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Nitric oxide sensitive fluorescent polymeric hydrogels showing negligible interference by dehydroascorbic acid

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ABSTRACT

A series of nitric oxide (NO) sensitive polymeric hydrogels have been synthesized and their properties described. The new materials are based on a poly(2-hydroxyethyl methacrylate) matrix entrapping the DAF-FM (4-amino-5-methylamino-2′,7′-difluorofluorescein) fluorescent probe. Some of the developed materials show sensitivities in the range ca. 50–75 nM towards NO dissolved in aerated phosphate buffer (PBS), being nitrous anhydride the reactive species under such conditions. Remarkably, the sensitivity of the fluorescent polymers is not influenced by dehydroascorbic acid, a molecule of biological importance, which has been reported to be a common interfering species capable to react with well known probes for NO.

1. Introduction

Nitric oxide (NO) is a small diatomic molecule playing numerous biological [1] and environmental roles [2]. Regarding the importance of NO for Biological Chemistry it can be mentioned that it is a neurotransmitter [3], a relevant species for the Immune System [4] and also a signaling molecule triggering the muscular relaxation response [5]. In the context of Environmental Chemistry, NO is a pollutant originated from the combustion engines of vehicles and hence a major concern for public authorities [6]. Hence, a great number of reports dealing with the measurement of NO in both contexts have been published during the last years. Electrochemistry [7] and fluorescence [8] based methodologies are the most popular approaches to the analytical determination of NO concentrations. Regarding the fluorescence based technologies, a big pool of molecular fluorescent probes with excellent sensitivity towards NO have been reported [9–13]. The majority of molecular probes are typically used in solution based applications; however some situations, e.g. environmental monitoring, require NO-sensitive probes to be incorporated into a solid material, in order to integrate them into a measuring device [14]. In comparison to the large number of molecular probes for NO, a limited number of fluorogenic and chromogenic solid materials sensitive to this species have been reported [15]. In this regard, we have developed recently new NO-sensitive composite materials based on organogels [16] and polymeric films [17–19].

One of the most popular fluorescent NO probes is the diamino fluorescein derivative DAF-FM (4-amino-5-methylamino-2′,7′-difluorofluorescein, Chart 1) [20], which has excellent sensitivity towards NO in oxygenated aqueous medium [21]. The selectivity of some diaminofluorescein and diaminorhodamine based probes were investigated by Sweedler and co-workers [22] who observed that biologically relevant compounds, in particular dehydroascorbic acid (DHAA), reacted to yield a fluorescent product that could be falsely identified as the NO adduct.

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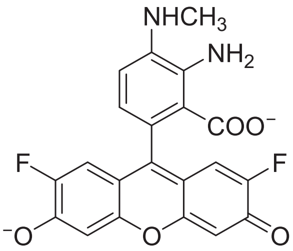

DAF-FM

Chart 1. Chemical structure of DAF-FM.

Therefore, the development of fluorescent NO sensors exhibiting low interference by DHAA, is highly desirable.

In this paper, we describe the encapsulation of DAF-FM into a series of polymeric hydrogels based on 2-hydroxyethylmethacrylate (HEMA) and the study of the spectroscopic and the analytical properties of the obtained materials. The new polymers are reactive to NO in oxygenated media (nitrosating nitrous anhydride formed in such conditions [1a]), displaying a fluorescent sensitivity analogous to the free probe in solution. Importantly, the new materials are insensitive to the potential interference of DHAA (up to 1 mM). Hence we have developed a novel and very effective strategy to avoid the interference by DHAA, which could be of potential interest for the development of more precise NO-sensitive devices.

2. Experimental section

2.1. Materials and methods

2-Hydroxyethylmethacrylate (HEMA), 3-(acrylamidepropyl)trimethylamonium chloride (AAPTEA$^+$), sodium 2-acrylamido-2methyl-1-propensulfonate (AAMPS$^-$), *N*-hydroxyethylacrylamide (HEAA), ethylenglycoldimethacrylate (EGDMA), polyethylenglycoldimethacrylate (PEGDMA), dehydroascorbic acid and sodium NONOate (sodium diethylammonium (Z)-1-(N,N-diethylamino)diazen-1-ium-1, 2-diolate) used in this study were obtained from Aldrich. Azobisisoburyronitrile (AIBN) was obtained from Fluka and DAF-FM from Invitrogen. Water used throughout the studies was Millipore Q quality.

UV–Vis absorption spectra were recorded with a Hewlett–Packard 8453 spectrophotometer. Fourier Transform Infrared (FT-IR) spectra were acquired using a FT/IR-6200 type A JASCO spectrometer, with 4 cm^{-1} resolution and 50 scan accumulation. Fourier Transform Raman (FT-Raman) spectra were recorded using a JASCO NRS-3100 dispersive laser Raman spectrometer, with 4 cm^{-1} resolution and 100 scans accumulation (λ_{ex} = 632 nm). Thermogravimetric Analysis (TGA) were carried out using a Mettler Toledo TG-STDA instrument (30–400 °C at a heating rate of 5 °C/min). Steady-state fluorescence spectra were recorded with a Spex Fluorog 3–11 equipped with a 450 W xenon lamp. Fluorescence spectra were recorded in the front face mode. Time-resolved fluorescence measurements were done with the technique of time correlated single photon counting (TCSPC) using an IBH-5000U apparatus. Samples were excited at 464 nm using a nanoLED with a FWHM of 1.4 ns and a repetition rate of 100 kHz. Data were fitted to a single exponential model after deconvolution of the instrument response function by iterative deconvolution using the IBH DAS6 fluorescence decay analysis fluorescence decay analysis software (monoexponential decays), where reduced χ^2 and weighted residuals serve as parameters for goodness of the fit. All the samples were measured under aerated conditions.

2.2. Preparation and characterization of polymers

The preparation of the dye-loaded sensitive films was carried out following the methodology previously described to prepare analogous materials [17,19,23]. The appropriate weight composition of monomers and cross-linking agents was mixed in a vial with 1% of AIBN (total weight). Then, DAF-FM (100 µg/mL) was added and the sample was stirred vigorously at room temperature until complete dissolution of the reagents. Subsequently, the polymerization mixture was placed in a mold, which consisted in two microscope glass slides separated by two thin lamellas (~120 µm thickness).

The polymerization reaction was conducted in an oven at 85 °C for 15 min. The films were then removed mechanically from the molds and washed with a solution of PBS buffer 0.1 M. All of the films were red-colored and highly transparent. The polymers were characterized by FT-IR, FT-Raman, UV–Vis absorption and fluorescence spectroscopies (see Electronic Supporting Information, ESI). **Film I**: FT-IR (cm^{-1}): 3378, 2949, 1713, 1453, 1244, 1152, 1072. FT-Raman (cm^{-1}): 2938, 1722, 1634, 1446, 1296, 972. UV–Vis (λ_{max}, nm): 501. Fluorescence (λ_{max}, nm): 522 (λ_{ex} = 500 nm). TGA (T_g, °C): 260 – 270. **Film II**: FT-IR (cm^{-1}): 3429, 2944, 1717, 1442, 1247, 1153, 1072. FT-Raman (cm^{-1}): 2948, 1718, 1639, 1455, 1283, 972. UV–Vis (λ_{max}, nm): 500. Fluorescence (λ_{max}, nm): 519 (λ_{ex} = 500 nm). TGA (T_g, °C): 250–255. **Film III**: FT-IR (cm^{-1}): 3386, 2945, 1711, 1637, 1449, 1256, 1153, 1075. FT-Raman (cm^{-1}): 2944, 1716, 1636, 1421, 1314, 1234, 1100. UV–Vis (λ_{max}, nm): 501. Fluorescence (λ_{max}, nm): 522 (λ_{ex} = 500 nm). TGA (T_g, °C): 330–340. **Film IV**: FT-IR (cm^{-1}): 3376, 2952, 1707, 1644, 1453, 1257, 1160, 1057. FT-Raman (cm^{-1}): 2936, 1723, 1642, 1419, 1317, 1241, 1005. UV–Vis (λ_{max}, nm): 498. Fluorescence (λ_{max}, nm): 520 (λ_{ex} = 500 nm). TGA (T_g, °C): 330 – 340**. Film V**: FT-IR (cm^{-1}): 3407, 2931, 1716, 1647, 1454, 1244, 1153, 1075. FT-Raman (cm^{-1}): 2942, 1718, 1642, 1419, 1311, 1241, 1106. UV–Vis (λ_{max}, nm): 496. Fluorescence (λ_{max}, nm): 516 (λ_{ex} = 500 nm). TGA (T_g, °C): 320 – 330. **Film VI**: FT-IR (cm^{-1}): 3379, 2951, 1705, 1647, 1450, 1159, 1081, 983, 844. FT-Raman (cm^{-1}): 2935, 1721, 1641, 1417, 1310, 1236, 1104. UV–Vis (λ_{max}, nm): 497. Fluorescence (λ_{max}, nm): 518 (λ_{ex} = 500 nm). TGA (T_g, °C): 320–330. **Film VII**: FT-IR (cm^{-1}): 3375, 2943, 1712, 1643, 1454, 1251, 1153, 1069. FT-Raman (cm^{-1}): 2942, 1717, 1636, 1414, 1317, 1241, 1106, 1046. UV–Vis (λ_{max}, nm): 498. Fluorescence (λ_{max}, nm): 520 (λ_{ex} = 500 nm). TGA (T_g, °C): 330 – 340. **Film VIII**: FT-IR (cm^{-1}): 3363, 2951, 1709,