CARBON CHARS AS OXYGEN AND NITRIC OXIDE SENSORS FOR BIOMEDICAL APPLICATIONS

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Introduction

The overall goal of this research is to develop paramagnetic carbon-based particulates, including synthetic chars, fusinite and other naturally occurring materials, for use as sensors in medical and biophysical research and diagnostic applications of *in vivo* EPR oximetry. This program has an immediate need to supply calibrated oxygen sensors for many different applications, and a longer-term goal of creating much improved sensors for new applications, including those involving the very low-frequency EPR instruments currently being perfected [1]. The use of EPR for oximetry has been an important and increasingly popular development because of the sensitivity and versatility of this methodology [2]. It usually has been based on the use of nitroxides as the paramagnetic probes, and with these probes it has been possible to measure the O_2 concentration more accurately and conveniently in many types of samples than has been possible with other techniques. As with NMR methods, this EPR oximetric technique makes use of the effect of molecular oxygen on spin relaxation (although in this case it is of electrons rather than nuclei), which is detected by changes in the EPR resonance line width (ΔB_{pp}). The method with nitroxides still has some significant limitations in applications to functioning biological systems such as living cells and tissues because of the low concentrations of oxygen that occur there (often less than 20 micromolar), the lower limits of oxygen detectible by the method, and bioreduction of the nitroxides.

Several years ago, we introduced a new class of oxygen-sensitive probes for EPR oximetry based on paramagnetic carbon particles processed from a fraction of coal called fusinite [3]. This new probe answered both the sensitivity and bioreduction problems encountered with nitroxides, and convinced us that more optimized materials based on this same principle would greatly advance the practice of oximetry. Preliminary synthetic experiments have confirmed our expectations [4]. Table I illustrates the sensitivity advantages of the new materials.

Unlike nitroxides, which dissolve in water to form solutions, fusinite and other carbon-based materials are particulates, with unpaired electrons stabilized in the aromatic carbon structure. These particulate chars can be used for the measurement of $[O_2]$ in the aqueous environment of cells and tissues with a sensitivity to $[O_2]$ that is higher than that of nitroxides over a broader range of $[O_2]$, as Table I illustrates.

Table I. Comparison of Oxygen Sensitivities of Paramagnetic Probes

Material	$d\Delta B_{pp}/d[O_2$	Minimum Detectible $[O_2]$
Nitroxide	0.0007 Gauss/μM	5 - 10 μM
Fusinite	0.09	0.5
Cellulose Char	1.9	0.1
Sucrose Char	8.1	0.05

Other advantages of carbon chars over nitroxides are: (i) lack of concentration broadening that is typical for nitroxides in solution, and which can lead to the calculation of incorrect oxygen concentrations, (ii) a high spin density, producing a better signal-to-noise ratio than nitroxides in the concentrations typically used in living systems, (iii) a single resonance line spectrum, (iv) absence of bioreduction to a non-paramagnetic species (e.g., hydroxylamines in the case of nitroxides), and (v) lack of toxicity in cells and tissues. Carbon-based oxygen sensors can be ground to subcellular dimensions (diameter < 1 μm). This versatility permits a useful degree of microscopic site-specificity and control for the measurements of $[O_2]$. It also allows the production of particles with dimensions suitable for incorporation into cells by phagocytosis, for example.

Experimental

Fusinite is obtained from Illinois #6 coal by hand selection, followed by grinding and acid washing. No additional thermal or chemical processing is performed. Carbon chars are produced from hard and soft wood, cotton, synthetic polymers, and polysaccharides, by low-temperature pyrolysis under controlled flowing gas atmospheres. A three-zone, digitally controlled furnace allows for programmed heating, heat soaking, and cooling, while maintaining temperature uniformity in the sample of better than ±1°C. The synthesis apparatus also allows for precise, digitally metered injection of low molecular weight hydrocarbons into the flowing gas stream at any point in the thermal cycle. After pyrolysis, samples are ground in an agate ball mill, and sieved into size fractions ranging from <5μm to 400μm.

Characterization of chars and fusinite is performed at various field strengths, making use of EPR spectroscopy. Typical measurements are made at 1 GHz and 9.5 GHz, with additional spectra made at 95 GHz. The oxygen or NO response of the materials is tested in dry vacuum and under aqueous conditions suspended in physiologic buffers. Gas mixtures of N_2 and O_2 are used to determine the oxygen response of each char. Additional physical characterization is provided by optical microscopy and SEM, as well as by SQUID magnetometry [5].

Experiments involving living cells make use of 1 GHz EPR in conjunction with gas-tight glass chambers of our own design [6].

Results and Discussion

Figure 1 illustrates a typical spectral response of a carbon char to two different oxygen gas pressures. Since the EPR spectra of all these materials consists of one rather sharp resonance line, the effect of oxygen can be measured simply by determining the peak-to-peak width of the first derivative absorption line, ΔB_{pp}. Analysis of the narrow, oxygen-sensitive resonance line from fusinite and synthetic chars reveals that it is nearly Lorentzian in the center, and broader than Lorentzian in the wings, a shape often encountered in spin exchange-narrowed systems [7]. Because the center portion of the line is precisely modeled by a Lorentzian lineshape, the peak-to-peak linewidth can be determined with good precision using a simulation technique. Under conditions of reasonable signal strength, ΔB_{pp} can be determined to ± 1%, which means that for carbon sensors with linewidths of about 2 - 10 Gauss, a precision of ± 20 - 100 mG in the peak-to-peak linewidth determination is expected. As we have shown, this affords a very high precision in the determination of oxygen concentrations, better than ±0.1μM [8]. The chars also respond similarly to concentrations of NO, thus providing an experimental route to study the kinetics of nitric oxide metabolism in living cells [9].

Conclusions

Synthetic carbon chars can be synthesized with oxygen or NO response characteristics that suite a wide variety of biomedical applications. By carefully controlling pore volume, surface area, size, and surface chemistry, the interactions of the particles with cellular and tissue environments can be controlled.

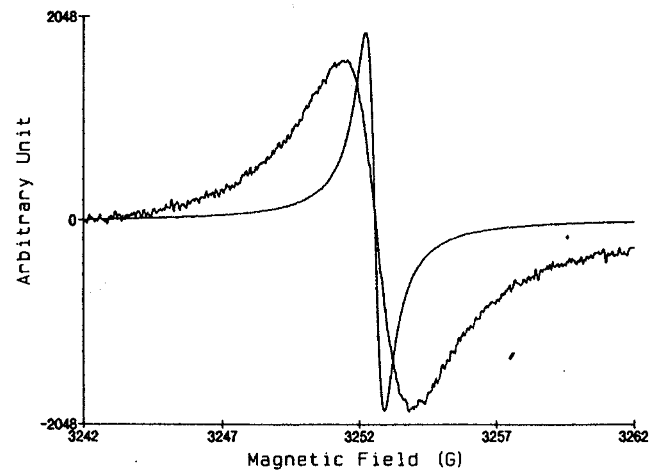

Figure 1. EPR spectra of a carbon char at two O_2 pressures. Low P_{O2} is indicated by a narrow line.

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