An EPR Characterisation of Stable and Transient Reactive Oxygen Species formed under Radiative and Non-Radiative Conditions

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ABSTRACT:

Electron paramagnetic resonance (EPR) spectroscopy is the ideal method of choice when detecting and studying the wide variety of paramagnetic oxygen centred radicals. For simple diatomic radicals, such as the super oxide ($O-_2$) or peroxyl (ROO·)(ROO·) species, the CW EPR profile (in particular the g-values) of these species can appear similar and indeed indistinguishable in some cases. Experiments using ^{17}O-enriched oxygen, revealing a rich ^{17}O hyperfine pattern, are therefore essential to distinguish between the two species. However, in many cases, particularly involving TiO_2 photocatalysis, the peroxy type (ROO·)(ROO·) radicals or other intermediate species such as the [$O-_2$…organic]-type adducts can be transient in nature and once again can produce similar g-values. In general terms, these reactive oxygen species (ROS) are formed and detected at low-temperature conditions. Hence, the application of EPR spectroscopy to studies of surface-stabilised oxygen-centred radicals must be performed under carefully selected conditions in order to confidently distinguish between the differing types of diatomic radicals, such as $O-_2$, ROO·ROO· or [$O-_2$…organic].

Keywords: Electron paramagnetic resonance (EPR), Reactive Oxygen, Radiative and non-radiative agents.

Introduction:-

Oxygen is one of the five basic elements of all life and is the third most abundant element, making up 21% of the earth's atmosphere. There are several forms of oxygen, but the most common and stable form is diatomic oxygen (known as molecular oxygen), which is a colourless, odourless, and tasteless gas. However, it is critical to the life processes of all living creatures, especially human beings, since 90% of the energy we utilize is derived from oxygen. Oxygen inspired from atmospheric air reaches the lungs, where it diffuses out of the alveoli into the blood stream. Hemoglobin, in the red blood cells, acts as the carrier for oxygen and it is about 95% saturated with oxygen under normal physiological conditions.

When radiation interacts with matter, some amount of energy is absorbed in the targets, which leads to ionization and excitation of some of its atoms and molecules. In the case of living matter, these processes initiate a complex chain of radiochemical and biochemical events involving free radicals, and leading to acute oxidative stress. Free radicals, the transient species with one or more unpaired electrons, exhibit both beneficial and harmful effects in living organisms. The most important free radicals are reactive oxygen / nitrogen species (ROS/RNS), such as superoxide ($\bullet O\text{-}_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO•), peroxyl (ROO•), alkoxyl (RO•), and peroxynitrite (ONOO•) radicals. These free radicals may be involved in the progression of a number of diseases including diabetes, hyperlipidemia, and neurodegenerative disease, reperfusion injury, pulmonary toxicity, cataractogenesis, and so on. Free radicals may be involved in the initiation and propagation of free radical chain reactions, which are potentially highly damaging to cells. Oxidative stress is one of the important factors in the progression of various chronic diseases including cancer, cardiovascular diseases, age-related muscular degeneration (AMD), and the aging process. Radiation-induced damage and oxidative stress are closely coupled. During oxidative stress the flux of free radicals increases significantly as compared to the corresponding physiological levels.

Investigations on free radicals and their linkage with oxidative stress can lead to the development of newer technologies, wherein both the qualitative and quantitative aspects of such free radicals are evaluated. Spectroscopic methods such as X-ray photoelectron spectroscopy (XPS) or absorption spectroscopy are the primary techniques used for the detection of free radicals. Measurement of the decrease in the amount of antioxidant enzymes, for example, super oxide dismutase (SOD), catalase, and glutathione peroxidase, is an indirect method for the detection of free radicals. EPR is an acronym for Electron Paramagnetic Resonance. EPR is an electromagnetic technique which is used for the study of paramagnetic species such as free radicals, transition metal ions, and so on. It is also known as electron spin resonance (ESR). The EPR spectroscopy technique has found its unique application in the detection and quantification of free radical-related processes and in the study of different oxidant products of biomolecules.

Principle of EPR

Electron Paramagnetic Resonance is the resonant absorption of microwave radiation by paramagnetic systems in the presence of an applied magnetic field. The applied magnetic field generates discrete orientation and this orientational difference is physically equivalent to a separation of energy levels. Resonance absorption of electromagnetic radiation (microwave energy) occurs when the applied microwave energy exactly matches the energy level separation. The degree of absorption is proportional to the number of free radicals in the material.

Modes of EPR measurement

EPR spectroscopy measurements can be performed in two different modes, viz. continuous-wave (CW) and pulse EPR (also known as time-domain EPR). In the CW mode, the sample is constantly irradiated with microwave energy at a low power, whereas in the pulse EPR mode, the sample is irradiated with short (nanosecond) pulses of intense energy (300 W) in the radio-frequency range. The signals generated, following the pulse, are used to obtain a frequency spectrum of the sample.

Experimental

Materials

$O-_2$ on MoO_3/SiO_2: The samples of supported molybdenum were prepared by a grafting method [21], where $MoCl_5$ was reacted with surface silanol groups in organic media according to the process:

$MoCl_5+nSiOH\rightarrow(SiO)nMoCl_5-n+nHClMoCl_5+nSiOH\rightarrow(SiO)nMoCl_5-n+nHCl$

After hydrolysis of the remaining chlorides, the catalyst was washed and dried in air at 120 °C. After transfer into quartz EPR tubes (4 mm thin wall, WilmadLabGlass), the samples were first preheated in oxygen at 600 °C then reduced at 600 °C under 100 Torr H_2 for 30 min and finally evacuated to $1 \times 10-^5$ Torr. Oxygen was adsorbed at 77 K at pressures of 10−2 to 10−1 using $^{16}O_2$ or isotopically enriched $^{17}O_2$ (63 atom % ^{17}O, Icon Isotopes) to produce the $O-_2$ superoxide ions.

$O-_2$ on Polypropylene: Powdered isotactic polypropylene was obtained from lCl Ltd.

The samples were evacuated for 48 h at 100 °C and $1 \times 10-^5$ Torr and then γ-irradiated in vacuo at room temperature with a 60 ^0C source to a dose of 70 Mrad.

The gaseous radiation products were evacuated and the samples transferred under vacuum in EPR tubes. Oxygen was added under 3 Torr to form the corresponding peroxy radicals. The labelled peroxy radicals were obtained by adsorption of O_2-enriched oxygen.

ROS on TiO_2: The polycrystalline TiO_2 (Degussa P25) was placed in an EPR cell and heated to 423 K under dynamic vacuum (10−4 Torr) overnight to remove any physisorbed water from the surface. The temperature was subsequently raised over 5 h to a final temperature of

773 K and left at this temperature for 1 h. This produced a non-stoichiometric, blue-coloured sample due to the excess of Ti3+ centres. The reduced sample was cooled prior to exposure to molecular oxygen and selected organic substrates. The organic substrates (including methanol, toluene and acetonitrile) were exposed to the sample at 77 K, followed by annealing to a series of elevated temperatures (200–260 K) for a period of 10 min. In some experiments, co-adsorbed mixtures of oxygen and the organic substrate were premixed in the vacuum manifold (with a ratio of 1:10) prior to exposure to the $_{TiO2}$ surface at 298 K. All UV irradiations were performed at 77 K using a broadband (250–2500 nm) 1000-W UV lamp (Oriel Instruments, 66021), incorporating a water filter for removal of infrared frequencies.

Conclusions

The superoxide radical anion ($O{-}_2$) is readily formed over MoO_3/SiO_2 by the exposure of the reduced sample to molecular oxygen, as widely reported. However, whilst the CW EPR spectrum of the unlabelled species is consistent with a surface $^{16}O{-}_2$ centre, analysis of the spectrum obtained using ^{17}O enriched oxygen is necessary to fully interpret the nature of the radical which reveals the presence of an end-on bonded $O{-}_2$ species. The same $^{17}O{-}_2$ species could be formed from $^{16}O{-}_2$ by the exposure of the sample to ^{17}O-enriched molecular oxygen in an isotope exchange reaction. In principle, whilst side-on and end-on bonded $O{-}_2$ species can exist on MoO_3/SiO_2, the latter species appear to dominate.

Paramagnetic oxygen-based radicals are also readily formed when molecular oxygen is exposed to γ-irradiated polypropylene. In this case, the CW EPR signal has notably broad line widths. The profile of the spectrum is analogous to that expected of a generic ROS entity, with $g_1 = 2.035$, $g_2 = 2.008$ and $g_3 = 2.002$, but once again only a definitive assignment to an end-on peroxy radical (ROO·)(ROO·) can be made using ^{17}O hyperfine pattern.

A number of oxygen-centred radicals have been proposed to play a key role in the degradation of organic substrates over TiO_2 under dark conditions or under UV irradiation. Here, we have shown how the decomposition of acetonitrile, methanol and toluene can occur over $O{-}_2/TiO_2$. The reaction of the organic substrates with $O{-}_2$ results in the production of a transient and thermally unstable (above 240 K) surface adduct, labelled [$O{-}_2$…organic]. These adducts are characterised by the EPR g-values of $g_1 = 2.031\text{-}2.025$, $g_2 = 2.010$ and $g_3 = 2.003$, and similar to previously published data appear to possess a side-on arrangement of the organic molecule with respect to the two equivalent oxygen atoms of $O{-}_2$. Additionally, a hydroperoxy (HO_2) radical was also formed under these experimental conditions. This [$O{-}_2$…organic] adduct can be formed regardless of how the $O{-}_2$ radical was originally generated.

Furthermore, a series of thermally unstable surface-organoperoxy-type radical intermediates were also identified following UV irradiation of a series of coabsorbed organic–O_2 mixtures. These transient radicals ($NCCH_2OO·$,$HOCH_2OO·$,$C_7H_7OO·$)($NCCH_2OO·$,$HOCH_2OO·$,$C_7H_7OO·$) produced after UV irradiation all possessed analogous g-values (g1 = 2.034, g2 = 2.007, g3 = 2.001). The signal intensities for the $HOCH_2OO·HOCH_2OO·$ and $C_7H_7OO·C_7H_7OO·$ radicals were much weaker compared to those of the $NCCH_2OO·NCCH_2OO·$ species (possibly due to the different stabilities of the respective radicals and the different competing reactions that can occur with these substrates over the TiO_2 surface).

The work presented in this paper demonstrates the complexity of organic substrate decomposition with ROS over TiO_2. It is clear that these substrates can react and degrade under dark conditions (non-radiative) via a reaction with surface $O{-}_2$ radicals forming transient [$O{-}_2$…organic] adducts, or via UV irradiation conditions where hole-mediated processes lead to the formation of ROO·ROO· species. In all cases, the nature of the transient reactive oxygen species (ROS) in TiO_2 photocatalysis is very important and highlights the need to perform low-temperature in situ measurements to obtain a complete picture on the oxidation radical steps.

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