Acetate Peroxyl Radicals, \( \text{O}_2\text{CH}_2\text{CO}_2; \): A Study on the \( \gamma \)-Radiolysis and Pulse Radiolysis of Acetate in Oxygenated Aqueous Solutions

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Dedicated to Professor Dr. Dr. h. c. mult. Günther Wilke on the occasion of his 60th birthday

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Acetic Acid, Peroxyl Radicals, Oxy Radicals, Superoxide Radicals, Pulse Radiolysis

Hydroxyl radicals from the radiolysis of \( \text{N}_2\text{O}/\text{O}_2 \) (4:1 v/v)-saturated aqueous solutions have been reacted with acetate ions (\( 10^{-2} \) M). As measured by pulse radiolysis, the resulting \( \text{CH}_3\text{CO}_2; \) radicals react with oxygen yielding the corresponding peroxyl radicals, \( \text{O}_2\text{CH}_2\text{CO}_2; \) (\( k = 1.7 \times 10^8 \text{ M}^{-1}\text{s}^{-1} \)).

These peroxyl radicals decay bimolecularly (\( 2k = 1.5 \times 10^9 \text{ M}^{-1}\text{s}^{-1} \)) giving rise to the products (G values in brackets) glyoxylic acid (2.7), glycolic acid (0.7), formaldehyde (1.4), carbon dioxide (1.4), organic hydroperoxide (0.7) and hydrogen peroxide (2.5). Oxygen is consumed with a G value of 5.3. Aided by data from pulse radiolysis it is concluded that the intermediate tetroxide formed upon the bimolecular decay breaks down by various routes to yield:

1. hydrogen peroxide and two molecules of glyoxylic acid (ca. 27%);
2. oxygen, glycolic acid and glyoxylic acid (ca. 25%);
3. hydrogen peroxide and two molecules of formaldehyde, carbon dioxide and \( \text{OH}^- \) (25%).

These reactions do not involve free radicals as intermediates;
4. There is some \( \text{O}_2; \) (G = 0.5) formed in the decay of the peroxyl radicals, which is attributed to the decay of intermediate oxyl radicals (tetroxide \( \rightarrow \text{O}_2 + 2 \text{OCH}_2\text{CO}_2; \)) by 1,2-H shift, oxygen addition and \( \text{HO}_2; \) elimination, a reaction sequence which gives rise to glyoxylic acid (10%);
5. The reaction of \( \text{O}_2; \) with the organic peroxyl radical yields the hydroperoxide (13%).

Reaction (iii) is a novel peroxyl radical reaction.

Introduction

In radiation-biological studies it has been shown that oxygen has a strong sensitizing effect on living cells [1], an effect which is also important in the radiotherapy of cancer. This oxygen effect is due to the radiation-induced formation of peroxyl radicals within the cells, very likely at the DNA. In order to elucidate the reactions that may occur in the biological systems, DNA and smaller model systems have been extensively investigated (for a review see [2]) but for detailed product and kinetic studies macromolecules such as DNA are very difficult to handle. Until recently knowledge about the reactions of peroxyl radicals in aqueous solutions was rather limited. We have investigated a large number of low-molecular-weight compounds (cf. Ref. [3] and references cited therein) in order to better understand the mechanistic details of the peroxyl radical reactions. We have now studied the formation and the decay of the acetate peroxyl radical \( \text{O}_2\text{CH}_2\text{CO}_2; \), and it will be shown that this study has again revealed a new reaction pathway of peroxyl radicals.

The acetate peroxyl radicals have been generated radiolytically in \( \text{N}_2\text{O}/\text{O}_2 \)-saturated aqueous solution of sodium acetate. Solvated electrons from the radiolysis of water (reaction (1)) are quantitatively converted to \( \text{OH} \) radicals by \( \text{N}_2\text{O} \) (reaction (2)).

\[
\text{H}_2\text{O} \rightarrow \text{e}^- + \text{OH}^- + \text{H}_2\text{O} (1)
\]

\[
\text{N}_2\text{O} + \text{e}^- \rightarrow \text{OH}^- + \text{OH}^+ + \text{N}_2 (2)
\]

The hydroxyl radicals react with the acetate anion by H abstraction to give the \( \text{CH}_3\text{CO}_2; \) radical [4] (reaction (3)).

\[
\text{CH}_3\text{CO}_2; + \text{OH} \rightarrow \text{CH}_2\text{CO}_2; + \text{H}_2\text{O} (3)
\]

Oxygen present in the solution adds rapidly to the \( \text{CH}_3\text{CO}_2; \) radicals to give the corresponding peroxy radicals [5—7] (reaction (4)) which then react with each other to yield the final products.

\[
\text{CH}_2\text{CO}_2; + \text{O}_2 \rightarrow \text{O}_2\text{CH}_2\text{CO}_2; (4)
\]

H atoms from reaction (1) react only slowly with acetate ions and are therefore effectively scavenged by oxygen to give the \( \text{HO}_2; \) radical (pKₐ = 4.7) [8].
The major products resulting from the reactions of the \( {\text{O}_2\text{CH}_2\text{CO}_2} \) radicals, namely, glyoxylic acid, glycolic acid, formaldehyde and carbon dioxide, have been reported in earlier studies [9–11]. However, these have been carried out when reactions of peroxyl radicals were not well understood. Their experimental results also lack the material balance between measured product yields and the known yields of the radiolytically formed precursor radicals. In the present work we have reinvestigated the sodium acetate system using both product analysis and pulse radiolysis which enable us now to present a more detailed scheme of the processes involved in the decay of the \( {\text{O}_2\text{CH}_2\text{CO}_2} \) radicals.

**Results and Discussion**

**Product studies**

The products and their G values in the radiolysis of sodium acetate in oxygenated solutions are listed in Table I. In oxygen saturated solutions, G\((\text{CH}_2\text{O})\) and G\((\text{CO}_2)\) drop to 0.7, i.e. half the value of that found in the presence of \( \text{N}_2\text{O}/\text{O}_2 \). In accordance with other peroxyl radical systems (cf. [12]), we think that the organic peroxidic material consists mainly of the hydroperoxide HOO\(\text{CH}_2\text{CO}_2 \). Within experimental error there is no dependence of the product yields on the dose rate or pH. No such organic peroxidic material is formed at the high dose rate of pulse radiolysis, neither in \( \text{N}_2\text{O}/\text{O}_2 \) nor in \( \text{O}_2 \)-saturated solutions at pH 7.8.

No carbon dioxide is formed in the absence of oxygen. This observation excludes an electron transfer of the acetate ion to the hydroxyl radical followed by a fragmentation of the so-formed methylcarboxyl radical into carbon dioxide and a methyl radical. This indicates that reaction (3) is the only reaction of OH with the acetate ion.

**Pulse radiolysis**

We have been able to confirm most of the earlier pulse radiolysis data concerning the \( \text{CH}_2\text{CO}_2 \) and \( \text{O}_2\text{CH}_2\text{CO}_2 \) radicals [4, 6, 7]. The \( \text{CH}_2\text{CO}_2 \) radical is observed immediately at pulse end in the pulse radiolysis of oxygen-free \( \text{N}_2\text{O} \)-saturated solutions of sodium acetate. It has a broad transient absorption spectrum centered at 350 nm \( (\varepsilon = 750 \pm 70 \text{ M}^{-1}\text{cm}^{-1}) \). The absorption at 350 nm disappears by second-order kinetics with \( 2k = (1.2 \pm 0.2) \times 10^8 \text{ M}^{-1}\text{s}^{-1} \).

In the pulse-irradiated \( \text{N}_2\text{O}/\text{O}_2 \) \((4:1 \text{ v/v})\)-saturated solutions of sodium acetate the absorption of the \( \text{CH}_2\text{CO}_2 \) radical around 350 nm decays rapidly to give rise to a new transient absorption with a maximum around 280–290 nm as \( \text{CH}_2\text{CO}_2 \) radicals are converted to the peroxyl radicals \( \text{O}_2\text{CH}_2\text{CO}_2 \) (reaction (4)). The rate constant of reaction 4 obtained from the buildup of absorption at 275 nm \( (k_4 = (1.7 \pm 0.4) \times 10^9 \text{ M}^{-1}\text{s}^{-1}) \) is in agreement with the value published by Abramovitch and Rabani [6]. The extinction coefficient of the \( \text{O}_2\text{CH}_2\text{CO}_2 \) radical at 280 nm is \( (730 \pm 70) \text{ M}^{-1}\text{cm}^{-1} \) after corrections for the absorption of \( \text{O}_2 \) radical \( (G(\text{O}_2) = 0.55) \) have been made.

The transient absorption of the peroxyl radicals \( \text{O}_2\text{CH}_2\text{CO}_2 \) at 280 nm decays by a second-order process with \( 2k = (1.4 \pm 0.3) \times 10^8 \text{ M}^{-1}\text{s}^{-1} \) at pH 7.8–10.0 for G(peredoxyl radicals) = 5.8. The rate constant is attributed to the bimolecular decay of the \( \text{O}_2\text{CH}_2\text{CO}_2 \) radicals (reaction (5)). A higher value \( 2k = 5 \times 10^8 \text{ M}^{-1}\text{s}^{-1} \) has been obtained by Josimovic et al. [7]. After this bimolecular decay there remains an absorption of about 30% of the original one. At pH 10.0 \( \text{O}_2 \) decays only very slowly [8]. The residual absorption of the pulse-irradiated acetate solution at 250–290 nm after the decay of \( \text{O}_2\text{CH}_2\text{CO}_2 \) taken 10 ms after the pulse should be a measure of the products including \( \text{O}_2 \). Since all the products listed in Table I have only negligible absorption in this wavelength region the absorptions observed 10 ms after the pulse can be attributed mainly to \( \text{O}_2 \). From the known extinction coefficients of \( \text{O}_2 \) at these wavelengths [8] an upper limit of \( G(\text{O}_2) \) of 1.5 is obtained which includes \( \text{O}_2 \) from the primary H atoms. In the earlier pulse radiolysis

<table>
<thead>
<tr>
<th>Products</th>
<th>( \gamma )-Radiolysis</th>
<th>( \gamma )-Radiolysis</th>
<th>Electron pulses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 7.8</td>
<td>10.5</td>
<td>7.8</td>
</tr>
<tr>
<td>Glyoxylic acid</td>
<td>2.7</td>
<td>2.5(^a)</td>
<td>2.6</td>
</tr>
<tr>
<td>Glycolic acid</td>
<td>0.7</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>1.4</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>1.4</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Organic (hydro)peroxide</td>
<td>0.7</td>
<td>n.d.</td>
<td>0.0</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>2.5</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Oxygen uptake</td>
<td>5.3</td>
<td>5.0</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

\(^a\) Value corrected for the fast decomposition of glyoxylic acid in the presence of \( \text{H}_2\text{O} \) in basic solutions; n.d. = not determined.
studies [6, 7] even less O_{2}^\cdot (G \leq 0.4) was found to result from the bimolecular decay of O_{2}CH_{2}CO_{2} radicals under similar conditions.

We have also determined the yield of O_{2}^\cdot formation from the bimolecular decay of O_{2}CH_{2}CO_{2} radicals using tetranitromethane (TNM). The latter is reduced effectively by O_{2}^\cdot to the nitroform anion (NF\cdot) which has a strong absorption band with a maximum at 350 nm. Both the CH\cdot\cdot\cdot and O_{2}CH_{2}CO_{2} radicals do not reduce TNM at observable rates under our pulse radiolysis conditions. Thus in the pulse-irradiated N_{2}O/O_{2}-saturated solutions of sodium acetate (pH 7.8-10) containing 0.06-0.2 mM TNM, the formation of NF\cdot at 300—370 nm was found to take place in two stages. The initial fast increase of absorption (6—10 \mu s after the pulse) can be accounted for by the reaction of primary H atoms (and some 3% of e_{aq}) reacting directly with TNM to give G(NF\cdot) = 0.6 plus the absorption of the peroxyl radical O_{2}CH_{2}CO_{2}. This fast process was followed by a slower buildup of NF\cdot with approximately the same rate constant as the bimolecular decay of O_{2}CH_{2}CO_{2}. At the final plateau level G(NF\cdot) = 1.2 was obtained. Thus the net yield of NF\cdot corresponds to G(O_{2}^\cdot) = 0.5 from the bimolecular decay of O_{2}CH_{2}CO_{2}; in good agreement with the earlier report [6, 7] of G(O_{2}^\cdot) \leq 0.4.

In order to ensure that the TNM method yields reliable data for intermediate O_{2}^\cdot it has been tested whether NF\cdot might react with peroxyl radicals. Using O_{2}^\cdot-saturated solutions containing acetate and TNM (10^{-4} M) resulted in the immediate formation of large yields of NF\cdot due to the high yield of O_{2}^\cdot present in that system. No depletion of NF\cdot was observed. Another important question is whether O_{2}^\cdot and acetate peroxyl radicals react with one another (cf. reaction (17)) at the relative concentrations attained in pulse radiolysis experiments. Using again oxygen-saturated solution and monitoring O_{2}^\cdot by its absorption at 260 nm it has been shown that no significant depletion is observed within 10 ms due to such a reaction while at the mean time the acetate peroxyl radicals (monitored at 300 nm) decayed bimolecularly with the rate constant stated above. This observation is in good agreement with the fact that under pulse radiolysis conditions no organic hydroperoxides are formed.

In pulse conductivity experiments (10^{-2} M acetate, pH 10.0 and 10.7) it was observed that directly after the pulse the conductivity dropped due to the formation of H\cdot and O_{2}^\cdot with subsequent neutralisation of the proton by OH\cdot (i.e. substitution of OH\cdot, λ_{0} = 198 \Omega^{-1} cm^{-1} equiv^{-1} by O_{2}^\cdot, λ_{0} = 65 \Omega^{-1} cm^{-1} equiv^{-1} at 25 °C). This drop was followed by an increase in conductivity (Fig. 1) which was of second-order kinetics (2k \approx 1.5 \times 10^{8} M^{-1}s^{-1} for G(RO_{2}) = 5.8 dose rate region 5—30 Gy per pulse). Subsequent to this buildup a first-order decrease in conductivity is observed (Fig. 2). The kinetics at the two pH values agree with that of the reaction of CO_{2} with OH\cdot [13].

A quantitation of these data taking the pK_{a} = 10.3 of

\begin{align*}
\Delta \chi / 10^{-8} \Omega^{-1} cm^{-1} & \\
\text{Time} & \\
\text{Pulse} & \\
0.76 ms & \\
\text{Fig. 1. Conductivity change following a 1 \mu s electron pulse of 25 Gy in a N_{2}O/O_{2} (4:1 v/v)-saturated 10 mM aqueous solution of sodium acetate at pH 10.7.} \\
\Delta \chi / 10^{-8} \Omega^{-1} cm^{-1} & \\
\text{Time} & \\
\text{Pulse} & \\
0.38 s & \\
\text{Fig. 2. Conductivity change following a 1 \mu s electron pulse of 26 Gy in a N_{2}O/O_{2} (4:1 v/v)-saturated 10 mM aqueous solution of sodium acetate at pH 10.7.}
\end{align*}
the bicarbonate ion into account yields $G(CO_2) = 1.4$ in agreement with the measured value (Table I).

**Reaction mechanism**

The products listed in Table I result essentially from the bimolecular self-termination of the $'$O$_2$CH$_2$CO$_2^-$ since it is the only major radical species present. In the Scheme (reactions (5)—(16)) we have presented the various reaction paths to explain the formation of the observed products.

It is generally accepted that the self-termination of peroxyl radicals results in the formation of a short-lived tetroxide which either decomposes to the products or reverts to the parent peroxyl radicals. The observed second-order rate constant ($2k = 1.5 \times 10^8$ M$^{-1}$s$^{-1}$) is therefore a composite of the forward (reaction (5)), reverse (reaction (−5)) and decomposition reactions (6)—(9). In the decay of the intermediate tetroxide two types of reaction are discussed, concerted reactions and reactions involving free radicals as intermediates. The so-called Russell mechanism which is considered to be a concerted process (reaction (6)) has also its free radical equivalent (reactions (9), (14)—(16) (cf. Ref. [16]). The yield equal amounts of glyoxylate, glycolate and oxygen. Since glyoxylate ($G = 2.7$) is formed in large excess over glycolate ($G = 0.7$), further reactions must take place, either by a concerted route (reaction (7)) (cf. Ref. [14, 15]) or its free radical equivalent (reactions (9), (14)—(16)) (cf. Ref. [16]). The free radical pathway involves a 1,2-H shift of the oxyl radical (reaction (14)) [16–20] followed by the addition of oxygen to the so-formed $\alpha$-hydroxyalkyl radical (reaction (15)). These $\alpha$-hydroxyalkylperoxyl radicals are well known to eliminate spontaneously or OH$^-$-induced HO$_2$/O$_2^-$ (reaction (16), for a review see Ref. [21]). Both processes can account for the excess glyoxylate, but they can be distinguished, because the free radical pathway yields HO$_2$/O$_2^-$ (which can be monitored) whereas the concerted process does not. Only very little O$_2^-$ is formed, hence the majority of the excess glyoxylate ($G = 2.0$) must be formed in the concerted process (reaction (7)).

A combination of the oxyl radicals (reaction (11)) must also be considered. This reaction yields dialkylperoxides which are often very difficult to determine. This pathway is usually not very important as has been shown in a case where the determination of the dialkyl peroxide was straightforward [12]. Material
balance considerations (see below) allow to conclude that this also applies to the present system.

In competition to reactions (10), (11) and (14) the oxyl radical could undergo β-scission (reaction (12)) giving rise to formaldehyde and the carboxyl radical. The latter is known to transfer rapidly an electron to oxygen yielding carbon dioxide and \( \text{O}_2 \) (reaction (13)). Thus this reaction might be another source of \( \text{O}_2 \) besides reaction (15). Since only small amounts of \( \text{O}_2 \) have been detected as the result of the bimolecular reaction of \( \text{O}_2 \text{CH}_2\text{CO}_2 \), \( G(\text{O}_2) \approx 0.5 \), which has to be shared between the two reactions (13) and (16), and the \( G \) values of carbon dioxide and formaldehyde are 1.4, there must be some other reaction pathway leading to carbon dioxide and formaldehyde. We therefore propose a new type of peroxyl radical reaction, a concerted process which leads directly to the desired products without the formation of \( \text{O}_2 \) (reaction (8)).

Such a reaction could be written as a concerted process with a six-membered transition state as shown by the protonated form of the tetroxide (A). This route would yield formaldehyde, hydrogen peroxide and carbon dioxide. The schematic representation of A is only a heuristic one. The process might well proceed stepwise and may involve an electron rather than an H-transfer. There is another possibility which involves a relay of water molecules (B). This pathway would result in the formation of bicarbonate ions instead of carbon dioxide. Pulse radiolysis with conductivity detection can distinguish between these two routes, because reaction (8) releases \( \text{OH}^- \) which causes the conductivity increase shown in Fig. 1. Only at the later stage \( \text{CO}_2 \) is converted to bicarbonate in a rather slow reaction (Fig. 2). The fact that identical \( G \) values for free \( \text{CO}_2 \) were obtained indicate that the decay of the tetroxide does not involve a relay of water molecules as depicted by B. However it is not possible to fully exclude a minor contribution (\( G < 0.5 \)) of reaction (13) to \( \text{CO}_2 \) formation on the ground of the observed net increase in conductivity (cf. Fig. 1). This is due to the fact that \( \text{O}_2 \) might not only be formed in reaction (13) but also in reaction (16). It is interesting to note that in the present system the fragmentation reaction does not (or only to a very minor extent) involve free oxyl radicals. This observation has its analogy in the cyclopentylperoxyl radical system where the important fragmentation process also does not involve free oxyl radicals [3].

A small fraction of \( \text{O}_2 \text{CH}_2\text{CO}_2 \) is probably scavenged by \( \text{O}_2 \) to give the organic (hydro)peroxide observed (reaction (17)).

\[
\text{O}_2 \text{CH}_2\text{CO}_2 + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{HOOCH}_2\text{CO}_2 + \text{O}_2 + \text{OH}^- \tag{17}
\]

Although initially only about 10% of the total radicals are \( \text{O}_2 \), some more \( \text{O}_2 \) are formed in reactions (13) and (16). At pH 7.8, \( \text{O}_2/\text{HO}_2 \) disappears only slowly by self-termination \( (k \approx 10^7 \text{ M}^{-1}\text{s}^{-1}, \text{cf. Ref. [8]}) \); under γ-radiolysis conditions \( \text{O}_2 \) can build up to a considerable steady-state concentration for reaction (17) to compete with reaction (5). The rate of reaction of \( \text{O}_2 \) with the organic peroxy radical must be slow \( (k < 10^7 \text{ M}^{-1}\text{s}^{-1}, \text{cf. also Ref. [22]}) \) because under pulse radiolysis conditions no organic peroxy is formed even if the \( \text{O}_2 \) concentration is considerably increased by irradiating \( \text{O}_2 \)-saturated instead of \( \text{N}_2\text{O}/\text{O}_2 \)-saturated solutions.

**Quantitative aspects**

Full materials balance has been achieved between the OH radical scavenged by the acetate ions \( G(\text{OH}) = 5.3 \) [23]) and the products. Following the mechanistic scheme one can calculate \( G(\text{H}_2\text{O}_2) \) and \( G(\text{O}_2 \text{ consumption}) \). These values agree within 10% with the measured values. This good material balance together with the information obtained from the pulse radiolysis study allows us to draw conclu-
sions with respect to the contribution of the various reaction routes. There is one uncertainty concerning the attribution of the small amounts of O$_2^·$ formed in the course of the bimolecular decay of the peroxyl radicals. If one attributes this to reaction (16) rather than to reaction (13) the following distribution is obtained: Of about equal importance are reactions (6) plus (10) (25%), reaction (7) (27%) and reaction (8) (25%); hydroperoxide formation (reaction (17)) contributes about 13% leaving 10% for reaction (16).

**Experimental**

Aqueous 10$^{-2}$ M sodium acetate (Merck, p.A.) solutions (pH = 7.8) which had been made up with triply distilled water were saturated with N$_2$/O$_2$ (4:1, v/v) or pure oxygen and irradiated at room temperature at a dose rate of 0.26 Gys$^{-1}$. For irradiations at high dose rates, a sequence of 1 μs electron pulses from a van-de-Graaff accelerator was used (2 GY/pulse, frequency 1 Hz). The absorbed doses were between 78—310 Gy, i.e. conversions were kept below 5%. Dose rates were determined by using the Fricke dosimeter.

Gas chromatographic analysis of glycolic acid as TMS ester was performed on the Packard 427 gas chromatograph equipped with a 25 m SE-54 capillary column and a flame ionization detector. The column was operated at 50—250°, at 5° min$^{-1}$. The carrier gas was H$_2$ at 1 ml min$^{-1}$; i.e. conversions were kept below 5%. Dose rates were determined by using the Fricke dosimeter.

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Total peroxide was determined iodometrically according to Allen et al. [27]. Organic (hydro)peroxides were determined by the same method after H$_2$O$_2$ had been decomposed by addition of catalase. Carbon dioxide was determined by gas chromatography after catalytic reduction to CH$_4$ [28]. Oxygen uptake was measured with an oxygen-sensitive electrode (Wiss. Techn. Werkst., Weilheim, Germany).

Pulse radiolysis was carried out using a 2.8 MeV van-de-Graaff electron generator. The electron pulses were of 0.4—2 μs duration with doses ranging from 5—40 Gy. Conductivity change was monitored by an AC-operated two-cell bridge as described by Asmus and Janata [29]. The frequency of the 40 V-applied voltage is 10 MHz. Dosimetry was performed with a 0.1 M tert.-butanol solution saturated with CH$_3$Cl for which G(HCl) = 3.1 [30]. The optical detection technique and data processing procedure have been described previously [31]. Dosimetry for optical measurements was performed with a N$_2$O-saturated 10 mM KSCN solution taking ε(480 nm) = 7600 M$^{-1}$cm$^{-1}$ and G = 6.0 for (SCN)$^-$.