is primarily identified then applied to nitric oxide detection in solution and this is followed by immobilising the reagent in an appropriate polymeric support. A number of reagents are available for the detection of NO in biological solutions. Three of the most common colorimetric methods include the Griess assay [11,12] involving the nitrosation of the griess reagent [sulfanilamide (SULF) and *N*-(1-naphthyl) ethylenediamine hydrochloride (NEDD)], the conversion of ferrocyanide to ferricyanide [11] and the ABTS (2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid)) assay [11,13]. All three methods can simultaneously measure NO and nitrite, which can lead to over-estimation of NO concentration. The oxyhemoglobin is another well-established method for NO detection [14,15]. The major drawback of the oxyhemoglobin assay is that oxyhemoglobin is not commercially available and the preparation of oxyhemoglobin involves oxygenation of the dithionite reduced heme protein followed by de-salting with a separation column. Chemiluminescence has shown exquisite sensitivity and selectivity for NO detection [16,17]. The most widely used fluorescence probes for NO detection include diaminonaphthalene (DAN), diaminofluoresceins (DAFs) and diaminorhodamines (DARs) [18]. All three fluorescent techniques are more sensitive than the colorimetric assays available but also have their disadvantages. DAN is poorly soluble in solution and employs UV excitation (375 nm), which can cause autofluorescence and serious damage to living cells [19]. In the search for fluorescence probes, which employ longer excitation wavelengths, DAFs and DARs were synthesised. DAFs are prone to photobleaching and instability around neutral pH [20] and although DARs are virtually unaffected by photobleaching, they still exhibit instability at neutral pH [21]. However, DAFs and DARs both exhibited low detection limits for NO of 3 and 7 nM, respectively. More recently, 1,3,5,7-tetramethyl-8-(3′,4′-diaminophenyl)-difluoroboradiaza-*s*-indacene (TMDABODIPY) was synthesised to overcome these problems and was shown to be photostable and pH independent over a wide range [22]. However, on approaching physiological temperatures, TMDABODIPY was quickly protonated, which interfered with its response to NO.

The sol–gel encapsulation of cytochrome *c* in the development of an optical fibre sensor for NO gas resulted in calibration curves with a linear range of 1–25 ppm with a detection limit of 1 ppm [23]. Due to interference caused by NO_2, cytochrome *c*′, extracted and purified from the bacterium *paracoccus denitrificans*, was also encapsulated in sol–gel matrices and was shown to be more selective to NO over NO_2 due to steric hindrance, which excluded NO_2 from the heme binding site [24]. A working range of 10–400 μmol l^{-1} NO was achieved. An NO selective optical ratiometric sensor was constructed using a fluorescein dye derivative (4-carboxy-2′,7′-difluorofluorescein-succinimidyl ester) attached to colloidal gold [25]. The sensor had a detection limit of 20 μM NO. Fluorescein dye-labelled cytochrome *c*′ attached to colloidal gold [26] resulted in an increase in sensitivity to NO and an improved detection limit of 8 μM. Further investigations using the previous sensor design utilised the fluorescently labelled heme domain of soluble guanylate cyclase (sGC) [27]. The sensor response was measured between 0 and 0.7 mM NO concentrations with a detection limit of 1 μM. One drawback of this method is the heme domain of sGC is fragile and is only useful at room temperature for a period of several hours.

In the identification of a suitable reagent scheme for NO quantification, the biological redox Cu(I/II) couple is of interest [28]. The Cu(II)–eriochrome cyanine R (Cu(II)–ECR) complex has been shown to react quantitatively with NO in aqueous solutions and with NO gas when immobilised in a silicone rubber matrices [29]. The Cu(II) complex of 2,9-dimethyl-1,10-phenanthroline (dmp) ($Cu(dmp)_2^{2+}$) (Fig. 1) can be reduced by nitric oxide in solution [30,31] and the formation of the cuprous complex was observed at 454 nm in the optical absorption spectrum by its characteristic metal to ligand charge transfer (MLCT) band. The Cu(II) complex is also pH independent between pH 5 and 9 [32] making it effective for use at physiological pH (pH 7.4).

This paper presents the use of the Cu(II)–dmp complex in aqueous solutions under aerobic conditions for NO detection. This reagent was then used in the development of a sensitive optical chemical sensor for NO by the electrostatic immobilisation of the complex in nafion followed by investigation of its reaction with nitric oxide gas. Nafion is a perfluorosulfonate resin in which hydrophilic perfluorinated ether side chains terminate with sulfate groups, which are periodically attached to hydrophobic perfluoroethylene backbone molecules. The fluorocarbon backbone provides exceptional chemical and thermal stability while the sulfate groups are responsible for ion exchange [33]. The sulfonate groups are assumed to be completely dissociated thus creating a strongly acidic medium [34].

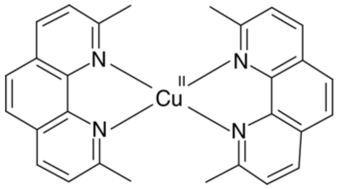

Fig. 1. Structure of the cupric phenanthroline complex.