University of Santiago
de Compostela

Iv.Javakhishvili
Tbilisi State University

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

Tamar Chachibaia1,2, Manuel Martín Pastor3.

1. Department of Analytical Chemistry, Food Science and Nutrition, Faculty of Pharmacy, University of Santiago de Compostela, Spain

2. Department of Public Health and Epidemiology, Faculty of Medicine, Iv.Javakhishvili Tbilisi State University, Tbilisi, Georgia

3. Magnetic Resonance Unit, CACTUS, University of Santiago de Compostela, Spain

Presentation on March 9, 2015

in the Institute of Experimental Pharmacology & Toxicology

Department of Biochemical Pharmacology

Slovak Academy of Sciences

Bratislava

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) sodiun 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]].

[1] S. Chang and S.H. Lamm. (2003). Human health effects of sodium azide exposure: a literature review and analysis. *Int. J. Toxicol.* **2222:**175–186.

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

Black, M. M., B. W. Zweifach and F. D. Speer (1954) “Comparison of Sodium azide in Normotensive and Hypertensive Patients.” Exp Biol Med 85:11.

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

NaN_3 in the content of Sartans

NaN_3 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.

[1]Subramanian N., Babu V., Jeevan R., Radhakrishnan G. (2009). Matrix Elimination Ion Chromatography Method for Trace Level Azide Determination in Irbesartan Drug. J. of Chromatographic Science, Vol. 47, 529-533.

[2] Santosh Kumar Bharti, Raja Roy (2012) Quantitative 1H NMR spectroscopy. Trends in Analytical Chemistry, 35, 5-25.

List of Sartans

Table 2.7 Sartans, originators and commercial relevance[55]

Sartan name	Originator	Biosteric functional groups[1)]	Patented since[55]	Dosage [mg/d][55]	Sales2006 [USD mn][7/56]	Drug / Marketed by
Candesartan	Takeda	BPT	1990	8–16	3864	Blopress® / Takeda; Atacand® / AstaZeneca
Elisartan	GE Healthcare	BPT				2)
Eprosartan	GSK	BPT	1989	300–400	119	Teveten® / Solvay; Emestar® / Trommsdorff
Fimasartan	Boryung Pharm	BPT	2001			2)
Forasartan	Pfizer	BPT	1991			3)
Irbesartan	Sanofi	BPT	1990	150–300	2336	Aprovel® / Sanofi-Aventis; Karvea® / Bristol-Myers Squibb
Losartan	DuPontMerck	BPT	1986	50–100	3163	Lorzaar® MSD
Milfasartan	Menarini	BPT	1991			3)
Olmesartan	Daiichi/Sankyo	BPT	1991	>20 mg	1237	Olmetec® / Sankyo; Votum® / Berlin-Chemie Mencord® / Menarini Pharma
Pratosartan	Kotobuki	BPT	1992			4)
Valsartan	Novartis	BPT	1990	80–160	4343	Diovan® / Novartis; Provas® / Schwarz Pharma; Cordinate® / AWDPharma
Tasosartan	Wyeth	BPT	1991			5)
Telmisartan	Boehringer Ingelheim	BPC	1991	40–80	1639	Micardis® / Boehringer Ingelheim; Kinzalmono® / Bayer
Zolasartan	SKB	PT	1992			6)

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]

[1] O'Dell, L.A. (2011). Direct detection of nitrogen-14 in solid-state NMR spectroscopy. *Progress in Nuclear Magnetic Resonance Spectroscopy,* 59 (4), 295–318.

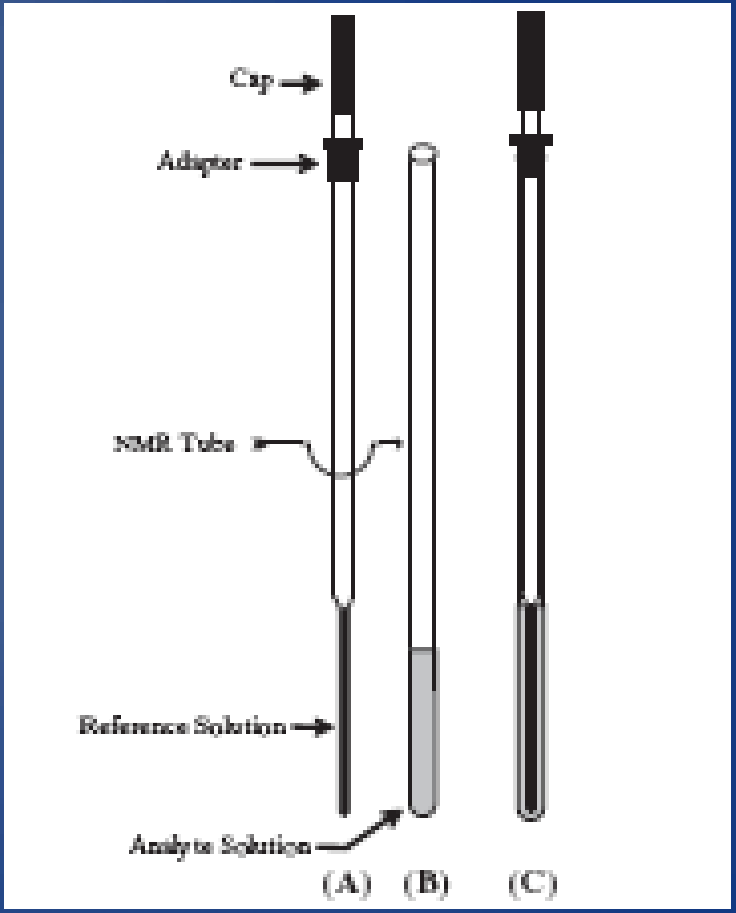
Sample preparation

A) co-axial insert tube - 100% nitromethane (CH_3NO_2) – 600 microliters

B) Sample (5 different concentrations of NaN_3 water solution (9:1 H20/D2)

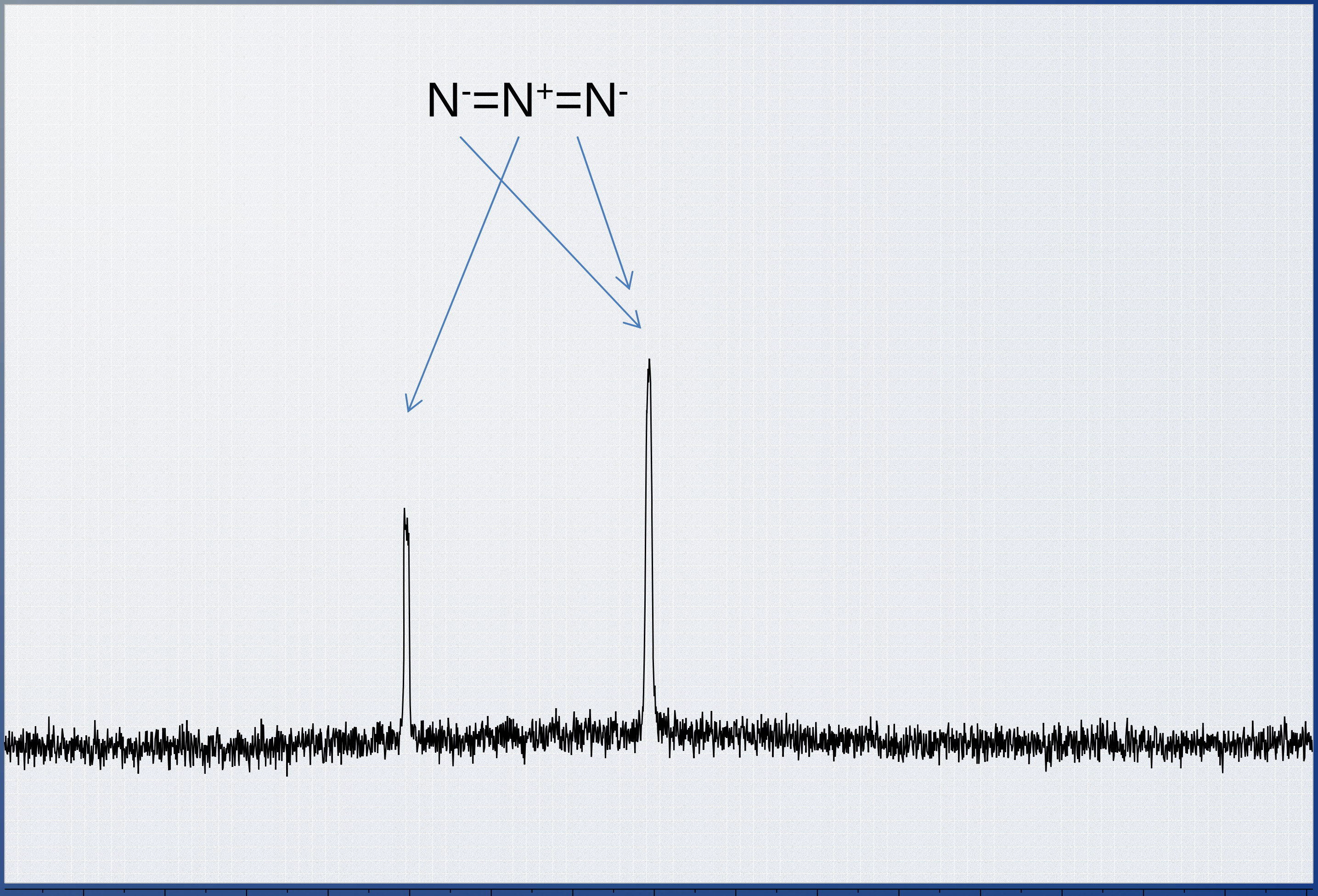
100 mM, 50 mM, 25 mM, 10 mM, 4 mM

C) Assembled for analysis



Sodium azide 100 mM

^{14}N NMR

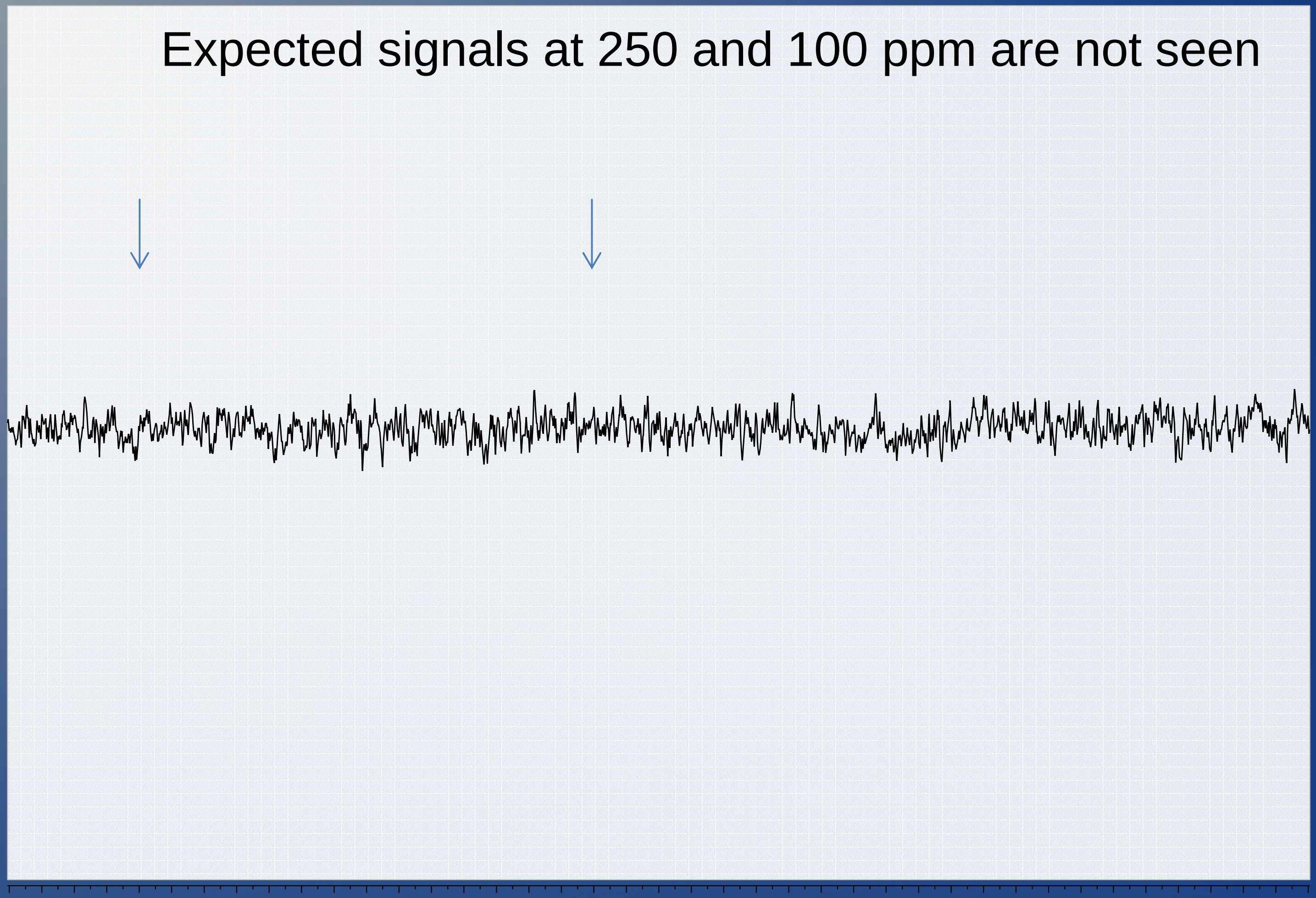


in 30 seconds with 64 scans

DRX-500, 300 K

Sodium azide 100 mM

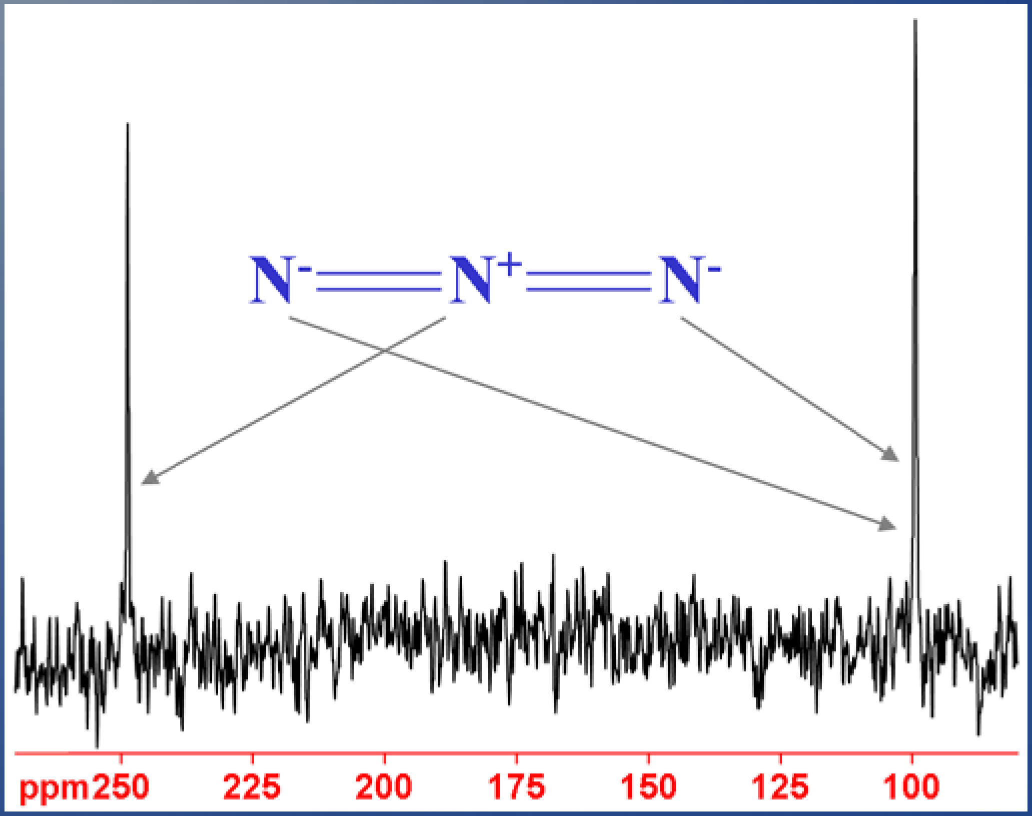
^{15}N NMR



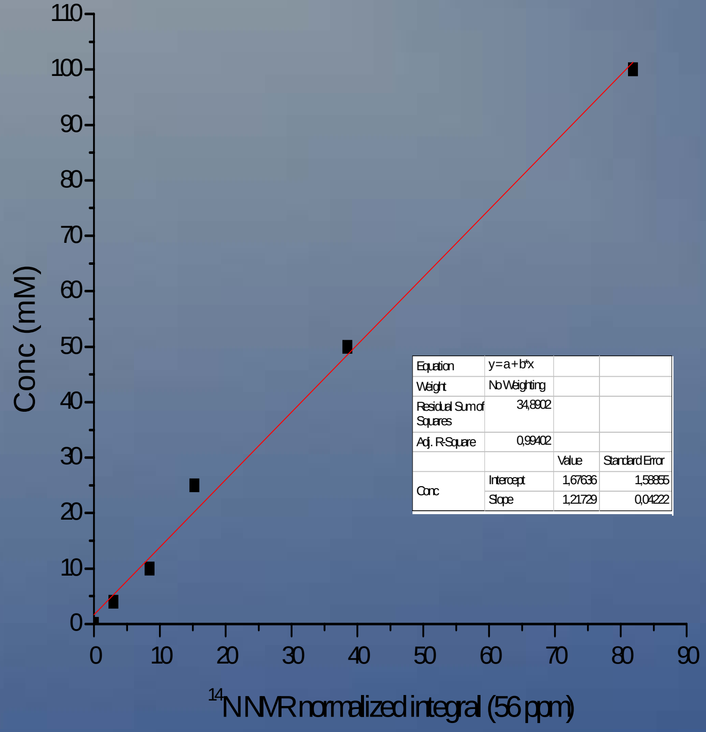
1 hour

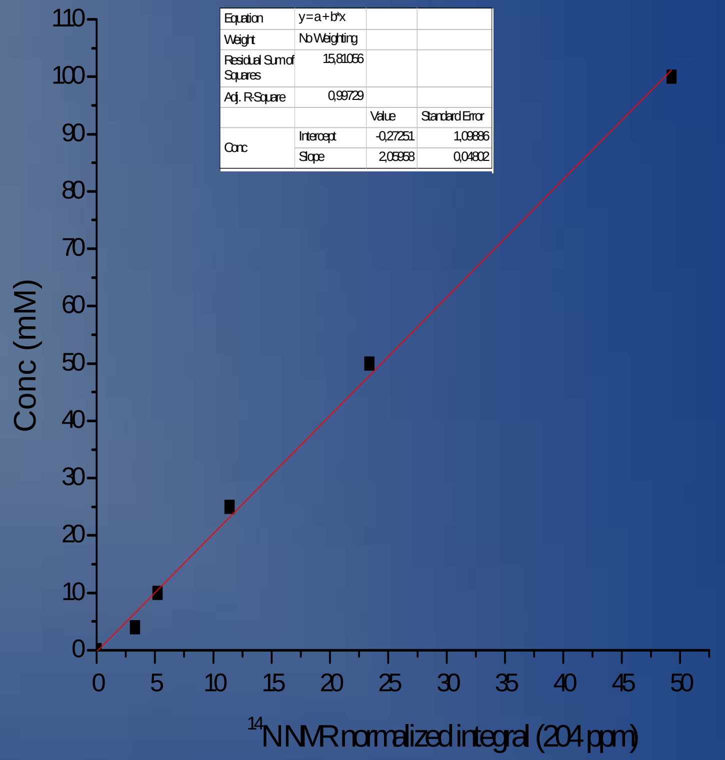
DRX-500, 300 K

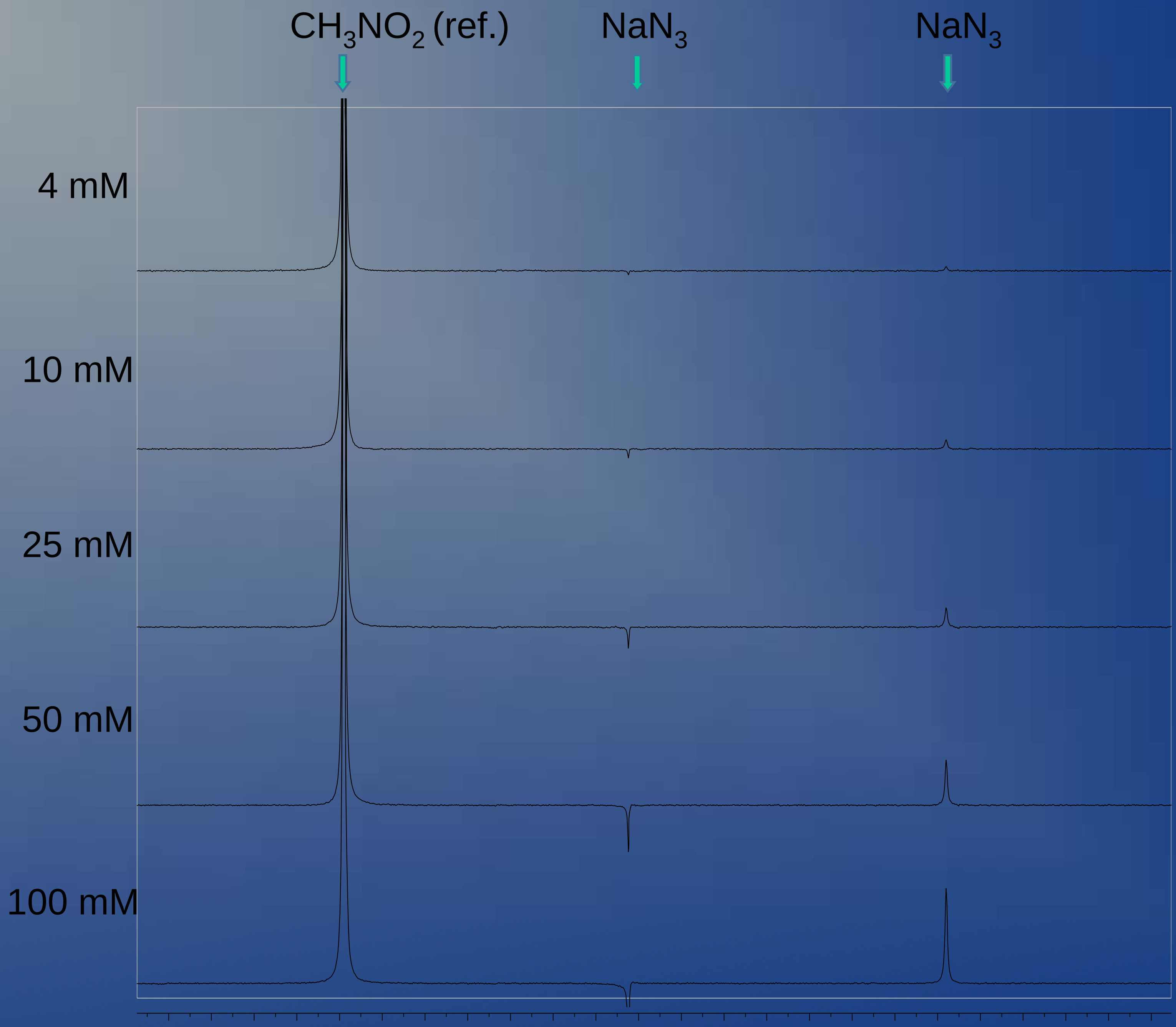
15N NMR spectrum of sodium azide (1M) in D2O



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


Conc (mM)
110
100
90
80
70
60
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90
14NMR normalized integral (56 ppm)
Equation y=a+b*x
Weight No Weighting
Residual Sum of Squares 34,8902
Adj. R-Square 0,99402
Value Standard Error
Conc Intercept 1,67636 1,58855
Slope 1,21729 0,04222


Conc (mM)
110
100
90
80
70
60
50
40
30
20
10
0
0 5 10 15 20 25 30 35 40 45 50
14NMR normalized integral (204 ppm)
Equation y=a+b*x
Weight No Weighting
Residual Sum of Squares 15,81056
Adj. R-Square 0,99729
Value Standard Error
Conc Intercept -0,27251 1,09886
Slope 2,05958 0,04802



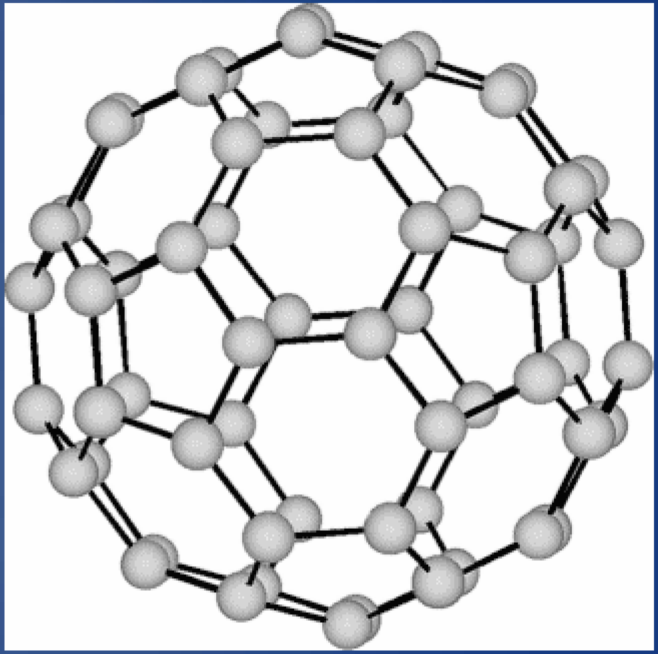
CH3NO2 (ref.)
NaN3
NaN3
4 mM
10 mM
25 mM
50 mM
100 mM

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.

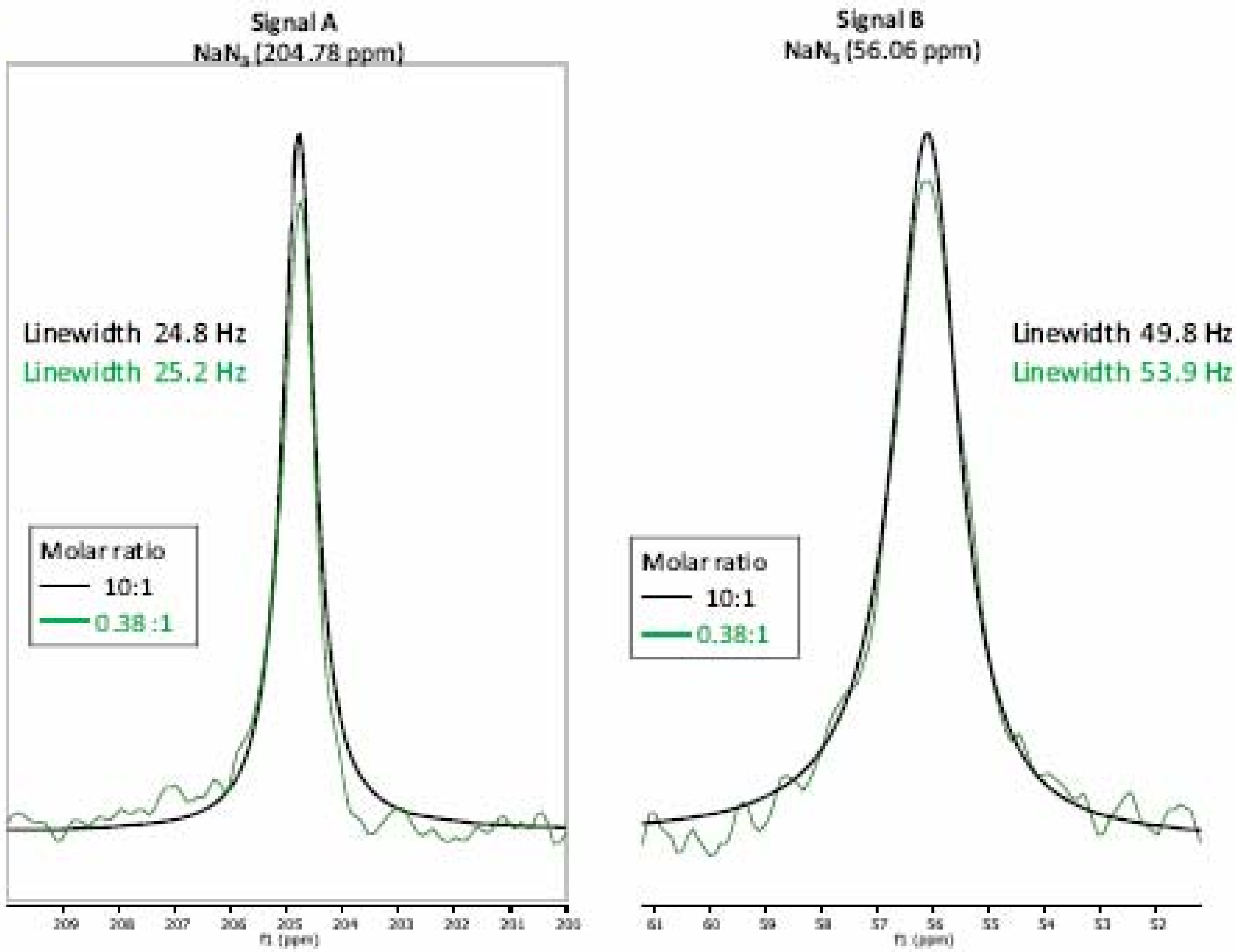


A. R. Akhmetov, A. R. Tuktarov, U. M. Dzhemilev, I. R. Yarullin, and L. A. Gabidullina (2011). **First example of the interaction of fullerene C60 with hydrazoic acid.** *Russian Chemical Bulletin, International Edition, 60 (9), 1885—1887.*

^{14}N NMR Titration study C60 fullerene + NaN_3 in water

Superimposition of two spectra

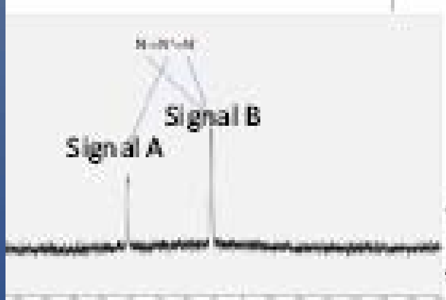
At low molar ratio NaN_3:C60 (<= 10 :1)
No appreciable
change of ^{14}N
chemical shift or linewidth occurs
for the peaks of NaN3

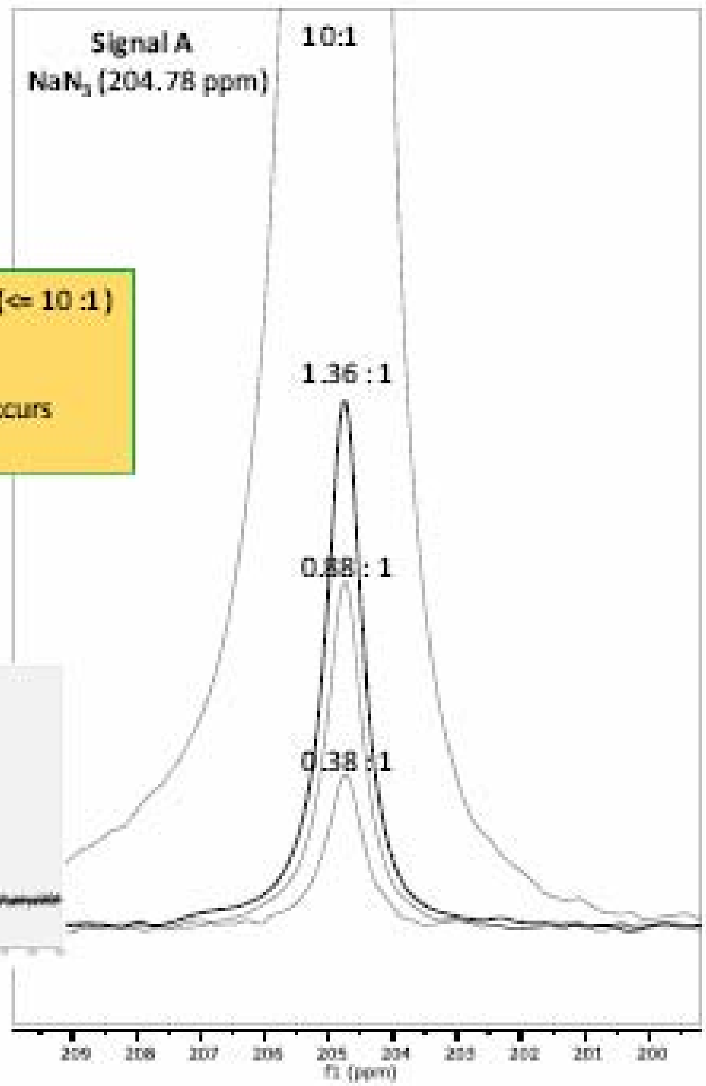


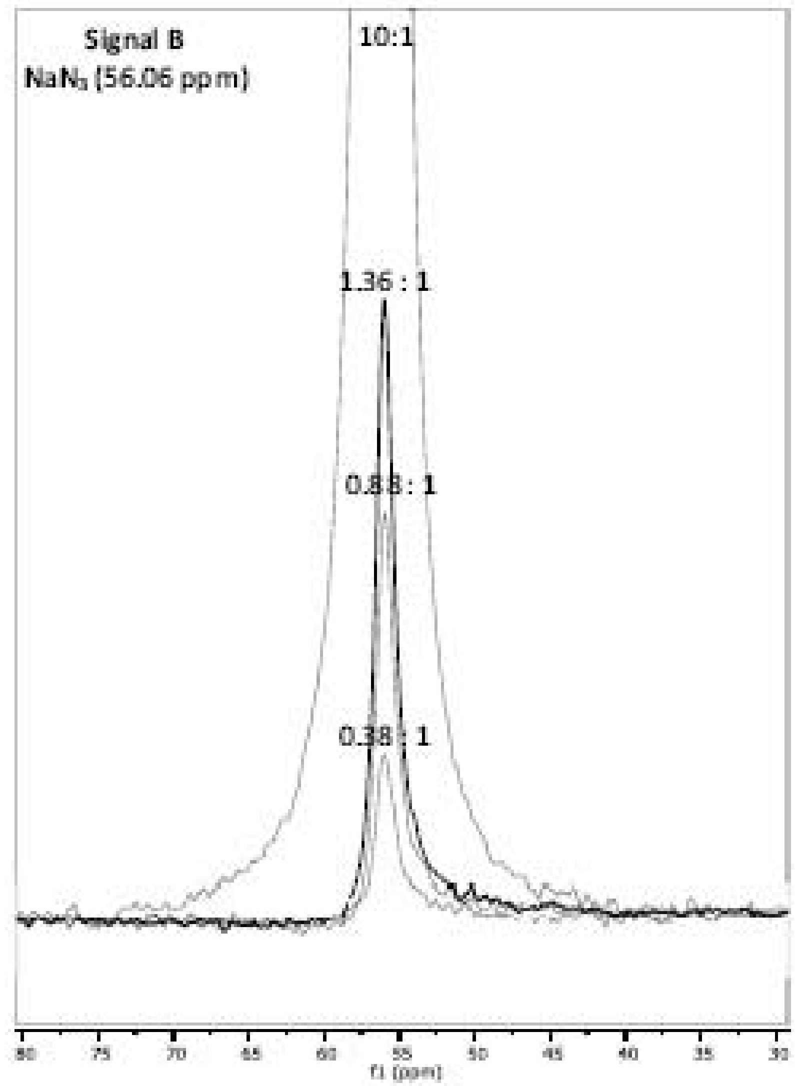
^{14}N NMR Titration study   C60 fullerene + NaN_3 in water

Superimposition of 4 spectra at low Molar ratios NaN_3: C60   (molar ratio <= 10:1)

At low molar ratio NaN_3:C60 (<= 10 :1)
No appreciable
change of ^{14}N
chemical shift or linewidth occurs
for the peaks of NaN3



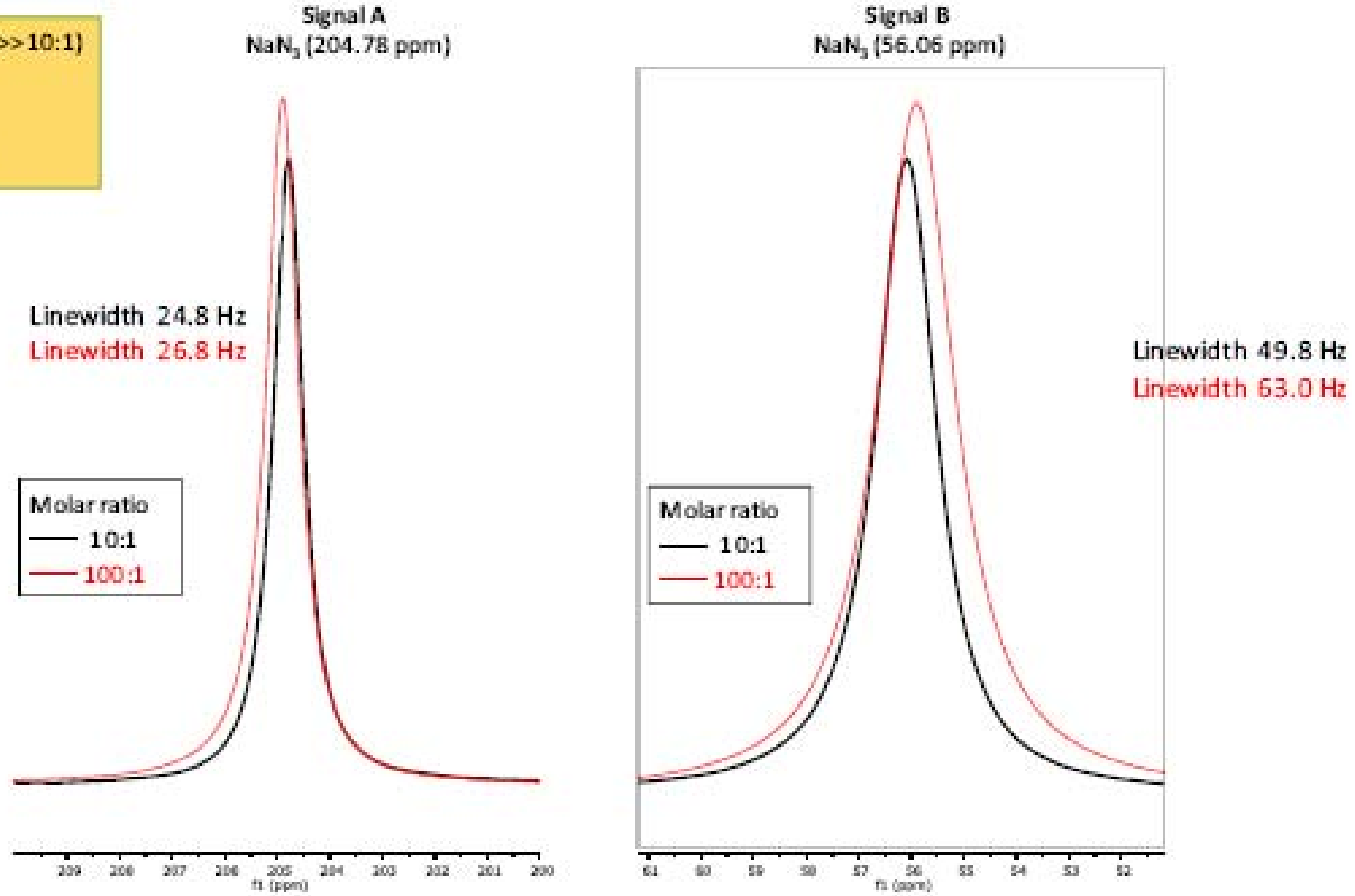




^{14}N NMR Titration study   C60 fullerene + NaN_3 in water

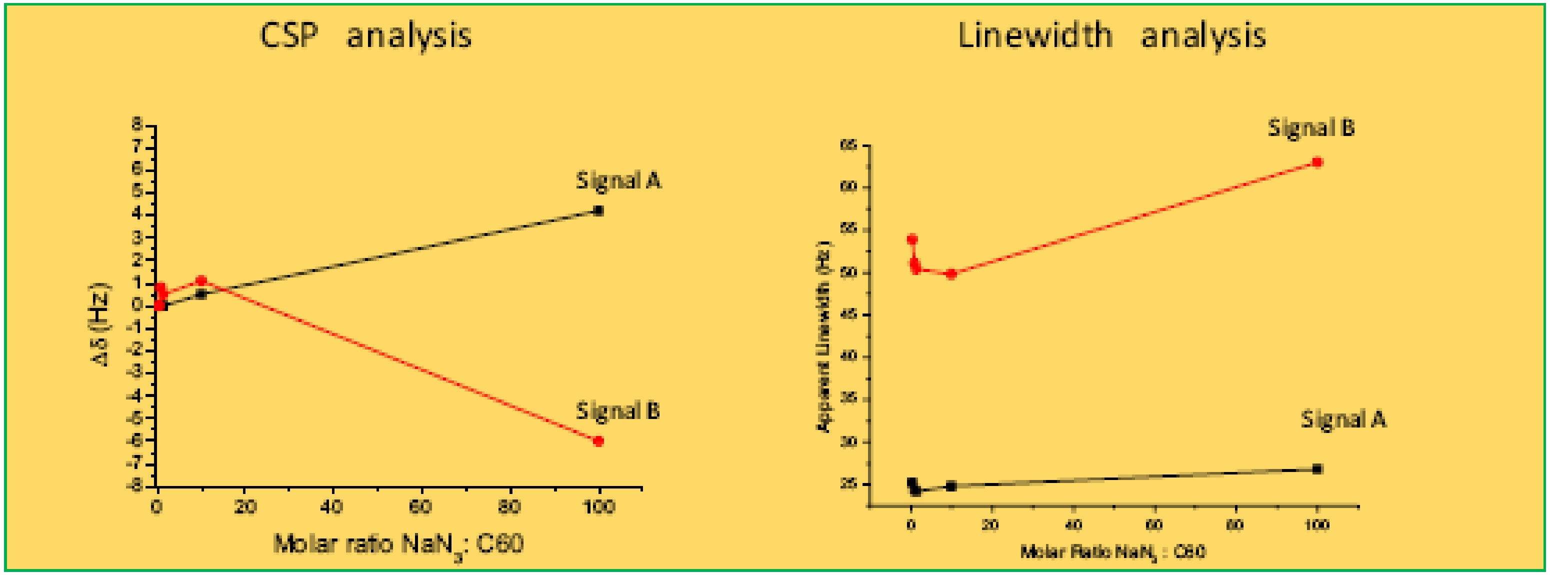
Superimposition of two spectra

At high molar ratio NaN_3:C60 (>> 10:1)
There are some subtle
changes of ^{14}N
chemical shift and linewidth
of both NaN3 peaks



^{14}N NMR Titration study C60 fullerene + NaN_3 in water

Chemical Shift Perturbations (CSP) and Linewidth study of ^{14}N peaks of NaN_3 at several molar ratios



→At high molar ratio NaN_3:C60 100:1. The 14N peaks of sodium azide have a 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.