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

László Kótai[a,b]*, Ágnes Gömöry[a] and Pradeep K. Sharma[c]

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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.

* Corresponding Authors
Fax: +36-23-376-456
E-Mail: kotail@chemres.hu
[a] Research Centre for Natural Sciences, Hungarian Academy of Sciences, H-1025, Pusztaszeri u. 59-67, Budapest, Hungary;
[b] Deuton-X Ltd., H-2030, Érd, Selmeci u. 89, Hungary
[c] J. N. V. University, Department of Chemistry, 342 005, Jodhpur, Rajasthan, India

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.

Experimentals

The samples were collected from the ceiling of a industry where pyrolysis of the poly(acryl nitrile)-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 CuK_a 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. N_2 cooled MCT-A ($Hg_xCd_{1-x}Te$) 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%O_2 flow. Ammonium content was determined with standard method[2] after treatment with sodium sulphide (Na_2S).

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, overnight culture of testers train 100 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[3] .

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 characteristics 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 ($\nu(CH_{aromatic})$ 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

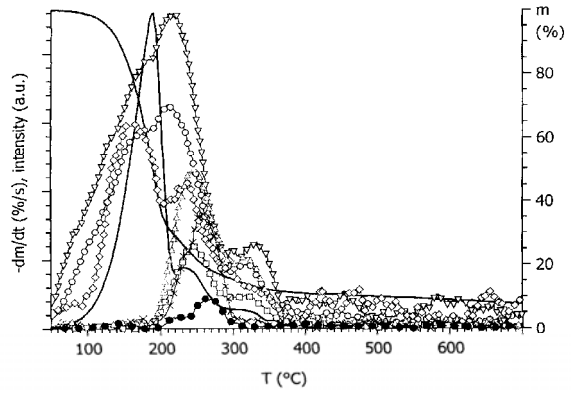
Solvent	Residue, wt.%	Dissolved part, wt. %
Water	38	62
Methanol	42	58
Ethanol	49	51
Toluene	59	41
Dichloromethane	44	56
Chloroform	45	55
Acetone	34	66
Acetonitrile	32	68

TG-MS studies on homogenized deposit sample were performed in Ar-10% O_2 (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.% O_2) atmosphere between 25 and 900 °C.

Conditions	$\Delta m_{25\text{-}400\ °C}$	$\Delta m_{25\text{-}700\ °C}$
Inert	89 %	92 %
Oxidative	90 %	99 %

Three peak pairs were occurred with *m/z*=17 and 16 fragment masses (NH_3 and NH_2 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 (N_2O or CO_2) peak both in the 160-170 and 220-250 °C temperature range. The peak with *m/z*=64 (SO_2) could be detected between 220 and 250 °C and 340-360 °C. Fragments with *m/z*=75 ($C_3H_3^+$) and *m/z*=100 ($C_6H_2(CN)_2^+$) appeared in the second decomposition step (220-250 °C) unambiguously showed that the organic component(s) decomposed in this temperature range.



G_0= 0.576 mg (0.587 mg), 10 °C/min, Ar: 140 ml/min

Curve	Maximum	Scale
G [%]	100.1	100.1
DTG [%/s]	0.2298	0.2321
m/z 16 (NH_2)[/mg]	65.01	92
m/z 17 (NH_3)[/mg]	91.93	92
m/z 44 (CO_2)[/mg]	28.87	45
m/z 48 (SO)[/mg]	7.710	30
m/z 64 (SO_2)[/mg]	14.94	30
m/z 75 [/mg]	2.169	6
m/z100 [/mg]	0.6321	6

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, $CaSO_4$ (anhydride), and two modifications of SiO_2 (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 [4] without formation of any solid residue: The formal summarized equation is:

$$(NH_4)_2SO_4 = 2NH_3 + H_2O + SO_2 + \text{'O'} \qquad (1)$$

Considering the available data about thermal decomposition of $(NH_4)_2SO_4$, 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_4HSO_4, and the second step together with NH_3, SO_2 and oxygen formation belongs to further decomposition of NH_4HSO_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_2O 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 origined from ammonium sulphate decomposition, thus it had to be belonged to N_2O 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.

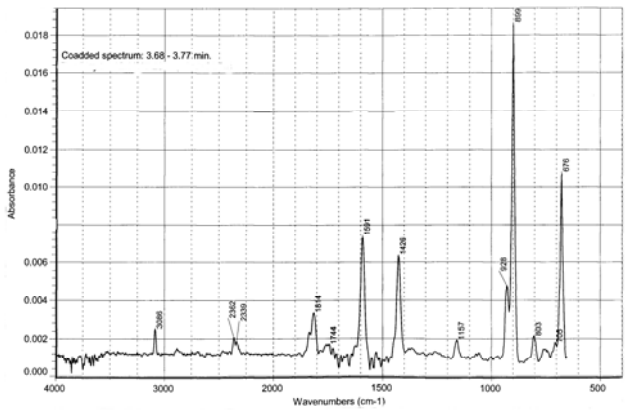


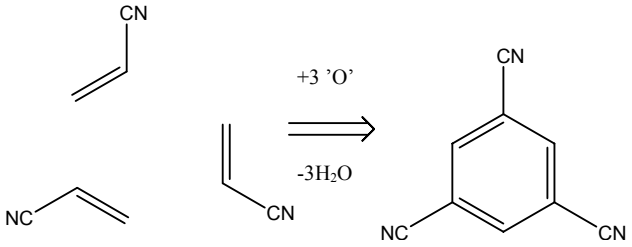
Fig.2 GC-IR of organic component found in the analyzed deposit material

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.

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

Sample			
Solid	Gas phase	Authentic	Assignation
459	-	461	CC,CN
674	676	674	CC, CN
910	899	910	CH
931	928	931	CN, CC
1430	1426	1429	CN
2249	2250	2250	CN
3083	3086	3086	CH

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 recrystallized 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.



The agreement between the authentic sample and the spectrum of the needle like crystals unambiguously showed the identity of the questionable monomer compound, which could be formed by oxidative cyclotrimerization of acrylnitrile monomers. Another possible method to synthesise this compound is a cyclotrimerization reaction of cyanoacetylene formed during the pyrolysis of the acrylnitrile containing polymers. Cyclotrimerization of cyanoacetylene, however, always led exclusively to 1,2,3 and 1,2,4- isomers, because these were formed from 1,2-dicyanocyclobutadiene di-radical intermediate, and 1,3,5-isomer could have been formed only from 1,3-dicyanobutadiene di-radical. The formation of 1,2-dicyanocyclobutadiene di-radical is more favoured and the di-radical formed more stable in its tautomer forms, in which the nitrile moieties are in vicinal and not in 1,3-positions, therefore the formation of the 1,3,5-tricyanobenzene in this reaction cannot be expected[6].

Ammonium sulphate is formed as a decomposition product of the ammonium peroxodisulphate used as an oxidant in iron(III) catalyzed surface oxidation of poly(acrylonitrile-co-methylmethacrylate) fibers. The presence of decomposition product together with the cyclotrimerization product of the assumed reducing agent (acrylonitrile) confirms our postulation about the presence of an oxidative chemical reaction between acrylonitrile and ammonium peroxodisulphate by the formation of ammonium sulphate and 1,3,5-tricyanobenzene.

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.

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