Development of nitric oxide sensor for asthma attack prevention

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Available online 15 December 2005

Abstract

The aim of this work is the development of a NO sensor for asthma control and medication monitoring. The transducer is a Molecular Controlled Semiconductor Resistor (MOCSER), which is a GaAs based heterostructure. Protoporphyrins IX, containing carboxylic groups to chemisorb on GaAs, were used as sensing molecules. Characterization of the protoporphyrin monolayers was held using Attenuated Total Reflection in Multiple Internal Reflection (ATR/MIR), High Resolution Electron Energy Loss Spectroscopy (HREELS) in the vibrational and electronic domain and X-ray Photoelectron Spectroscopy (XPS). Degreasing and etching of the GaAs substrates were accomplished before adsorption. Interfacial bonding investigated by ATR/MIR shows that protoporphyrin adsorbs to the GaAs (100) through a unidentate complex and remains mostly vertically oriented. The electronic domain of the HREELS spectra exhibits the Q band with \( \alpha \) and \( \beta \) components on the same position as in the UV/Vis spectrum. Soret band is blue shifted showing a face to face stacking of the protoporphyrin molecules on the GaAs substrates. XPS spectra reveal the presence of Cobalt in monolayers prepared with \( 8 \times 10^{-5} \) M CoPP solutions. Kinetics is best fitted by an Elovich equation, showing some hindrance due to the previous adsorbed molecules. Thickness found from XPS data ranges from 1.3 to 1.5 nm, which fits with the molecular dimensions. Using the GaAs preparation methods developed here, an NO sensor prototype was assembled and tested for NO sensitivity and repeatability. Relative to NO, tests reveal a good sensitivity between 1.6 and 200 ppb. NO sensitivity was also measured towards CO, CO\(_2\) and O\(_2\). Pure nitrogen sweeps NO from the porphyrin layer, opening the possibility of the sensor reutilization.

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Keywords: ATR/MIR; HREELS; XPS; GaAs (100); Protoporphyrin; NO sensor

1. Introduction

The past decade has seen an explosion of studies related to nitric oxide (NO). Previously seen as a pollutant and graded as a Class A poison, it was realised that a mechanism existed for “in vivo” synthesis of NO. The discovery of biological functions related to NO arose from conjectures of Robert Furchgott in 1980 regarding a circulating signalling molecule, which he called endothelium-derived relaxation factor that caused vascular smooth muscle to relax [1]. Subsequent to Ferid Murad’s discovery that a gas was capable of regulating important cellular functions, Louis Ignarro confirmed that Furchgott’s endothelium-derived relaxation factor was, in fact, nitric monoxide NO. The biology of NO advanced rapidly to a point where, in 1992, NO was named “Molecule of the Year” by Science magazine [2]. The 1998 Nobel Prize in Physiology or Medicine was awarded to Robert Furchgott, Louis Ignarro and Ferid Murad for their outstanding discoveries concerning NO as a signalling molecule in the cardiovascular system [3]. It is well-known today that NO acts in the human body as a signalling molecule of key importance in the nervous system as a weapon against infections, as a regulator of blood pressure and as a gatekeeper of blood flow to different organs. It is also established that NO concentration in the exhaled air increases in inflammatory conditions of the airways. In particular, in asthmatic patients it was shown that NO concentration is proportional to the degree of infection: in the exhaled air one could measure 70–80 ppb for sick people and around 10 for
healthy people [4]. The study here presented is related to the
development of a specific pocket sensor to detect the
imminence of an asthma attack and to dose the medication in
function of the needs. This sensor should be sensitive, NO
selective, reusable, low-cost maintenance and cheap.

The construction of this sensor is based on a new device,
the Molecular Controlled Semiconductor Resistor (MOC-
SER), a GaAs based resistor [5]. The carrier current is
controlled by the recognition of the NO by a molecular layer.
Due to the high carrier mobility, gallium arsenide (GaAs) is
obviously a promising material for building fast response
electronic devices for chemical sensing. MOCSEs can be
used as a new type of sensor, when coated with specially
synthesized bifunctional organic molecules chemically bound
to the surface of the device and containing molecular
recognition functions. For this purpose, a GaAs based
heterostructure is modified, in a way that carriers are confined
in a depth less than 50 nm from the surface to improve the
sensitivity of the device. The interaction of the recognising
molecules with the analyte varies the carrier current in the
semiconductor. Any change in the charge distribution in the
vicinity of the surface, as for instance the interaction of NO
with porphyrin, implies a variation on the carrier current.
Also, the thickness of the oxide layer as well as eventual
contaminations must be reduced. Several wet-etching proce-
dures were compared leading to a HF etching procedure
selected as the most efficient method of the surface
preparation [6].

As functional materials, porphyrin and its derivatives
exhibit remarkable properties for applications in different
fields such as oxygen storage, electron transfer, redox
catalysis and gas sensing devices [7]. Two molecules were
used in this study: the ferriprotoporphyrin IX chloride (hemin)
and the cobaltic protoporphyrin IX chloride (CoPP). Proto-
porphyrins IX have three kinds of substituents: two lateral
propionic groups, two vinyl groups and four methyl groups.
The acidic groups enable their chemical adsorption on the
residual oxide layer remaining after etching on the GaAs
(100) surfaces.

The nature of the interfacial bonds as well as the fractional
coverage of the porphyrin layer, crucial to stabilize the GaAs
surface and to ensure the properties of response of the sensor,
was studied. Propoporphyrins also possess side groups namely
propionic groups, which can be used to bind the porphyrin to
the GaAs substrate. The surface and the interface of the
porphyrin layer were analysed using several techniques having
different analysis probing depths: of the order of 10 nm for X-
ray Photoelectron Spectroscopy (XPS) and 1 nm for High
Resolution Electron Energy Loss Spectroscopy (HREELS) in
impact regime. The kinetics of adsorption was studied by
Fourier Transform Infrared Spectroscopy using Attenuated
Total Reflection in Multiple Internal Reflections (ATR/MIR)
with 1500 nm of depth analysis.

Characteristic parameters of the chemisorption were ana-
ysed. In fact, besides the adsorption time, several other
parameters can play an important role in defining the layer
adsorption efficiency as for instance the nature of the solvent or
even the fact of stirring or not the solution during adsorption.
This will be analysed elsewhere.

2. Experimental

2.1. Materials

GaAs wafers were undoped semi-insulating single crys-
tals with orientation (100). Acetone and ethanol (anhydric
and analytical grade) used for degreasing were purchased
from SDS and used without further purification. Hydro-
fluoric acid (40%) puriss. pro-analysis (p.a.) was delivered
by Sigma-Aldrich and diluted to 1% with deionized water
with a resistivity of 18.2 MΩ cm furnished by a Millipore
system. Dimethylformamide (DMF) was a Fluka HPLC
grade (less than 0.02% H2O). Ferric protoporphyrin IX
chloride (hemin) and cobaltic protoporphyrin IX chloride
(CoPP) were purchased from Sigma Aldrich and used as
received. Fourier Transform Infrared transmission spectra of
the free molecules of both porphyrins were recorded using
KBr pellets.

After degreasing in anhydric acetone and ethanol (5’each),
GaAs substrates were etched with a 1% solution of hydro-
fluoric acid following a procedure described in Ref. [6]. The
GaAs substrates, still containing a very thin oxide layer, were
immersed for 18 h in a 1 mM solution of CoPP in DMF.
Other samples were prepared with solutions containing slight
amounts of water. Adsorption was held with or without stirring
always in the dark. After adsorption, samples were rinsed in
pure DMF and then in acetonitrile and dried under an argon
flux. ATR/MIR samples were all gently sonicated.

2.1.1. ATR/MIR

The GaAs (100) are highly transparent to the infrared
radiation in the spectral range 650–4000 cm−1. Major faces
of the ATR/MIR elements, 800 nm thick, were cut in 40 × 15 mm
rectangles and optically polished in 45° bevelled edges in an
isosceles trapezoidal configuration. ATR/MIR spectra were
recorded using a FTIRS Magna-IR Nicolet 850 equipped with
a MCT detector. Spectral resolution is 4 cm−1. Kinetics
experiments were performed using a homemade Teflon liquid
cell provided with a hole for the introduction and the extraction
of the solution during the analysis. Spectra were automatically
recorded overnight at predetermined intervals. Background was
recorded immediately after the solution introduction. In ATR/
MIR, the transmitted infrared beam is reflected internally in the
GaAs element about 50 times providing submonolayer
sensitivity. In each total reflection, an evanescent wave probes
the layer with a characteristic penetration depth l depending on
the wavelength and the index of refraction of the molecular
layer and the substrate. In our experimental conditions, l is
roughly 1500 nm [8], much larger than the thickness of a
monomolecular layer.

2.1.2. XPS

The XPS spectrometer used is an XSAM800 (KRATOS)
operated in Fixed Analyzer Transmission (FAT) mode, with a
pass energy of 20 eV and the non-monochromatised MgKα and AlKα X-radiation (hν = 1253.7 and 1486.7 eV, respectively). A current of 10 mA and a voltage of 13 kV were used. Samples were analysed in an ultra-high-vacuum (UHV) chamber (∼10⁻⁷ Pa) at room temperature, using 0° and 60° analysis angles relative to the normal to the surface. Samples were transferred from the last rinsing solution into the fast introduction chamber under argon atmosphere and introduced under a pure nitrogen flux. Spectra were recorded by a Sun SPARC Station 4 with Vision software (KRATOS) using a step of 0.1 eV. A Shirley background was used for baseline subtraction and curve fitting for component peaks was carried out with Voigt profiles (Gaussian and Lorentzian product). No charge compensation (flood-gun) was used.

2.1.3. HREELS

Experiments were performed using two different spectrometers: a Leybold-Heraeus ELS-22 and a LK Technologies 2000R. Spectra were recorded under ∼10⁻⁸ Pa. Incident electron energy was 5 eV for all spectra. Electrons are here backscattered in broad lobes and impact and dipole interactions only can be distinguished through energy analysis. Samples were prepared using a fixed protocol and were carried to the UHV chamber under argon and introduced under a pure nitrogen flux. All spectra were recorded at room temperature.

3. Results and discussion

Fig. 1 displays the comparison between a FTIRS transmission spectrum of the hemin in a KBr pellet with the corresponding ATR/MIR spectrum of the hemin adsorbed on a GaAs (100) ATR element after 18 h interaction. The assignments of main features are summarized in Table 1. It is clear, from the comparison of both spectra, that most of the features of the spectrum of free molecules also exist in ATR/MIR spectrum.

One can also remark that some bands present significant shifts, specifically those related to the propionic groups, and more particularly to C–O (at 1428 cm⁻¹ in the free molecule and at 1368 cm⁻¹ after absorption) and C=O (1707 cm⁻¹ in the free molecule and 1662 cm⁻¹ after adsorption). This shift indicates that the atomic environment of these bonds is clearly modified after adsorption. On the other hand, it is well-known in literature that the difference between the frequencies ν(C=O) and ν(C–O) (here referred as Δ) correlates to the type of the complex formed during the molecular interaction, as indicated in Table 2. In the case of Ferriprotoporphyrin IX chloride (hemin) on GaAs (100) surfaces we found Δ = 294 cm⁻¹, which indicates a unidentate coordination. Moreover, one can see that in the ATR/MIR spectrum only a peak of much lower intensity at 1707 cm⁻¹ remains, showing that most carboxyl groups were bound to the substrate. A shift of the same order of magnitude of that of hemin also appears with adsorption of CoPP with a value of Δ = 282 cm⁻¹.

Table 1
Assignments of main features appearing in transmission infrared spectrum of the Ferriprotoporphyrin IX chloride [9–12]

<table>
<thead>
<tr>
<th>cm⁻¹</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>720</td>
<td>Pyrrolic cycle in-plane deformation.</td>
</tr>
<tr>
<td>838</td>
<td>Pyrrolic cycle in-plane deformation.</td>
</tr>
<tr>
<td>940</td>
<td>Porphyrin macrocycle in-plane deformation.</td>
</tr>
<tr>
<td>1120</td>
<td>C=C; C=N pyrrolic.</td>
</tr>
<tr>
<td>1240</td>
<td>Ligand deformation “breathing”</td>
</tr>
<tr>
<td>1368</td>
<td>CH3 deformation. sym</td>
</tr>
<tr>
<td>1428</td>
<td>CH3 deformation. asym</td>
</tr>
<tr>
<td>1550</td>
<td>C=C; C=N stretching</td>
</tr>
<tr>
<td>1707</td>
<td>C=O stretching</td>
</tr>
<tr>
<td>2916</td>
<td>CH3 stretching</td>
</tr>
<tr>
<td>3087</td>
<td>C–H vinyl stretching</td>
</tr>
</tbody>
</table>

Fig. 2. ATR/MIR spectra obtained during the interaction of a CoPP with a GaAs (100) element. First spectrum was recorded after 2 min interaction and the last one after 15 h. Background was recorded with the solvent (ACN containing 2% DMF) without CoPP.
The kinetics of adsorption of CoPP was followed by using a Teflon cell associated to the ATR element. Spectra recorded at different moments of the adsorption between 1200 and 2000 cm$^{-1}$ are presented in Fig. 2. The solution here was 1 mM in acetonitrile containing 2% (vol.) DMF. The comparison of the evolution of the intensity of peaks situated at 836 and 1670 cm$^{-1}$ presented in Fig. 3 clearly shows a very different behaviour.

The linear behaviour of 836 cm$^{-1}$ feature indicates an increasing accumulation of molecules towards the hydrophilic substrate of GaAs (100) covered by the thin layer of oxide. In contrast, C=O peak is characteristic of a molecular adsorption as it is shifted for lower wavenumbers. The fact that the intensity of this peak vs. time is well fitted by a Langmuir kinetics law until 165 min after the beginning of interaction. The other peak located at 836 cm$^{-1}$, corresponding to the in-plane deformation of the pyrrolic ring, follows a linear law.

accomplishment of adsorption of molecules associated by hydrogen bonding [17].

Another evidence of the chemical adsorption of the CoPP on the GaAs (100) is presented in Fig. 4 where a HREELS spectrum of CoPP (1 mM in DMF) adsorbed on GaAs (100) surface is compared with the FTIRS spectrum of the corresponding free molecules in KBr pellets. HREELS spectrum was recorded using incident electrons of 5 eV in impact geometry where the incidence angle is different from the one of analysis. One can remark in Fig. 4 that all peaks of the infrared spectrum have an equivalent structure in the HREELS spectrum excluding that of C=O bonds (1707 cm$^{-1}$ in the free molecule). This indicates that the C=O bond is not located at the extreme surface of the film but mostly buried near the substrate and therefore not detected by the electron beam. An analysis of the nature of the losses corresponding to the in-plane deformations of the CoPP privileges a dipolar interaction indicating that vibrations are excited perpendicularly to the surface. These experimental observations tend to confirm that molecules are mostly standing with the propionic groups chemically bound to the GaAs substrate.
The electronic domain of the HREELS spectrum was also investigated and is presented on Fig. 5. Different samples prepared with 1 mM solutions of CoPP in DMF (with and without water, sonicated and nonsonicated) were studied. The Q-band, observable in the UV–Vis spectrum, also appears in the same position in the HREELS spectra with the $a$ and the $b$ component around 2.19 and 2.33 eV, respectively. These bands are not influenced by a change in the stacking interaction. In contrast, Soret band appearing in the UV/Vis spectrum at 2.96 eV is blue shifted to 3.14 eV. The analysis of the normalized intensity as a function of the incident energy reveals that the first excitation has a dipolar character and thence corresponds to an optically allowed transition but the second one seems to be a mixture of several allowed and forbidden transitions. The shift of the Soret can be associated to a face-to-face stacking of porphyrin molecules on the substrate, which is consistent with the report that the Soret state exciton interactions are must larger than the Q state interactions, being proportional to the oscillator strength [18]. Consequently, the Soret band is far more sensitive to change from monomer to dimmer than is the Q band [19,20].

XPS of one of the samples prepared using a solvent mixture of 90% acetonitrile, 10% water and a concentration of $8 \times 10^{-5}$ M revealed the presence of the porphyrin undoubtedly attested by the Co spectral region. The other elements, which come exclusively from porphyrin, nitrogen and chlorine, are less easy to quantify since N 1s is superposed to an Auger structure (Ga L$_2$M$_4$M$_4$) when excited by Al X-ray and Cl 2p is in the tail of the As 3s peak. However, even the Co 2p region (the most intense cobalt photoelectron) overlaps with a Co Auger structure when excited with Al X-radiation and with an oxygen Auger structure when excited by Mg X-radiation. Therefore, the C1s peak may have some contamination contribution, the main calculations made for thickness layer estimations were made through the $C/(Ga + As)$ ratio. Other subsequent studies were performed using DMF as solvent. The adsorption isotherm for 18 h interaction with a solution in DMF shows that the thickness of the adsorbed layer reaches a plateau for a concentration of 1 mM. The kinetic study of 1 mM CoPP solutions in DMF on GaAs (100) is displayed in Fig. 6. The best fitting was achieved using the Elovich equation [21]:

$$\frac{d\theta}{dt} = kf(\theta) \exp\left(\frac{-E_0 - a\theta}{RT}\right) = b(1 - \theta) \exp\left(\frac{-a\theta}{E_i}\right)$$

$$t = -\frac{1}{b} \left[ E_i(-a + a\theta) - E_i(-a) \right]$$

where $\theta$ is the covered surface fraction, $b = k \exp(-E_0/RT)$, $E_0$ is the activation energy for adsorption, $E_i$ is the Exponential Integral Function, $t$ is the time of interaction, $R$ is the ideal gas constant and $T$ is the absolute temperature and $k$, $a$, $b$ are constants.

Values found for $a$ and $b$ were 16.3 and 20.578, respectively. Therefore, $a = 16.3RT$. It is interesting to notice...
that the logarithmic function also provides a good fitting. This is not astonishing as the logarithmic function is the solution of the Elovich equation when $f(\theta) = 1$:

$$\frac{d\theta}{dt} = k f(\theta) \exp\left(\frac{(-E_0 - a\theta)}{RT}\right) = b \exp\left(-\frac{a\theta}{RT}\right) \Rightarrow \theta = \ln(abt + 1)/a \quad (2)$$

$a$ and $b$ keeping the same order of magnitude: $a = 20.3$ and $b = 28876$. This means that the main barrier to the adsorption is caused by the presence of other molecules, and the increase they cause to the activation energy of adsorption, and not by the decrease of the number of available sites on the surface. This is compatible with an adsorption perpendicular to the surface. The thickness of the organic layer in all the samples ranges from 13 to 15 Å, which is compatible with the molecular dimensions of CoPP.

4. Application

Using the GaAs preparation obtained from the methods investigated above, an automated test system to detect nitric oxide using the MOCSER as the sensing device was built [22], including:

- Chip on a ceramic carrier with wiring;
- Printed circuit board;
- Mechanical holder allowing controlled gas flow and environment over chip;
- Gas flow and mixing system;
- Connection to electrical test equipment;
- Software to run test equipment and data acquisition.

The MOCSER was activated for NO detection with a monolayer of CoPP. A picture of a fully assembled and
activated device is shown in Fig. 7. On this chip there are about 20 different configurations of MOCSER devices.

The activated MOCSER was then tested for sensitivity, selectivity and repeatability. Fig. 8 shows the sensitivity of one particular device for NO concentration in N₂. It can be seen that level of 1.6 ppb NO is easily detected by the device.

Fig. 9 shows the selectivity of the MOCSER activated with CoPP. Selectivity was tested between NO and other major components of exhaled air, i.e., CO₂, O₂ and CO.

Finally, the repeatability of the sensor for pulses of NO in N₂ is shown in Fig. 10. This feature is critical for performing tests in real environment when the patient cannot exhale for a long time.

5. Conclusion

The study of surfaces and interfaces of layers of ferric and cobaltic porphyrins shows that a monomolecular layer can be created on GaAs (100) surfaces after etching with molecules chemically adsorbed stacking face-to-face and vertically oriented. GaAs samples are previously etched in a HF 1% solution and then immersed in a 1mM solution of protoporphyrin in DMF and then rinsed with the solvent. Kinetics followed by ATR/MIR in situ proofs the existence of a monolayer. On the other hand, the examination of HREELS spectra in the vibrational domain shows that molecules are vertically oriented. Besides, the electronic domain of HREELS spectra confirms that molecules are stacked face-to-face. Best fitting for kinetics obtained by XPS is the Elovich equation, which takes into account the volumic hindrance of the adsorbed molecules relatively to the coming ones.

Using these methods, a fully fabricated MOCSER was activated with a monolayer of CoPP. Detection limit as low as 1.6 ppb of NO was demonstrated, with NO relative sensitivity better than 10⁶ towards CO₂ and O₂, and 10⁴ towards CO.

Acknowledgments

This work is part of the Growth project no GRD2-2000-30012 SENTIMATS financed by EU. We also acknowledge the “Financiamento Plurianual” from FCT, Portugal and the project GRICES/Ambassade Française in Portugal.

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