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Cross-linked poly(2-hydroxyethylmethacrylate) films doped with 1,2-diaminoanthraquinone (DAQ) as efficient materials for the colorimetric sensing of nitric oxide and nitrite anion

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Abstract—A series of cross-linked poly(2-hydroxyethylmethacrylate) (polyHEMA) films doped with 1,2-diaminoanthraquinone (**DAQ**) are described. The synthesis of the **DAQ**-containing polymers does not require any previous derivatization of the diamine. The films can be used as solid-state colorimetric sensors for nitrite anion as well as for aqueous and gaseous nitric oxide. Polymers show no appreciable leaching of **DAQ** even after six months of storage in water.
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Nitric oxide (NO) is a gaseous free-radical playing important roles in environmental chemistry[1] and biomedicine.[2] Since the discovery that NO is produced endogenously by the body,[3] NO has been found to play many bio-regulatory functions, leading the research in such area to the award of the Nobel Prize in Physiology and Medicine in 1998 to Murad, Furchgott and Ignarro.[4–6] When bio-synthesized in the vascular endothelium, NO is crucial for the regulation of blood flow and pressure.[7] Synthesized by neurons it can act as a neurotransmitter.[8] Besides, NO is generated in large quantities by activated macrophages playing hence a defensive function for the immunological system.[9]

On the other hand, nitrite (NO_2^-) is known to be associated to gastric cancer since in acidic medium it can participate in the nitrosation of secondary amines ingested in food to yield carcinogenic nitrosamines.[10] Besides, nitrite is also linked to infantile methahemoglobinemia since nitrate in drinking water contaminated with bacteria can be reduced to nitrite, which then can oxidize Fe(III) in oxyhaemoglobin to Fe(II) (methahaemoglobin).[11]

According to the former considerations, the development of methods to monitor NO and NO_2^- in vivo and in vitro has become an area of intensive research and great effort has been taken from the synthetic viewpoint to develop sensors for those species.[12–19] A common strategy to detect NO and NO_2^- has been the use of aromatic *ortho*-diamines as reactive probes. Those compounds, in the presence of NO and NO_2^-, are converted into benzotriazoles (having different spectroscopic features). A common intermediate in this process is the nitrous anhydride (N_2O_3).[12] Thus, it is well known that in oxygenated aqueous solutions NO is rapidly converted into the reactive nitrosating species N_2O_3, and analogously, acidified nitrite has been employed as nitrosating medium for amines, provided the formation of N_2O_3 as well (Scheme 1).[20] Hence, many probes reported so far can be used either for sensing NO or NO_2^-. This is the case of 1,2-diaminoanthraquinone (**DAQ**)[13,14] and 2,3-diaminonaphthalene (**DAN**)[15] depicted in Chart 1. Also in Chart 1 a series of *ortho*-phenylenediamines sensitive to NO is shown, which could potentially be supported by the methodology described here (**DAF-FM**,[16] **DAR-4M**,[17] **DAMBO-P^{H}**[18]).

As a part of our research on dye-doped functional materials,[21] we were interested in the development of a solid-state colorimetric sensor for the *naked-eye* detection of nitric oxide and nitrite. Here we describe the

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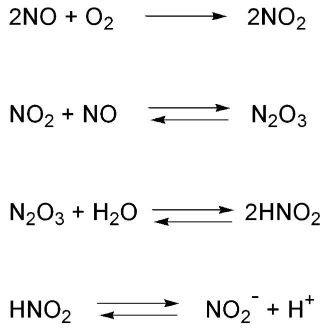
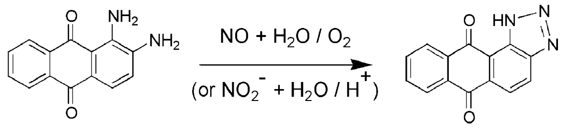


Scheme 1. Chemical equilibria in oxygenated water.

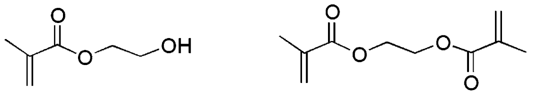
incorporation of a well-known solution sensor for both nitric oxide and nitrite, like **DAQ**, into a water permeable hydrogel film. To our knowledge, this is the first time that such a sensor has been efficiently supported in a polymer matrix for sensing purposes.[22] The main advantages of the synthesized polymers are as follows: (1) it is not necessary to synthesize a functional derivative of **DAQ** for the anchoring to the polymeric matrix, since the probe is just dissolved in the monomer/cross-linker solution and then polymerized; and (2) despite not being covalently linked to the organic matrix no leaching out of **DAQ** from the polymers takes place even for long periods of storage in water (up to six months tested).

The electronic spectrum of **DAQ** is characterized by the existence of a charge-transfer (CT) absorption band at 520 nm from the HOMO centered at one of the amino groups to the LUMO centered at the carbonyl moiety.[23] Reaction of the two *ortho* amino groups either with NO in aerated water or with NO_2^- under acidic conditions lead to the disappearance of such a band (due to the formation of a colourless triazole as described in the literature,[13,14] see Scheme 2). As a matter of fact, when solutions of **DAQ** in water were bubbled with gaseous NO complete decolouration occurred in 2–3 min.



DAQ

Scheme 2. Reaction between **DAQ** and NO or NO_2^-.



HEMA **EGDMA**

Chart 2. Hydrophilic monomer (HEMA) and hydrophobic cross-linker (EGDMA) used in the synthesis of the optically responsive films.

Table 1. Chemical description of films loaded with **DAQ**

Polymer[a]	**HEMA** (%)	**EGDMA** (%)	**DAQ** (mg/g)[b]	λ_{max} (nm)
A75	75	25	0.2	520
A90	90	10	0.2	520
B75	75	25	0.6	520
C75	75	25	1.5	520

[a] Polymerized at 85 °C with 1% AIBN as radical initiator.
[b] mg of **DAQ** per g of polymer.

Polymeric films loaded with **DAQ** were synthesized by dissolving a certain amount of sensor in a mixture of the hydrophilic monomer 2-hydroxy ethylmethacrylate (HEMA) and the hydrophobic cross-linker ethyleneglycoldimethacrylate (EGDMA; Chart 2). Radical polymerization with AIBN as thermal initiator yielded a series of films with different loadings of sensor and with quantitative yields (Table 1). The synthesized polymers have high transparency (non-doped films showed no absorbance above ca. 300 nm and straight baseline between ca. 300 and 800 nm), big size (ca. 2 × 3 cm), and good mechanical properties for the manipulation, since bending and cutting to the appropriate sizes and shapes was possible without any special instrumentation. Besides, very reproducible films can be made according to this procedure (mean thickness was 142 µm and std. dev. = ±0.006 mm from 36 measurements using six different films). FT-IR and FT-Raman analysis of the polymers showed complete polymerization by the absence of signals corresponding to olefinic vibrations.[24,25]

Films **A75** and **A90** were introduced into spectrophotometric cuvettes containing air-equilibrated water (pH 7.4, 10 mM HEPES, 0.1 M NaCl). Then 15 mL of NO (g) was bubbled into the sealed cells in order to obtain saturated solution of NO in water, corresponding to a concentration of 1.9×10^{-3} M (according to the literature[12]). Gradually, the colour of the films faded out, being the decolouration process faster in the **A90**

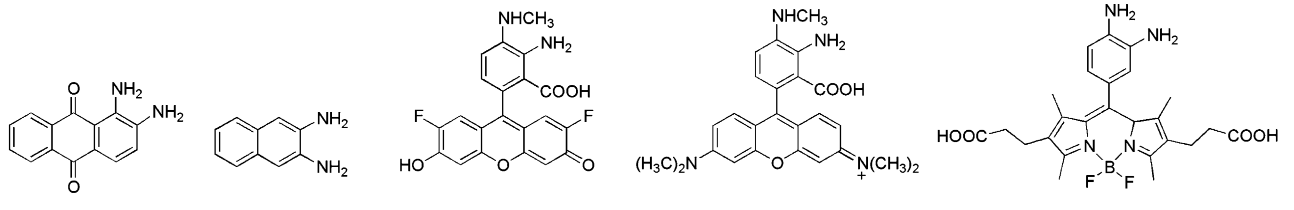


Chart 1. Some representative probes, described in the literature, based on the *ortho*-phenylenediamine moiety, including the sensor immobilized in the present work (**DAQ**).

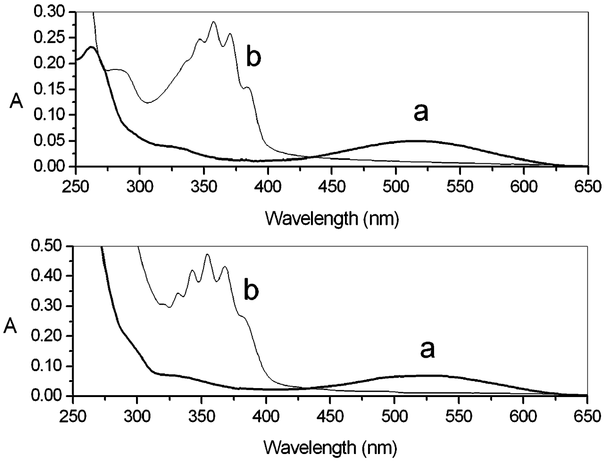


Figure 1. Absorption spectra before (a) and after (b) bubbling NO (gas). Top: free **DAQ** in aqueous buffer (pH 7.4; with 0.2% DMSO from concentrated stock in DMSO). Bottom: film **A75** immersed in aqueous buffer (pH 7.4).

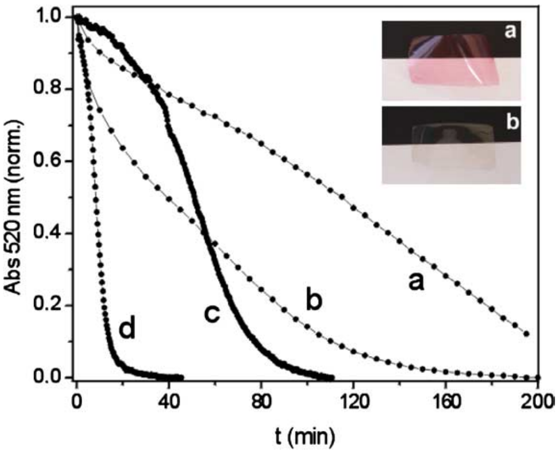


Figure 2. Evolution with time of the absorbance at 520 nm of the entrapped **DAQ** in different films exposed to aqueous and gaseous NO. (a) **A75** and NO gas; (b) **A90** and NO gas; (c) **A75** and NO aq; (d) **A90** and NO aq. All absorptions are normalized. Inset: pictures of a film before (a) and after (b) exposure to NO (gas).

film than in the **A75**. UV–vis spectra of films before and after reaction with NO can be compared to the spectra recorded using free **DAQ** (Fig. 1).

Differences are minimal between the absorption of the free and encapsulated probe, suggesting that **DAQ** is located in a polar environment inside the films, that is surrounded by the polymeric hydroxyl groups or by molecules of water. As it can be seen in Figure 1, the resulting product of reaction displays a fine vibronic structure in both the free and the entrapped formats, indicating that molecules in the cross-linked poly(HEMA) network display the same degree of mobility than in solution.

More interestingly, films were found to be sensitive not only to dissolved NO (in the presence of oxygen) but also *directly* to the NO gas. Thus, when films were placed in a pure NO gas atmosphere ($P = 1$ atm.) inside a sealed UV–vis cuvette, the same decolouration was noticed although at lower rates than in solution. For the sake of visual illustration, a series of pictures were taken before and after exposure to NO (g) (inset in Fig. 2).

Figure 2 shows the plots corresponding to the absorbance of the CT band versus time for **A75** and **A90** films. As it can be seen, curves a and b, corresponding to the NO (g) experiments, decay slower than c and d (solution measurements). It can also be seen that the films with higher percentage of hydrophilic monomer HEMA (**A90**, curves d and b) responded to NO faster than **A75** (curves a and c). Analogous behaviour of films **B75** and **C75** towards NO was found, although slower decolouration could be noticed for the films with higher concentration of **DAQ**.

The ability of this kind of films to signal the presence of NO is related to the hydrophilic nature of the films. It is well recognized that the reaction of NO with amines requires the concurrence of water and O_2.[26] Thus it seems that the ability of films **A75** and **A90** to retain water is crucial, specially to interpret the results with gaseous NO. As a matter of fact, when a hydrated sample (12 h soaked) of **A75** was dried in silica-gel during 12 h, a weight loss of 12% was measured. The same experiment afforded a decrease of 28% in the weight of the **A90** film. This higher *hydrophilicity* of **A90** is compatible with the faster response of this film if compared to the **A75** polymers. However, it must be noted that the water losses above mentioned represent upper limits for the water that each polymer is able to admit. Since films exposed to NO (g) were not previously soaked but equilibrated with the ambient conditions (23 °C and 70–80% of relative humidity) a microgravimetric determination was done in order to determine the water content at ambient conditions. The differences in weight between ambient-equilibrated and silica-dried films were measured, resulting in a weight loss of 0.1% for **A75** and a decrease of 6.6% for **A90**.

As anticipated, this kind of polymers were found to be sensitive not only to NO but also to NO_2^- in acidic solution. Several films of the **B75** series were tested. As it can be seen in Figure 3, the films are quite sensitive to nitrite, specially at the micromolar scale, where a linear correlation in the range 0–120 μM was found. It is worth mentioning that a linear fit has been previously described by Imperial et al.[14] for DAQ in solution at low pH as nitrite sensor (up to 50 μM).[27]

In relation with the solution experiments, one of the main drawbacks found with entrapped dyes in polymers or sol-gel derived materials is the eventual leaching of the entrapped molecules out of the insoluble matrices, during the first hours (or even minutes) of operation.[28] This phenomenon can severely limit the practical applications of such systems. Nevertheless, this problem is not present in **A75**, **A90**, **B75** or **C75**. Thus, films stored for six months in buffered water (pH 7.4) showed no detectable leaching of the dye, which is one of the main advantages of this kind of materials, opening the possi-

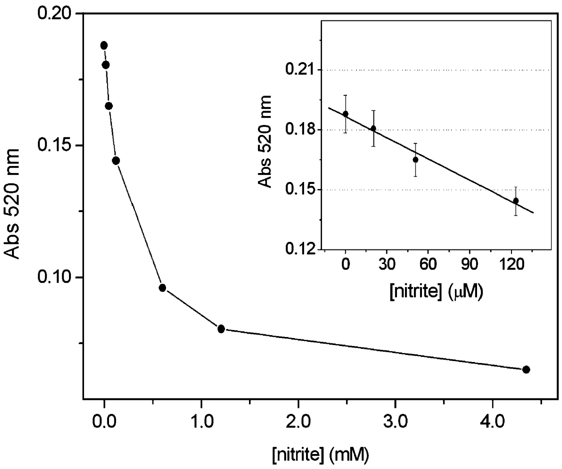


Figure 3. Optical response of films **B75** to sodium nitrite in water (1% AcOH, 30 min incubation time for each independent point).

bility of using this poly(HEMA) films for long-time accumulative measurements.[29] This absence of leaching could be attributed to the hydrophobicity of **DAQ**. In fact, one of the main problems associated with **DAQ** as a probe is its poor aqueous solubility.[13] In our case, this seems to be an advantage since it prevents the escape from the films.

In summary, a well-known organic probe like **DAQ** has been encapsulated into a poly(HEMA) matrix to obtain a solid-state colorimetric sensor for the detection of nitrite and nitric oxide (in the latter case for dissolved and gaseous forms). Future work will be done in order to expand this methodology to other sensors of interest.[30,31]

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