The determination of carbonyls as their 2,4-dinitrophenylhydrazine (DNPH) derivatives in peroxide-containing polymers

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The interference of allylic hydroperoxides in the reaction of 2,4-dinitrophenylhydrazine (DNPH) with oxidized polyisoprene was investigated. During the DNPH analysis, hydroperoxides were susceptible to acid-catalysed Hock cleavage, producing carbonyl compounds which subsequently reacted with DNPH to form additional hydrazone derivatives. Prior reduction of the hydroperoxides with triphenylphosphine (PPh_3) appeared to be a satisfactory method of eliminating this problem, giving more accurate carbonyl levels.

(**Keywords: peroxides; carbonyls; Hock cleavage; 2,4-dinitrophenylhydrazine; polyisoprene**)

INTRODUCTION

2,4-Dinitrophenylhydrazine (DNPH) has been used extensively to measure carbonyl levels in oxidized polymers and edible fats and oils[1–5]. However, Boon[6] recently questioned the validity of using this technique in the presence of allylic hydroperoxides. He demonstrated that the allylic hydroperoxides of 2,6-dimethylocta-2,6Z-diene undergo acid-catalysed Hock cleavage to form carbonyl compounds under typical DNPH reaction conditions. In addition, recent research has showed that in peroxide-containing oils more realistic carbonyl levels can be obtained by reducing hydroperoxides before carrying out the DNPH reaction[7]. This paper reports on the reaction of DNPH with rubber-bound hydroperoxides and assesses the effect of prior reduction with triphenylphosphine (PPh_3) before the DNPH analysis.

EXPERIMENTAL

Materials

A purified soluble natural rubber was prepared from the enzymic deproteinization of Malaysian HA latex[8] followed by extraction of the soluble rubber with petroleum ether (b.p. 30–40°C) and precipitation in methanol. This purification procedure resulted in a soluble deproteinized rubber (sol-DPNR) with a nitrogen content of <0.01 wt%.

Chloroform, dichloromethane, toluene and THF were all HiPerSolv grade (BDH). THF was further purified by passage through an activated alumina column followed by storage over deperoxidizing molecular sieve (Fluka). Traces of carbonyls were removed from toluene by percolation through a celite column impregnated with 2,4-DNPH followed by distillation. Isopropanol was refluxed over stannous chloride and distilled.

Preparation of hydroperoxidized DPNR

Hydroperoxidized DPNR was prepared via singlet oxidation using methylene blue as photosensitizer. In a typical preparation DPNR (3 g) was dissolved in CH_2Cl_2 (300 ml) and methylene blue (20 mg) added. The solution was placed in a water-cooled reaction vessel. A sodium lamp was used as a light source. The oxidized rubber samples could be reduced by the addition of an equimolar amount of PPh_3 (based on moles of polyisoprene repeat unit), which after 1 h at room temperature resulted in the complete reduction of the hydroperoxides as determined by the method below.

Determination of carbonyls in hydroperoxidized DPNR

Hydroperoxidized DPNR (0.1 g), DNPH (0.05 g) and conc. HCl (2 drops) were added to a 1:1 THF–toluene mixture (10 cm^3) and heated under nitrogen for 3 h. Unreacted DNPH was removed by four successive precipitations of the rubber from THF into ethanol. The rubber was dried to constant weight in vacuo at 30°C. Samples could be reduced by swelling an equimolar amount of PPh_3 (based on moles of polyisoprene repeat unit) into the rubber in THF–toluene overnight. The DNPH and acid were then added and the procedure was followed as above. The absorbances of the hydrazone derivatives were measured at 360 nm in THF and a control (DPNR substrate) was used as a reference. Although the molar extinction coefficient was measured for a variety of saturated and unsaturated carbonyl DNPH derivatives, a value of 21289 (DNPH derivative of butanal) was used, since allylic hydroperoxides do not produce α,β-unsaturated carbonyl compounds when they decompose by Hock cleavage. PPh_3 and $OPPh_3$ were found not to interfere in the analysis.

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Determination of hydroperoxides in oxidized DPNR

To a refluxing solution of potassium iodide (0.5 g), propan-2-ol (20 ml), chloroform (20 ml) and glacial acetic acid (2 ml) was added the rubber sample (0.1 g). After refluxing for a further 30 min, the solution was cooled to room temperature and made up to 100 ml with propan-2-ol. The absorbance of this solution was measured at 360 nm using a control (rubber treated as above except no sodium iodide present) in the reference cell. The I_3^- ion was measured at 360 nm using the value of 20643 for the molar extinction coefficient which was obtained for *tert*-butyl hydroperoxide.

RESULTS AND DISCUSSION

During the preparation of hydroperoxidized DPNR, aliquots of oxidized rubber were removed. Half of an aliquot was precipitated in methanol and the other half reduced with PPh_3 for 1 h before precipitation. The samples were then dried at room temperature under vacuum. Hydroperoxide and carbonyl levels were obtained for all samples (*Figure 1*). The hydroperoxide and carbonyl levels in the non-reduced samples increased with increasing reaction time. In samples that were reduced with PPh_3, negligible amounts of hydroperoxides and carbonyls were detected. This suggested that the carbonyls detected in the non-reduced samples did not result from hydroperoxide decomposition during the singlet oxidation reaction.

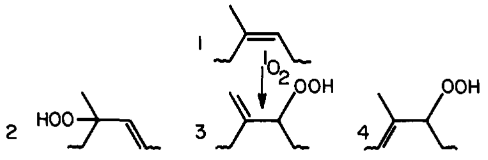
PPh_3 is a commonly used reducing agent and readily converts hydroperoxides to their corresponding alcohols:

$$ROOH + PPh_3 \rightarrow ROH + OPPh_3$$

To confirm that PPh_3 did not react with carbonyls in NR, a selection of carbonyl compounds were dissolved in deuterated 1:1 THF–toluene and their ^1H 300 MHz n.m.r. spectra recorded. An equimolar amount of PPh_3 was then added, the solution was heated at 50°C for 3 h and a further n.m.r. spectrum was recorded to allow any loss to be measured. *Table 1* confirms that the model compounds did not react to any considerable extent under the experimental conditions employed. It is therefore unlikely that PPh_3 reacted with carbonyl groups in the oxidized rubber, so this does not explain the lack of carbonyls in the reduced samples.

These findings agree with those of Chiba *et al.*[7], who reported the quantitative measurement of carbonyls (as their DNPH derivatives) in peroxide-containing oils after reduction with PPh_3.

A closer examination of the results for the non-reduced samples indicates that the concentration of carbonyls was about twice that of peroxides. This is good evidence that the decomposition of the rubber-bound hydroperoxides occurred by Hock cleavage, *Figure 2*, as two carbonyl fragments arise from the decomposition of one hydroperoxide. Boon[6] observed that the tertiary hydroperoxide (2,6-dimethyl-2-hydroperoxyocta-3E,6Z-diene) reacted quantitatively under typical DNPH reaction conditions but that the secondary hydroperoxide (2,6-dimethyl-3-hydroperoxyocta-1,6Z-diene) reacted more slowly. The singlet oxidation of *cis*-polyisoprene, 1, gives the three allylic hydroperoxides 2, 3 and 4[9,10]:



Initial 400 MHz ^1H n.m.r. studies indicated that the three isomers 2, 3 and 4 were formed in the ratio 3:1:1. The results presented here suggest that both secondary and tertiary hydroperoxides undergo Hock cleavage, with the reservation that the carbonyl levels could be slightly lower if a higher value for the molar extinction coefficient were used. It is also appreciated that precipitation of the rubber to remove unreacted DNPH during the carbonyl analysis may also have resulted in the loss of low molecular weight rubber, which may have affected the results.

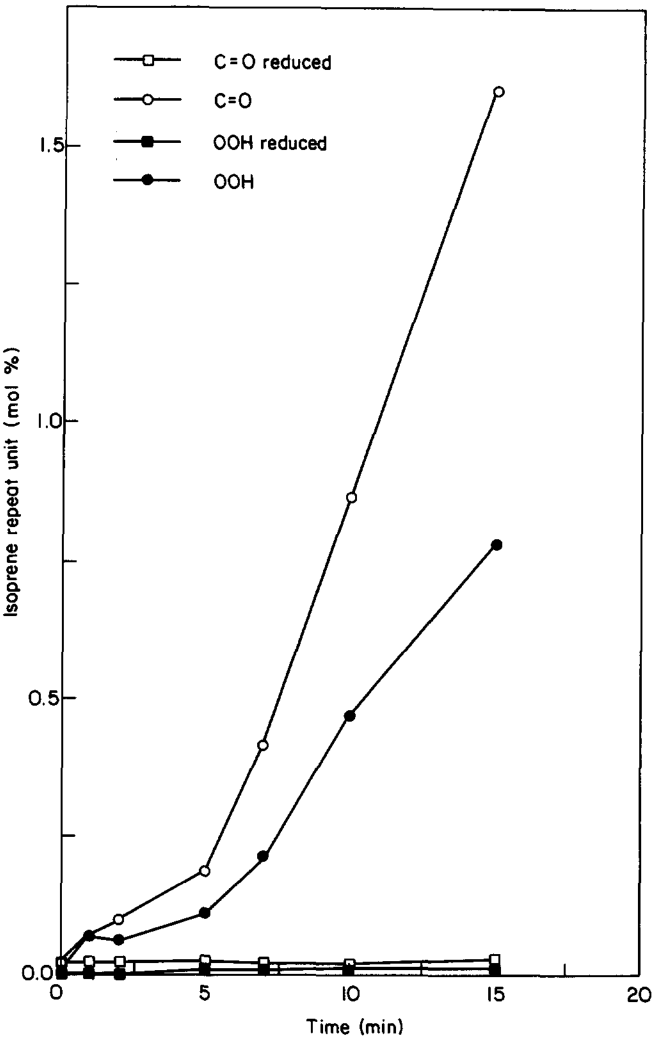


Figure 1 Effect of PPh_3 reduction on carbonyl and hydroperoxide levels of hydroperoxidized polyisoprene (based on moles of isoprene repeat unit)

Table 1 Reactivity of selected carbonyl compounds to PPh_3

Compound	Carbonyl recovered after treatment with PPh_3 (%)[a]
3-Methyl-2-cyclohexene-1-one	98.7
2-Methyl-3-pentanone	99.5
2-Methyl-2-pentenal	99.8
Butanal	100.0

[a] Samples heated for 3 h in deuterated THF–toluene; PPh_3 added at equivalent molar concentration to carbonyl compound

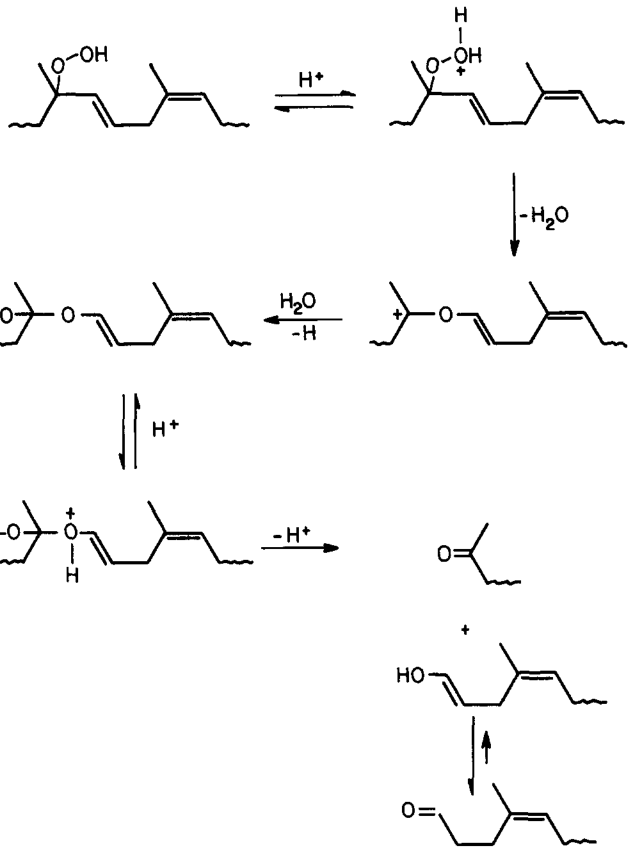


Figure 2 Hock cleavage of the tertiary isomer of hydroperoxidized DPNR. Both secondary isomers decompose by an analogous mechanism

Table 2 Effect of PPh_3 reduction on carbonyl and hydroperoxide levels of nominally unoxidized sol-DPNR

Sample	C=O (mol%)[a]	OOH (mol%)[a]
Sol-DPNR 1	0.038	0.060
Sol-DPNR 1 reduced	0.010	nd[b]
Sol-DPNR 2	0.037	0.061
Sol-DPNR 2 reduced	0.008	nd[b]

[a] Based on moles of isoprene repeat unit
[b] Not detected

To determine the relevance of the above results to nominally unoxidized rubbers, the carbonyl and hydroperoxide levels of two sol-DPNR samples were determined with and without prior reduction with PPh_3. The results, *Table 2*, indicate significant differences between the carbonyl and hydroperoxide levels of reduced and non-reduced samples and can best be explained in terms of peroxide decomposition by Hock cleavage during analysis. These results clearly show that inaccurate carbonyl levels will be obtained if the reduction of hydroperoxides is not undertaken before the DNPH derivatives are synthesized.

CONCLUSIONS

The results demonstrate that rubber-bound allylic hydroperoxides are susceptible to acid-catalysed Hock cleavage under typical DNPH reaction conditions. Reduction of the peroxides with PPh_3 appears to be a satisfactory method of avoiding this problem and should give more reliable carbonyl levels. Although this work was carried out on polyisoprene, the results should be applicable to other polymeric systems.

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