Electron Paramagnetic Study of Spin Labelled Molecule on Colloidal Surfaces[*,‡]

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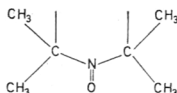*Chemistry Department, Princeton University, Princeton, N. J., U.S.A.*

Spin labelling techniques have been used to determine the adsorption characteristics of a nitroxide on colloidal gold. The number of molecules adsorbed per surface atom was determined and the desorption of adsorbed molecules on coagulation of gold noted.

INTRODUCTION

Recent publications have shown the value of EPR (electron paramagnetic resonance) spectroscopy to study the molecular characteristics of the environment.[1,2]

The use for this purpose of the free radical, 2,2-diphenyl-1-picryl hydrazyl DPPH (whose magnetic resonance has been disclosed by one[3] of us) has been handicapped by the reactivity of the unpaired electron on the nitrogen atom. The introduction of a whole series of nitroxide derivatives[4-7] in which the reactivity of the unpaired electron is shielded by four methyl groups on the neighboring carbon atoms

has opened up a whole field of »spin labelling« investigation. A »spin label« is defined as »a synthetic organic free radical that can be incorporated within, or attached to a molecule or a system of biological interest, to provide information concerning structure, conformation changes or chemical reactions.«[8] Since the unpaired electron is about 80% on the nitrogen and because of its interaction with the nitrogen nucleus with nuclear moment of 1, the electron paramagnetic resonance line has a hyperfine structure of three lines.

In a single crystal, because of the anisotropy of the free radical molecule, the EPR characteristics of the position of the central line and of **A**, the measure of the separation between the hyperfine components, vary with the orientation of the crystal in the magnetic field. These are characterized by the principal values of the tensors **g** and **A**, which for the case of the di-t-butyl nitroxide are $g_{xx} = 2.0089$, $g_{yy} = 2.0061$ and $g_{zz} = 2.0027$ and $A_{xx} = 6$ gauss, $A_{zz} = 32$ gauss.[9] Thus the position of three lines of *equal* intensity and shape and their mutual

separation change as the crystal assumes different orientations with respect to the magnetic field.

In powdered form or when the radical molecule is immobilized in a frozen solvent, the free radical assumes all possible orientations with respect to the magnetic field. The EPR spectrum is an average of all the single crystal spectra and the clean-cut appearance of a single crystal signal assumes a distorted form.

In solution where the free radical freely tumbles, the **g** and the **A** values assume a single average value of **g** = 2.0059 and **A** = 14.7 gauss. Three equally spaced lines are obtained of the same intensity and width. The oxygen, nitrogen and the two carbon atoms attached to the nitrogen are in the same plane of the molecule. The unpaired electron is to a large extent in the $2p\pi$ nitrogen orbital, perpendicular to the plane of the four atoms referred to above. The values of **g** and **A** vary slightly from compound to compound. Of particular interest is the observation that the **g** and **A** values vary with the nature of the solvent. Thus for 2,2,6,6-tetramethyl-4-piperidon-1-oxyl the nuclear splitting varies from 14.5 gauss for benzene to 16.6 gauss for water.[10] This can be used to investigate the solvent interaction as we have shown in the case of water--dioxan solutions, a break in the values of **g** and **A** with concentration representing a reorganization on a molecular basis of the solvent molecules.[11]

Intermediate cases, in which the rotation or tumbling of the molecule is restricted, can be used effectively to study variation of interesting phenomena characterizing molecular environment. In the case of restricted motion of the nitroxide radical, the widths $T_2(M)$ of the individual hyperfine lines characterized by nuclear quantum numbers $M = -1, 0, 1$ vary and their ratio is given by

$$\frac{T_2(0)}{T_2(M)} = 1 - [\tau \frac{4}{15} \Delta\gamma H_0 M - \frac{1}{8} b^2 M^2] T_2(0)$$

where $\dfrac{T_2(0)}{T_2(M)}$ is the experimentally determined ratio of the width of the central hyperfine line to the one with nuclear quantum number M and τ is the correlation time characteristic of the nuclear environment. This may be related to the local viscosity η by the well-known Stokes formula for macroscopic viscosity.[12]

$$\tau = \frac{4\pi \eta r^3}{3 k T}$$

where r is the particle radius.

The H_0 in the above expression is the applied magnetic field in gauss, b is anisotropy in the hyperfine constants

$$b = \frac{4\pi}{3} [A_{xx} - 1/2 (A_{yy} + A_{zz})]$$

and $\Delta\gamma$ is the anisotropy in g values.

$$\Delta\gamma = \frac{2\pi \beta}{h} [g_{zz} - 1/2 (g_{xx} + g_{yy})]$$

where β is equal to the value of the Bohr magneton and h is Planck's constant.[13,16] Variation in the relative intensities of the hyperfine lines have been used to determine the correlation times in the range of 10^{-12} s to 10^{-8} s, for a variety

of systems: changes in the conformation of proteins with changes in pH,[16] the course of enzyme action,[17] the allosteric transition of hemoglobin on oxygenation,[18] and micelle formation in detergents.[19,20,21]

In the course of the Senior Thesis work carried out in our laboratories by Ron Radius on spin labelling[22] of chicken intestine cells, both normal and neoplastic, grown in tissue culture and labelled by the nitroxide, it was found that on its adherence to the cell membrane, there was not only a change in the nature of the nitroxide signal but also a loss in signal intensity. It was therefore decided to study the adsorption of spin labelled nitroxide radicals on colloidal particles whose properties were well characterized. The system chosen was colloidal gold whose preparation and properties have been studied by one of us for many years.[23]

EXPERIMENTAL

Colloidal gold was prepared by the method of Turkevich, Stevenson and Hillier.[23] 100 ml of $HAuCl_4$ solution containing 50 mg of gold were added to 850 ml of distilled water boiling in a 2 liter flask. When the solution began to boil again 50 ml of 1% sodium citrate solution were added, stirring vigorously. After boiling for thirty minutes, the solution was allowed to cool and was made up to 1 liter. The particle size distribution and degree of agglomeration were checked by electron microscope examination. A formvar membrane on a 200 mesh grid was left for ten minutes in 0.1% gelatin solution. It was washed, allowed to dry and then introduced into the gold solution for ten minutes. The colloidal gold particles adhered to the membrane in the state in which they were in solution. The membrane was then washed, dried and examined using the RCA EMU-2 electron microscope. The above procedure gave monodisperse 200 Å particles (standard deviation from the mean of 12%). There was minimal clustering with doublets being less than a tenth percent of the singlets. Particles of 150 Å were prepared by modifying the synthesis conditions as described in above cited publications.[23]

The nitroxide (tetramethyl piperidone oxide isothiocyanate obtained from Varian Associates) was first dissolved in distilled water by stirring the solution for an hour. The solution was diluted with water to give a blank and with a colloidal solution of known concentration and size of gold particles to study the adsorption process. The solutions were measured immediately after mixing in the X band ESR spectrometer (Varian V4500) using a flat cell specially designed for measuring aqueous solutions.

RESULTS AND DISCUSSION

The EPR spectrum of the aqueous solution of tetramethyl piperidone oxide isothiocyanate consisted of three hyperfine lines centered on $g = 2.0057$ with a separation of 16.8 gauss and a width at half height of the individual hyperfine lines of 1.8 gauss. The intensities of the hyperfine lines were not quite the same, permitting us to calculate a correlation time for the tumbling of the free radical in water, a value of 2.5×10^{-11} s. The anisotropy values used were those of Stone et al.[16] The relaxation times calculated according to Poole[24] assuming Lorentzian shape of lines and homogeneous broadening are $T_1 = 3.6 \times 10^{-7}$ s and $T_2 = 3.5 \times 10^{-8}$ s.

The nitroxide is not very soluble in water, the solubility being less than 10^{-3} mol dm^{-3}. Below a concentration of 10^{-4} mol dm^{-3}, the solution shows a linear dependence of the intensity of signal on concentration. In the region of 1 to 3×10^{-4} mol dm^{-3} there is a drop of intensity from the extrapolated linear value. This effect seems to be real since there is an increase in the ratio of the central line intensity to that of its satellites. This may be due to a loose clustering of nitroxide molecules in an almost saturated solution, the clustering restricting the tumbling of the individual nitroxide molecules.

The gold colloid-nitroxide systems showed the same EPR signal characteristics as the water-nitroxide system. There was a significant decrease in the signal intensity due to the adsorption of the nitroxide on the colloidal gold. This permitted us to construct an adsorption isotherm of the nitroxide on the colloidal gold particles.

The loss of spins is proportional to the nitroxide concentration and in 2.5×10^{-5} mol dm^{-3} nitroxide solution was 0.7 nitroxide molecules per surface gold atom. The adsorption increases with decrease in particle size of the colloid, being 3 nitroxide molecules per gold atom for the 150 Å gold and two for the 230 Å gold at a nitroxide concentration of 10^{-4} mol dm^{-3}.

The loss of nitroxide concentration on interaction with gold surface may be due to a number of causes: electron transfer between the nitroxide radical and gold, broadening of the nitroxide signal by the surface field of the gold metal or finally very slow tumbling of the 200 Å gold particle whose correlation time according to the Stokes formula would be 1×10^{-5} seconds — too long to be observed by EPR techniques in our concentration range. Elucidation of these factors will be pursued using sols of uniform particles of different chemical composition and smaller size.

The kinetics of the adsorption process are of some interest. It was observed that the loss of EPR signal on mixing colloidal gold solution with nitroxide solution was slower for the dilute nitroxide solution than for the concentrated one. For the latter, the process occurs during ten minutes. The gold nitroxide colloid solution is not stable as is shown by change of its color from red to blue and the regeneration of the EPR signal in thirty minutes to its strength in pure water. The color change indicates coagulation of the gold particles into aggregates with change in their electronic structure. This produces a decrease in adsorption of the nitroxide. This is consistent with the previous experiments with 150 and 230 Å gold particles which showed that adsorption of nitroxide decreases with increasing particle size.

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SAŽETAK

Istraživanje molekule spinskog označivača na koloidnim površinama elektronskom paramagnetnom resonancijom

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Snimljeni su EPR-spektri nitroksidnog radikala tetrametil-piperidinoksid-izotiocijanata adsorbiranog na koloidnim česticama zlata. Topljivost radikala u vodi je manja od 10^{-3} mol dm^{-3}, a do 10^{-4} mol dm^{-3} je intenzitet EPR-signala linearno ovisan o koncentraciji. Promjene u spektrima opažene pri koncentracijama 1 do 3×10^{-4} mol dm^{-3} mogu se pripisati rahloj asocijaciji molekula nitroksida u gotovo zasićenoj otopini. Intenzitet EPR-signala opada zbog adsorpcije nitroksida na koloidno zlato, iz čega se može odrediti adsorpcijska izoterma. U $2,5 \times 10^{-5}$ mol dm^{-3} nitroksidnoj otopini 0,7 molekula nitroksida bilo je vezano na jedan površinski atom zlata. Adsorpcija je veća za manje čestice koloidnog zlata, tj. 3 molekule nitroksida po atomu zlata za čestice od 150 Å i 2 molekule za čestice 230 Å. Gubitak intenziteta signala kod adsorpcije u razrijeđenim otopinama nitroksidnog radikala polaganiji je nego li u koncentriranim otopinama, u kojima uspostavljanje ravnoteže traje oko 10 minuta. Sistem nije stabilan zbog koagulacije čestica zlata, tj. intenzitet nitroksidnog signala povećava se s vremenom do postizanja vrijednosti u čistoj vodenoj otopini.

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