Detection of Sodium Azide by Heteronucleus 14N NMR spectroscopy and binding to Fullerene C60

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Aim

The aim of our study is to propose 14N NMR heteronucleus spectroscopy as valuable chemical analytical method for detection of sodium azide, which is used as a starting substance for the synthesis of many drugs and APIs, or investigational drugs and compounds and not only limited to these.
Quantitative NMR spectroscopy in pharmaceutical applications

Sodium azide is acute poison similar to cyanide. Due to its attractive chemical and physical properties it is widely used in many spheres including automotive industry, medicine, pharmaco-chemistry and even everyday life.

Detection of sodium azide becomes more demanding nowadays than several decades ago. We propose to use of 14N NMR spectra to detect and quantify sodium azide in aqueous solutions and extrapolate calibration results for real time detection of unknown concentrations. The results of this methodology relying in measurement of 1D 14N NMR spectra at the lowest concentration of sodium azide aqueous solutions.
Hazards and risks associated with Sodium Azide

1) Explosive – used in airbags and detonators.

2) Acute poison similar to cyanide. Inhibiting mitochondrial cytochrome C oxidase (CO) causing cerebral hypoxia and death; NaN3 is contributing to fast elaboration of nitric oxide (NO) with concomitant collapse.

In human intake of 0.7–2 g (10 mg/kg) sodium azide can lead to death within half an hour, and oral ingestion of lower doses (0.004–2 mg/kg) of NaN3 cause harm to human health, and chronic exposure to very low doses – dementia, e.g. at workplace area [[1]].

Spheres of application NMR spectroscopy for NaN3 detection

- Pharmaco-chemical analysis
- Occupational workplace monitoring
- Forensic tests
- Environmental safety
- Food and beverage quality control
- Security (detection of explosives)
1954s clinical study of sodium azide for its hypotensive effect


Although was demonstrated lowering of arterial blood pressure by NaN3, but due to neuro degenerative deleterious side effect was not approved for clinical use.
NaN3 in the content of Sartans

NaN3 is widely used as starting molecule in the synthesis of Sartans, containing tetrazoles. [1]

List of some sartans: Candesartan, Irbesartan, Losartan, Valsartan.

While the pharmaceutical companies extensively apply qNMR in drug discovery and development they mostly use HPLC in routine quality analysis rather than qNMR. [2]

Even though one- and two-dimensional NMR spectroscopy and qNMR are capable of the quality evaluation of drugs the number of applications in international pharmacopoeias, e.g. the European Pharmacopoeia (PhEur) and United States Pharmacopoeia (USP) is limited.


## List of Sartans

<table>
<thead>
<tr>
<th>Sartan name</th>
<th>Originator</th>
<th>Bioisoteric functional groups</th>
<th>Patented since</th>
<th>Dosage [mg/d]</th>
<th>Sales 2006 [USD mn]</th>
<th>Drug / Marketed by</th>
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<tbody>
<tr>
<td>Candesartan</td>
<td>Takeda</td>
<td>BPT</td>
<td>1990</td>
<td>8–16</td>
<td>3864</td>
<td>Blopress® / Takeda; Atacand® / AstaZeneca</td>
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<td>Eprosartan</td>
<td>GE Healthcare</td>
<td>BPT</td>
<td>1989</td>
<td>300–400</td>
<td>119</td>
<td>Teveten® / Solvay; Emetast® / Trommsdorff</td>
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<td>Fimasartan</td>
<td>Boryung Pharm</td>
<td>BPT</td>
<td>2001</td>
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<td>Forasartan</td>
<td>Pfizer</td>
<td>BPT</td>
<td>1991</td>
<td></td>
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<td>Irbesartan</td>
<td>Sanofi</td>
<td>BPT</td>
<td>1990</td>
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<td>Losartan</td>
<td>DuPontMerck</td>
<td>BPT</td>
<td>1986</td>
<td>50–100</td>
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<td>Lorzaat® MSD</td>
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<td>Milfasartan</td>
<td>Menarini</td>
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<td>Olmesartan</td>
<td>Daiichi/Sankyo</td>
<td>BPT</td>
<td>1991</td>
<td>&gt;20 mg</td>
<td>1237</td>
<td>Olmetec® / Sankyo; Votum® / Berlin-Chemie Mencord® / Menarini Pharma</td>
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<td>Pratosartan</td>
<td>Kotobuki</td>
<td>BPT</td>
<td>1992</td>
<td></td>
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<td>Valsartan</td>
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<td>BPT</td>
<td>1990</td>
<td>80–160</td>
<td>4343</td>
<td>Diovan® / Novartis; Provas® / Schwarz Pharma; Cordinate® / AWDPharma</td>
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<td>Tasosartan</td>
<td>Wyeth</td>
<td>BPT</td>
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<td>40–80</td>
<td>1639</td>
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<td>Telmisartan</td>
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<td>Micardis® / Boehringer Ingelheim; Kinzalmon® / Bayer</td>
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14N and 15N

Nitrogen is a nucleus of considerable chemical and biological importance. However, despite its high isotopic abundance (99.63%), 14N has always been a nucleus difficult to observe in NMR. It is a spin-1 nucleus.

15N is a spin-1/2 nucleus and thus can be studied with relatively high resolution even in the solid state, but it suffers from a low natural abundance (0.37%), which translates to a poor sensitivity.

While the number of published 15N NMR papers is disproportionately small relative to the importance of nitrogen, studies of 14N isotope are even scarcer. [1]

Sample preparation

A) co-axial insert tube - 100% nitromethane (CH3NO2) – 600 microliters

B) Sample (5 different concentrations of NaN3 water solution (9:1 H2O/D2)
100 mM, 50 mM, 25 mM, 10 mM, 4 mM

C) Assembled for analysis
Sodium azide 100 mM

$^{14}\text{N NMR}$

N$\equiv$N$^+$=N$^-$

in 30 seconds with 64 scans

DRX-500, 300 K

USC. CACTUS. 19 Nov. 2013
Sodium azide 100 mM

$^{15}\text{N} \text{NMR}$

Expected signals at 250 and 100 ppm are not seen

1 hour

DRX-500, 300 K

USC. CACTUS. 19 Nov. 2013
15N NMR spectrum of sodium azide (1M) in D2O

http://chem.ch.huji.ac.il/nmr/techniques/1d/row2/n.html#n14properties
Properties of Fullerene C60

C60 is like any electron-deficient molecule can accept from 1 to 6 electrons and C60 is converted into anion.

In the role of donors will serve external electrical charge, alkali metal ions or organic molecules.

Like alkenes fullerene could be involved in the reaction of azide-alkyne cycloaddition, with the formation of triazole rings.

$^{14}\text{N}$ NMR Titration study  C60 fullerene + NaN$_3$ in water

Superimposition of two spectra

At low molar ratio NaN$_3$:C60 ($<=$ 10:1) No appreciable change of $^{14}$N chemical shift or linewidth occurs for the peaks of NaN$_3$.
$^{14}\text{N} \text{ NMR Titration study} \quad \text{C60 fullerene + NaN}_3 \text{ in water}$

Superimposition of 4 spectra at low Molar ratios NaN$_3$: C60  \quad (molar ratio $\leq 10:1$)

At low molar ratio NaN$_3$:C60 ($\leq 10:1$) No appreciable change of $^{14}\text{N}$ chemical shift or linewidth occurs for the peaks of NaN$_3$
$^{14}$N NMR Titration study  C60 fullerene + NaN$_3$ in water

Superimposition of two spectra

At high molar ratio NaN$_3$:C60 ($\gg$ 10:1) there are some subtle changes of $^{14}$N chemical shift and linewidth of both NaN$_3$ peaks.

Signal A
NaN$_3$ (204.78 ppm)
Linewidth 24.8 Hz

Signal B
NaN$_3$ (56.06 ppm)
Linewidth 49.8 Hz

Molar ratio
- 10:1
- 100:1
At high molar ratio $\text{NaN}_3$ : C60 100:1. The $^{14}$N peaks of sodium azide have observable CSPs and changes in Linewidth. The two effects are stronger for the two external nitrogens of sodium azide (signal B) than for the central nitrogen (signal A).
Results

The results demonstrate that there are changes in the chemical shift position and line-broadening related to the molar ratio NaN3:C60 in the sample (100:1).

These results can be interpreted as binding interaction occurring between NaN3 and C60 molecules.

As you will see in the attached figure, from the two 14N peaks of NaN3, the one that is more affected is the one that resonates at aprox. 56 ppm, which corresponds to two external nitrogen atoms.