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FORMATION OF RADICALS BY MECHANICAL PROCESSES

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The application of e.s.r. spectroscopy to the study of radicals formed by mechanical means (grinding, shearing, bending, cutting, etc.) is discussed. Two mechanisms are considered, one being the homolytic breaking of main-chain bonds in polymers, and the other being charge-transfer (tribo-electric effect). The former is thought to occur for high polymers whilst the latter may be important for certain inorganic materials especially ionic solids.

Specific examples are given for the generation of radicals by cutting finger nails and by grinding bone. In both cases, well-defined e.s.r. spectra were obtained for which identifications are offered. Possible implications of the mechanical formation of radicals in bone are considered.

KEY WORDS: ESR Spectroscopy, radicals, mechanical degradation, nails, bone.

INTRODUCTION

Probably the first demonstrations of radical formation during the mechanical fracture of polymers were by Bresler *et al.*[1,2] and Butyagin *et al.*[3-6] These early studies, reviewed by Campbell[7] were all concerned with high polymers, largely synthetic. Our first involvement in this type of work was in the early sixties, in a study of the formation of radicals during the milling of flour, which may well have some significance in the baking industry. At about the same time, we attempted to relate radical formation with the accumulation of charge (static electricity). However, using a range of electron-donor and -acceptor solids, it proved to be impossible at that time to accumulate enough charge in the systems studies to give any significant e.s.r. signals.[8] At that time we confirmed, for our own satisfaction, much of the earlier work on the mechanical generation of radicals in polymers. We concluded that, for organic polymers, there did not seem to be any clear link between tribo-electric effects and radical generation. (However, see below).

Radicals in Polymers

Since those early experiments, e.s.r. spectroscopy has played a leading rôle in the study of mechanically generated radicals in polymers. This can be judged from the large number of reviews on this topic.[9-14] There seem to be two main approaches. In one, deformation involving bending or stretching is studied, often *in situ*, at or not far below room temperature. Many of the materials studied in this way give low con-

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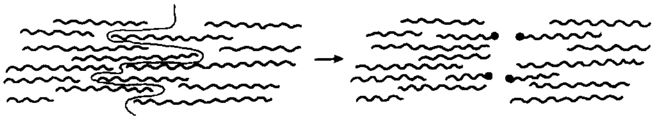
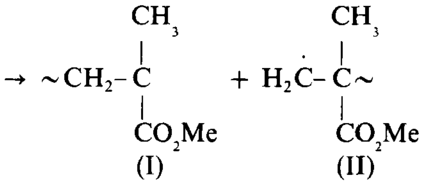


FIGURE 1 Schematic representation of a shearing process in which fibrous molecules pull apart with occasional homolytic bond breakage.

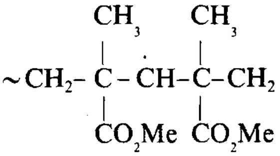
centrations of radicals, often quite transient. Also, if oxygen is not removed, peroxy radicals ($RO_2\cdot$) are usually the major products detected by e.s.r. spectroscopy.

In the other procedure, samples are cooled, usually to *ca.* 77 K, and are ground, generally using a ball mill. Most materials are very hard at these temperatures, so grinding is the most satisfactory method for radical generation and trapping. In this way, high yields of radicals having well-defined e.s.r. spectra can be built up, and often identified. Most workers are agreed that, initially, main chain breakage occurs, particularly in cross-linked polymers. I imagine that as slippage between molecules occurs at a weak point, some long-chain molecules are forced to suffer a strand-break because the total intermolecular process holding the strands are greater than the bond strength (Figure 1). Often, secondary reactions occur to give more stable radicals on annealing.

Poly(methyl methacrylate). The well-known "4 + 5" line spectrum also found in irradiated samples was clearly detected (Figure 2). I originally assigned this spectrum to radical I, which is the most stable of the two radicals, formed by chain scission (1).[15]



$$\rightarrow \sim CH_2-\underset{\underset{(I)}{CO_2Me}}{\overset{CH_3}{C}} \quad + \quad H_2\dot{C}-\underset{\underset{(II)}{CO_2Me}}{\overset{CH_3}{C}}\sim \qquad (1)$$



$$\sim CH_2-\underset{CO_2Me}{\overset{CH_3}{C}}-\dot{C}H-\underset{CO_2Me}{\overset{CH_3}{C}}-CH_2\sim$$

(III)

The expected radical II was not detected, but a relatively unstable doublet characteristic of *one* coupled α-proton is also present at low temperatures. This is probably III formed from II by H-transfer. In the presence of oxygen, $RO_2\cdot$ radicals are formed. I stress that radical cations and anions formed in this polymer should have been detectable had they been trapped. Electron return may be facile (they are not detected on irradiation), in which case an excited state could be formed which might give rise to the observed radicals. However, it seems to me that mechanical breakage is a more probable mechanism. Pilar and Ulbert claim to have detected II as well as I and III on grinding at 77 K, but the evidence for II is not strong.[16]

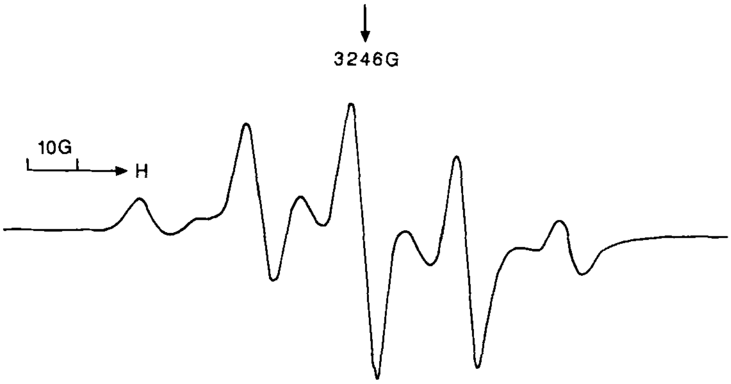


FIGURE 2 First derivative X-band e.s.r. spectrum for poly(methyl-methacrylate) after grinding at *ca* 0°C, showing features assigned to $RCH_2\dot{C}(CH_3)R'$ radicals.

Spin-Trapping This technique is of no great advantage in low temperature milling, since the polymer radicals are trapped as such. However, it could be used to advantage in room temperature studies, and especially in studies of shearing in melts. However, this technique has not been widely exploited. It is well established that aqueous solutions of DNA undergo strand-breaks on stirring at room temperature. We have attempted to study this using various spin-traps and carefully deoxygenated solutions.[17] However, nitroxide radicals were not detected. Sakaguchi *et al.* have recently suggested that *ca.* 40% of the chain scissions formed in polypropylene by mechanical fracture are actually formed by heterolysis rather than homolysis.[18] This seems to be improbable for polypropylene, but may be more reasonable for DNA, especially in fluid water. Obviously, further study is required before we can draw any firm conclusions.

Elastomers

Mechanical degradation of elastomers, especially tyres, is clearly of great importance. E.s.r. spectra have been detected in many such systems during deformation and other forms of mechanical stress. For example, spectra assignable to allyl and peroxy radicals were detected during deformation of cross-linked polyisoprene and polybutadiene elastomers.[19] The results can be linked to other studies of failure from mechanical stress.

Human Nails

We have recently shown that when nails are cut at room temperature two types of radicals are generated (Figure 3). One, which is remarkably well defined, comprises g_x, g_y and g_z features split into characteristic 8.5 G doublets [the g_z lines are partially hidden beneath features for the second radical]. These features are completely characteristic of sulphur containing radicals which used to be formulated as $RCH_2S\cdot$, but which are now thought to be either $RCH_2SS\cdot$,[20,21] or $RCH_2S^{\cdot}S(H)CH_2R$ σ^* radi-

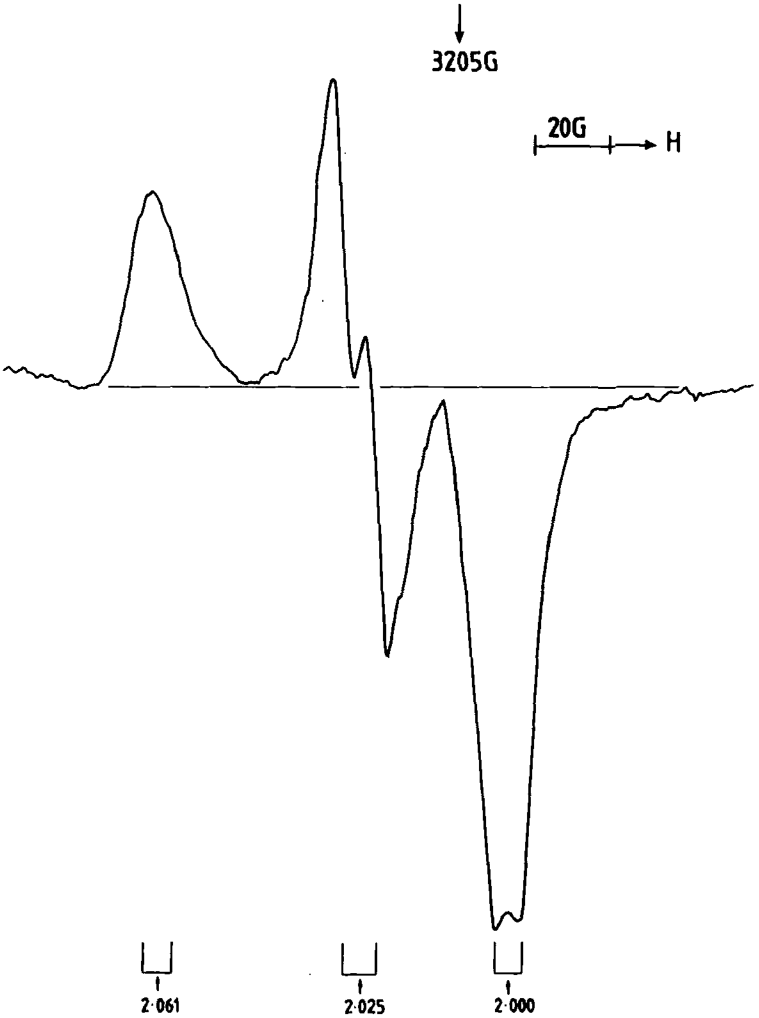


FIGURE 3 First derivative X-band e.s.r. spectrum for human finger nails after cutting into very small pieces measured at 77 K. The three sets of doublets are shown at $g = 2.061$, $g = 2.025$ and $g = 2.00$.

cals.[22,23] I have recently reviewed the arguments for the against these alternatives,[24] and this is not the place for further comment. Suffice it to say that there are strong arguments in favour of both structures, and it seems possible that, by coincidence, they both give rather similar spectra. Using the σ^* formulation one can see that this frequently encountered species can be formed either from RSH compounds (2,3) or from RS-SR derivatives (4,5). On the other hand, RSS· radicals

$$RSH(-e^-) \rightarrow RS\cdot + (H^+) \quad (2)$$

$$RS\cdot + RSH \rightarrow RS\dot{-}S(H)R \quad (3)$$

$$\mathrm{RSSR(+e^-)} \rightarrow \mathrm{RS\overset{\cdot}{-}SR^-} \qquad (4)$$

$$\mathrm{RS\overset{\cdot}{-}SR^-} + \mathrm{(H^+)} \rightarrow \mathrm{RS\overset{\cdot}{-}S(H)R} \qquad (5)$$

could possibly be formed simply by homolysis of the R-S carbon-sulphur bond.

The other radical which is always formed in cut nails has a less well defined spectrum, but the marked shift to high average g-values strongly suggests the formation of $\mathrm{RS\overset{\cdot}{-}SR^-}$ radicals (4). These results can be considered in terms of main chain homolysis or charge-transfer.

The major consituent of nails is the fibrous protein α-keratin. This contains many cross-linking disulphur bridges which are clearly responsible for the e.s.r. spectra. There are several problems. If bond homolysis were important during cutting, I would have expected that the weak S-S bond would break rather than the adjacent C-S bond. No $\mathrm{RS\cdot}$ radicals were detected, and it is difficult to understand how they could be converted into $\mathrm{RSS\cdot}$ or the α^* radicals, bearing in mind the rigidity of the matrix.

On the other hand, an electron-transfer process is more attractive. Electron-capture is expected to occur with considerable specificity at S-S bonds giving $\mathrm{RS\overset{\cdot}{-}SR^-}$ anions.[25] This is because electron trapping in proteins is thought to be specific, the electrons migrating to sites of high affinity such as RS-SR units.[26] Thus, formation of $\mathrm{RSSR^-}$ anions is quite reasonable. Since no such mobility is found for electron-loss centres,[26] I tentatively suggest that *both* sulphur entres are formed from electrons, the $\mathrm{RS\overset{\cdot}{-}S(H)R}$ species (if correctly formulated!) being formed by simple protonation of $\mathrm{RS\overset{\cdot}{-}SR^-}$ radical anions. In that case, the electron-loss centres, which are probably diverse, are not detected by e.s.r. spectroscopy. If these ideas are correct, this may be a good example of charge-transfer damage rather than homolytic damage. There remain many unsolved problems which we are currently trying to unravel.

Inorganic and Ionic Compounds

Simple non-ionic compounds such as water do not give rise to radicals on low temperature grinding. However, the three-dimensional polymer, SiO_2, gives a very narrow line with a *g*-value slight less than the free-spin value (*ca.* 2.0010) either on griding, or on explosive shock.[27] The most obvious assignment is to $\equiv\mathrm{(O)_3Si\cdot}$ radicals which are a well-known defect in silica, probably formed by electron-capture at –SiOH defect sites.[28] These centres could be formed either by bond homolysis or by electron transfer: in either case, the hole-centre on oxygen is not apparent in the e.s.r. spectra, which is curious. Also, a search for ^{29}Si satellite features was not successful.[28] Two types of ionic crystals are known to undergo major decomposition under pressure or shearing conditions, namely potassium ferricyanide, and silver chloride. The former study did not involve the use of e.s.r. spectroscopy, but seems to be a very well known phenomenon which was first studied by Lea many years ago.[29,30] It seems that reaction (6) occurs in a dry atmosphere, and that HCN and HOCN are formed in the presence of water.[31] The le$^-$ transfer (7) is suggested but

$$\mathrm{2Fe(CN)_6^{3-}} \rightarrow \mathrm{2Fe(CN)_5^{3-}} + \mathrm{C_2N_2} \qquad (6)$$

$$\mathrm{Fe(CN)_6^{3-}} \rightarrow \mathrm{Fe(CN)_5^{3-}} + \mathrm{\cdot CN} \qquad (7)$$

there was no direct evidence for $\cdot$CN formation. If it is involved, it seems unlikely that it could be trapped long enough for e.s.r. study.

Silver Halides. It is well established that photographic materials are very sensitive to pressure, abrasion, or torsion,[32–36] In particular, pressure desensitises film, and it is generally argued that this is a consequence of an increase in *internal* sensitivity rather than surface sensitivity, the latter being essential for developing. This is envisaged as an increase in internal nucleating sites resulting from deformation of the relatively soft silver halide crystals.

However, this concept alone is not enough to account for the appearance of 'fog' or of a number of other observations all of which suggest that a tribo-electric component is also involved.[36–40] In particular, a photo-induced e.s.r. signal from shallowly trapped electrons[41] at *ca.* $g = 1.89$ is greatly enhanced by high pressure.[40] Thus it seems probable that plastic deformation coupled with electron-transfer is involved in the complex pressure effects for silver halides.

Bone

It occurred to me that, since radicals are strongly implicated in inflammatory joint diseases,[42,43] it is worth considering the possibility that grinding itself can give rise to active radicals. It has been established that the synovial fluid, which acts as a joint lubricant, has a greatly reduced viscosity in diseased joints. This is a result of breaking down of the highly polymeric polysaccharide, hyaluronic acid.[44] Pressure on joints such as the knee joint can be momentarily very high indeed, especially for athletes. This occurs during motion and could lead to mechanical formation of radicals. These might possibly attack molecules of the synovial fluid causing bonds to break. The resulting fall in viscosity would then lead to an increase in such grinding thereby catalysing the effect. I do not suggest that this is an alternative to the autoimmune effects characterised by inflamation, but it might help to explain the rather obscure origins of these diseases.

There have been several e.s.r. studies of the generation of radicals on grinding bone.[45–47] There seems to have been some confusion because of Cu^{II} uptake and also because of the generation of carbon centres on heating.

As with most bio-minerals, the structure of bone is determined by the organic 'templates' from which the mineral grows. There are two major types of bone, compact bone which consititutes the major structural regions, and spongy bone, found for example, inside the ends of long bones at the joints. The mineral phase is largely hydroxyapatite, $Ca_{10}(PO_4)_6(OH)^2$, but crystallites of apapite are also occasionally present. The crystallites of hydroxyapatite, stemming from the bone cells are arranged in long fibrils intimately associated with fibrils of collagen. Larger cavities between groups of these fibrils are packed with fat and with collagen.

We find that grinding bone samples at ambient temperatures gives rise to weak signals comprising signlets at *ca.* $g = 2.002$ which decayed rapidly. However, milling at 77 K followed by e.s.r. measurements at this temperature reveal the generation of intense e.s.r. signals due to at least two different types of trapped radicals[48] (Figure 4). One appears to be a doublet species ($A \sim 38$ G) which is lost initially on warming above 77 K, leaving a second species which is an asymmetric singlet ($g_\parallel = 2.033$, $g_\perp = 2.002$). The former species has an apparent g-value of *ca.* 2.003, but some asymmetry may be present, the maximum possible value being $g_{max} = 2.018$, assuming that $A(^{31}P)$ is isotropic.

The doublet centre has the properties expected for a $PO_4{\cdot}^{2-}$ type radical.[49,50] We

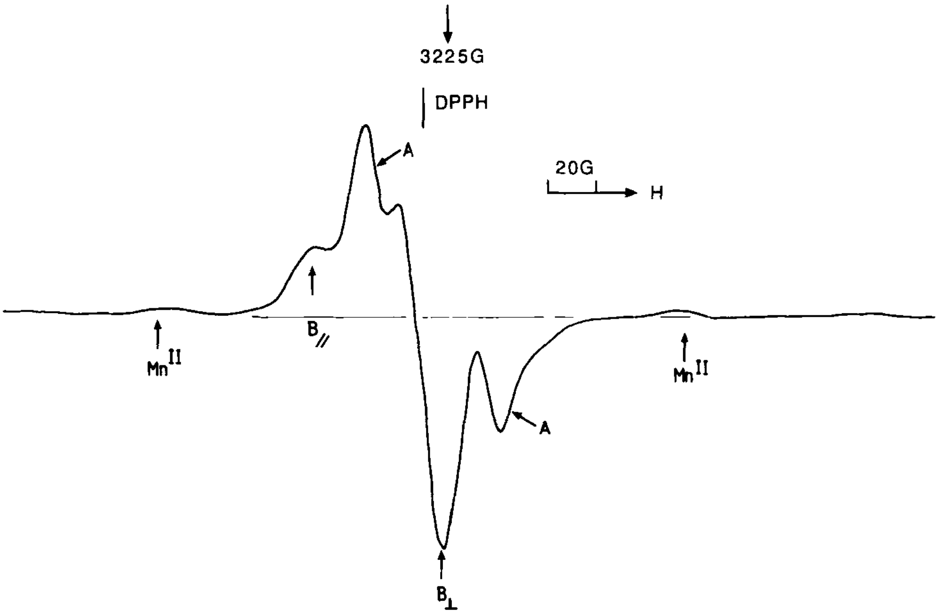


FIGURE 4 First derivative X-band e.s.r. spectrum for a bone sample after grinding at 77 K, and measuring at 77 K without allowing the sample to warm. This sample was almost completely free of any e.s.r. signal prior to grinding. The doublet signal (A) was lost more rapidly than the asymmetric singlet (B) on annealing. Some of the outer features assigned to Mn(II) are indicated.

hope to be able to establish its e.s.r. parameters more firmly using computer subtraction and Q-band spectroscopy.

The asymmetric singlet is characteristic of peroxy radicals, $RO_2\cdot$. These are probably formed from organic radicals, but could conceivably be $O_3PO\dot{O}^{2-}$ type radicals similar to the $O_3SO\dot{O}^-$ radicals formed from persulphate ions.[51] Both radicals decay at room temperature leaving the weak singlet discussed above.

One very curious aspect of these results is the appearance of an unusual sextet of lines probably due to Mn^{2+} ions, but having an unusually low hyperfine splitting (71 G). These features were completely absent prior to grinding, and were not induced on grinding at room temperature. These results are curious since the most likely form of manganese is Mn^{II}, taking the place of Ca^{2+} in the bone structure. The most probable form of manganese that is not e.s.r. active is Mn^{III}, and this could certainly capture electrons to give Mn^{II} on grinding. In that case, the source of manganese is probably the organic components. For example, manganese superoxide dismutase contains Mn^{III}.

I conclude that grinding generates radicals in both the inorganic and organic phases of bone. These are reactive, and are only trapped at low temperatures. It is conceivable that such radicals, being formed on surfaces, could interact destructively with their environment and hence could possibly be a source of molecular damage in joints under extreme mechanical stress.

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