1,3,5-TRICYANOBENZENE – A MUTAGENIC BY-PRODUCT OF CARBON FIBER PRODUCTION IN PYROLYSIS OF POLY(ACRYLONITRILE-CO-METHYL METHACRYLATE)

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A solid by-product formed as deposits in the pyrolysis process of poly(acrylonitrile-co-methyl methacrylate) was analyzed. Two main components, namely ammonium sulphate and 1,3,5-tricyanobenzene were identified by IR, GC-IR, TG-MS and XRD techniques. The major organic components, 1,3,5-tricyanobenzene was crystallized from acetone extract as colourless needles. 1,3,5-Tricyanobenzene proved to be a mutagenic active agent by bacterial (Salmonella typhimurium TA98 and TA1537) reverse mutation assay.

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Introduction

Pyrolysis of poly(acrylonitrile-co-methyl methacrylate) derivatives is the most common/simple method for carbon fibre manufacture[1]. A sponge-like pale yellow compound with unknown composition and beneficial effect, deposited on ceiling of the polymer pyrolysis industry, has been analyzed to get an insight about its chemical composition and biological effects.

Experimental

The samples were collected from the ceiling of a industry where pyrolysis of the poly(acrylonitrile)-co-poly(methyl methacrylate) fibres were performed. The yellow coloured solid has polyurethane-foam like characteristics were collected and extracted with various solvents, and the evaporation residues were analyzed with TG-MS, IR and XRD techniques.

10 g of each sample was extracted with 200 ml of solvent (water, methanol, ethanol, toluene, dichloromethane, chloroform, acetone and acetonitrile) with rigorous stirring at room temperature for 2 h followed with a reflux for 10 min. The extracts were evaporated and the residue was studied by IR and XRD techniques. The evaporation residue was recrystallized with acetone. The nest-like aggregates of long needles were formed. The organic residue was dissolved in acetone and GC-IR and GC-MS studies were done on this solution.

X-ray powder diffraction measurements were performed by means of a Philips PW-1050 Bragg-Brentano parafocusing goniometer, equipped with a secondary beam graphite monochromator and proportional counter; scans were recorded in step mode by using CuKα radiation at 40 kV and 35 mA tube power. Evaluation of the diffraction patterns have been obtained by full profile fitting techniques.

Solid state IR spectra have been obtained by a Biorad-Digilab FTS-45 FT IR spectrometer in the 4000-400 cm⁻¹ region in KBr pellet at room temperature. GC-IR measurements were performed on a Thermo Finnigan TRACE GC combined with a Nicolet Magna 750 FTIR instrument supplied with a liq. N₂ cooled MCT-A (Hg₉Cd₁₋₉Te) detector. The measurements were performed under isotherm conditions (200 °C) or with temperature programmed mode (40-200 °C with 40°C min⁻¹ heating rate) on an RTX-5 crossbond Restek (30 m, 0.32 mm, 1 µm, 5% diphenyl-95% dimethylpolysiloxane) column.

TG-MS measurements were accomplished by a STD 2960 Simultaneous DTA/TGA (TA Instruments) + Thermostar GSD 200 Q-MS (Balzers) device with 10 °C heating rate in Ar or Ar-10%O₂ flow. Ammonium content was determined with standard method[2] after treatment with sodium sulphide (Na₂S).

GC-MS measurements were performed on a VG ZAB2-SEQ Tandem mass spectrometer (VG Analytical, UK) coupled with a HP 5890A gas chromatograph. Column parameters: Restek Rtx-5Am (low polarity phase; crossbond 174; 5% diphenyl/95% dimethyl polysiloxane), 30mx0.25 mm with 0.25 µm film thickness. The measurements were done under isothermal conditions (200 °C) or with a 65–220 °C temperature range with 10 °C min⁻¹ heating rate, using He as carrier gas with 2 ml min⁻¹ flow rate. Mass spectra were scanned with 1.5 sec cycle time in mass range of m/z 25-520. Electron impact (70 eV) ionization was used. The samples were analyzed in acetone solution.
Mutation tests were performed by a standard plate incorporation procedure as an initial mutation test. The experiments were carried out using aqueous as well as DMSO extracts and histidine requiring auxotroph strains of Salmonella typhimurium TA98, TA100, TA1535, TA1537 and tryptophan-requiring auxotroph strain of Escherichia coli WP2 uvrA. Bacteria were exposed to the test item both in the presence or absence of an appropriate metabolic activation system. Molten top agar was prepared and kept at 45 °C. 2 ml of top agar was aliquoted into individual test tubes (3 tubes per control or concentration level). The equivalent number of minimal glucose agar plates was properly labelled. The test item and other components were prepared fresh and added to the overlay (45 °C). Each tube contained top agar (2000 µL, solvent or dilution of test item extracts or positive controls 50 µL, phosphate buffer (pH=7.4) or S9 mix (post mitochondrial supernatant) 511 µL. This solution was mixed and poured on the surface of minimal agar plates. For activation studies, instead of phosphate buffer, 0.5 ml of the S9 mix was added to each overlay tube. The entire test consisted of non-activated and activated test conditions and each of them with the addition of negative and positive controls. The plates were incubated at 37 °C for 48 h. The colony numbers on the control, positive control and the test plates were determined. The mean number of revertants per plate, the standard deviation and the mutation factor values were calculated for each dilution “concentration” level of the test item and also for the controls.

Discussion

The pale yellow coloured sponge-like, light-weight material so collected was non-uniform in character. The deposit showed porous structure with holes of different sizes (small and large) and distribution of well-defined heterogeneity could be observed in the tissue structure.

Identification of chemical nature

The IR spectroscopic results of the powdered deposit sample showed characteristic bands belong to ammonium ion (ν(as)(NH) 3232 cm⁻¹; δ(NH) 1415 cm⁻¹, ρ(NH) 616), sulphate ion (ν(as)(SO) 1092), aromatic CH groups (ν(CHaromatic) 3083) and covalently bound cyano groups (ν(CN) 2249 cm⁻¹). The bands of expected triazines and nitrates could not be detected at all. Extraction experiments were carried out with a series of solvents and the results of leaching can be seen in Table 1.

Table 1. Leaching results of homogenized deposit samples in various solvents at room temperature

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Residue, wt.%</th>
<th>Dissolved part, wt.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>38</td>
<td>62</td>
</tr>
<tr>
<td>Methanol</td>
<td>42</td>
<td>58</td>
</tr>
<tr>
<td>Ethanol</td>
<td>49</td>
<td>51</td>
</tr>
<tr>
<td>Toluene</td>
<td>50</td>
<td>41</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>44</td>
<td>56</td>
</tr>
<tr>
<td>Chloroform</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>Acetone</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>32</td>
<td>68</td>
</tr>
</tbody>
</table>

TG-MS studies on homogenized deposit sample were performed in Ar-10% O₂ (oxidative) and in pure argon (inert) atmosphere and the results can be seen in Fig.1. and summarized in Table 2.

Table 2. Mass changes during thermal decomposition of homogenized deposited sample in inert (Ar) or oxidative (Ar-10 vol.% O₂) atmosphere between 25 and 900 °C.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Δm25-400 °C</th>
<th>Δm25-700 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inert</td>
<td>89 %</td>
<td>92 %</td>
</tr>
<tr>
<td>Oxidative</td>
<td>90 %</td>
<td>99 %</td>
</tr>
</tbody>
</table>

Three peak pairs were occurred with m/z=17 and 16 fragment masses (NH₃ and NH₂ or OH and O, respectively) at TG-MS curve (Fig.1) in inert atmosphere. Two un-separated sharp peaks and a separated one were observed with maximums between 160-170 °C, 220-250 °C and 330-340 °C, respectively. The appearance of m/z=17 and 16 peaks were almost simultaneously with the appearance of m/z=44 (N₂O or CO₂) peak both in the 160-170 and 220-250 °C temperature range. The peak with m/z=64 (SO₂) could be detected between 220 and 250 °C and 340-360 °C. Fragments with m/z=75 (C₃H₅) and m/z=100 (C₆H₂(CN)₂) appeared in the second decomposition step (220-250 °C) unambiguously showed that the organic component(s) decomposed in this temperature range.

Fig.1. TG-MS curves of yellow sponge-like deposit material in Ar

In oxidative atmosphere, the organic component(s) were almost completely ignited whereas the inorganic component(s) were decomposed without the formation of important amount residues. The XRD study on the 1% residue observed, showed the presence of CaO, CaSO₄ (anhydride), and two modifications of SiO₂ (quartz and cristobalite) which probably derived from the upper layer of the ceiling deposits.

Evaporation of the aqueous extract resulted a colourless crystalline mass identified unambiguously as ammonium sulphate by IR and XRD. The ammonium sulphate content of various deposit samples collected was varied between 20-34 %. Ammonium sulphate decomposition proceeds in a complicated reaction route via consecutive decomposition steps \(^5\) without formation of any solid residue: The formal summarized equation is:

\[
\text{(NH}_4\text{)}_2\text{SO}_4 = 2\text{NH}_3 + \text{H}_2\text{O} + \text{SO}_2 + \text{‘O’}
\] 

(1)

Considering the available data about thermal decomposition of (NH\(_4\)\(_2\)SO\(_4\), the \(m/z\)=17 and 16 fragment pairs appeared in the first two decomposition steps (160-170 °C and 220-250 °C) belong to NH\(_3\) and NH\(_2\) fragments, however, the \(m/z\)=17 and 16 signals occurred at 350 °C probably belong to OH and O fragments. The first decomposition step is a simple NH\(_3\) loss with formation of NH\(_4\)HSO\(_4\), and the second step together with NH\(_3\), SO\(_2\) and oxygen formation belongs to further decomposition of NH\(_4\)HSO\(_4\). Sulphonation by-reaction of the aromatic ring by SO\(_3\) formed in situ by decomposition of ammonium pyrosulphate intermediate\(^5\) may also be occurred. This could explain the presence of HO and O fragments without H\(_2\)O peaks at 330-340 °C due to desulphonation of the C-SO\(_2\)-OH fragment. Since no decomposition of organic compound(s) occurred at 160-170 °C, the \(m/z\)=44 band probably orginied from ammonium sulphate decomposition, thus it had to be belonged to N\(_2\)O formation. The \(m/z\)=44 peak at 220-250 °C, however, probably belong to CO\(_2\) because the oxidation of the organic component(s) can be occurred by the oxygen formed (Eqn. (1)) from the decomposition of ammonium sulphate, furthermore, other organic fragments can also be occurred in this temperature range.

\[
\begin{align*}
\text{Solid} &
\begin{array}{c}
\text{Gas phase} \\
\text{Authentic} \\
\text{Assignation}
\end{array} \\
459 & \text{ - } & 461 & \text{C,C,CH} \\
674 & 676 & 674 & \text{C,C,CH} \\
910 & 899 & 910 & \text{CH} \\
931 & 928 & 931 & \text{CNC,C} \\
1430 & 1426 & 1429 & \text{CN} \\
2249 & 2250 & 2250 & \text{CN} \\
3083 & 3086 & 3086 & \text{CH}
\end{align*}
\]

Table 3. IR spectral data of organic component found in deposit material and 1,3,5-tricyanobenzene.

The vibrational bands found in the IR spectrum of the only component separated by GC, was unambiguously show the presence of an aromatic ring and cyano groups. The residue formed by evaporation of the extract was recrystalized with acetone when a nest-like mass of colourless needles were formed. The IR spectrum of this compound completely agree/match with the IR spectrum of authentic 1,3,5-tricyanobenzene. The amount of this compound was varied between 64-78 % in each sample. The main characteristic bands of the isolated crystalline compound and its gaseous form can be seen in Table 3.

![Fig.2 GC-IR of organic component found in the analyzed deposit material](image)

Although acetonitrile proved to be the best solvent to remove the organic components, however, in order to avoid of interferences between the spectroscopic signals of nitrile group in MeCN and nitrile group of the unknown organic component, in the next experiments acetone was used. Surprisingly only one peak could be observed on the GC-MS chromatogram of the acetone extract at 2.51 min retention time (isothermal study), or at 13.7 min in the temperature programmed measurement. Similarly, only one peak was appeared in the GC-IR around 3.7 min (isothermal measurement) retention time.

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\text{DOI: 10.17628/ecb.2012.1.94-97}
\]
Results of mutagenecity studies

Mutation tests carried out with DMSO (1,3,5-tricyanobenzene) extracts of the deposit material using histidine-requiring auxotroph strains of *Salmonella typhimurium TA98* and *TA1537* exhibited mutagenic effect. The DMSO extracts contained 1,3,5-tricyanobenzene induced gene mutations by frameshifts in the genome of the used *Salmonella typhimurium TA98* and *TA1537*.

Conclusion

Based on IR, XRD, GC-MS, GC-IR and TG-MS methods the deposited material formed during pyrolysis of poly(acrylonitrile-co-methyl methacrylate) proved to be a mixture of ammonium sulphate 20-34 % and 1,3,5-tricyanobenzene 64-78 %. 1,3,5-Tricyanobenzene probably formed in the redox reaction of the liberated acrylonitrile and ammonium peroxodisulphate as a result of an oxidative cyclotrimerization reaction. 1,3,5-tricyanobenzene shows mutagenic activity on the growth of *Salmonella typhimurium TA98* and *TA1537* strains.

References


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