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^{14}N NMR Spectroscopy Study of Binding Interaction between Sodium Azide and Hydrated Fullerene

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Abstract: The presence of human pharmaceutical compounds in surface waters is an emerging issue in environmental science. Low levels of many active pharmaceutical ingredients are detected in the aquatic environment as a result of pharmaco-chemical industrial waste spill-offs in draining water. In the manufacturing of pharmaceutical drug substances azides are used as reagents or when they are generated somehow in the synthesis, it may be necessary to demonstrate that these impurities are sufficiently removed to levels below an appropriate safety threshold. Sodium azide is an example of an azide for which the environmental exposure limits have been reasonably well characterized. The treatment of waste and industrial water can be conducted by removing dissolved materials and ions in water using membrane separation technology with ultra- and nanofiltration (NF) and reverse osmosis (RO) membranes. To achieve better effluent water quality, tertiary treatment with activated carbon adsorption is used. To analyze the risk of pharmaceuticals in the environment, a proposed validated methodology by NMR spectroscopy will support the evaluation of the eco-toxicological hazards during the early development process of pharmaceuticals.

Keywords: sodium azide; fullerene; 14N NMR spectroscopy; nanofiltration.

1. Introduction

The presence of pharmaceutical active compounds (PhACs) in the surface, drinking, and wastewaters is an emerging issue in environmental science. [1,2,3,4,5,6,7,8,9]. Low levels of many pharmaceutical active compounds are detected in the aquatic environment as a result of pharmaco-chemical industrial waste spill-off in draining water. It may be necessary to demonstrate that these impurities are sufficiently removed to levels below an appropriate safety threshold.

Sodium azide is an example of an azide for which the environmental exposure limits have been reasonably well characterized.

In the manufacturing of pharmaceutical drug substances azides are used in the synthesis, or they are generated as intermediate substance. Sodium azide (NaN_3) is widely used as starting molecule in the synthesis of Sartans, for the treatment of hypertension since the 1990s [10]. Some of these products have reached a market volume of several 100 t/a with an upward trend and are therefore considered as blockbusters.

Besides Sartant it is used in the synthesis other pharmaceuticals, such as Alfentanil (analgesic), Azosemid (diuretic), (anti-inflammatory) Broperamol and others.

There are required regulations for the analysis of pharmaceutical wastewater treatment and removal using membrane bioreactors (MBR). The waters treatment can be conducted by removing dissolved materials and ions in water using membrane separation technology with ultra- and nanofiltration (NF) and reverse osmosis (RO) membranes. [11]

To achieve better effluent water quality, tertiary treatment with activated carbon adsorption is used [12]. Activated carbon filters, which may contain fullerene retains extremely effective by mechanical filtration effect [13,14,15,16]. In 2009 by Chae and coworkers was developed technology of membrane coating by hydrated fullerene for improvement of filtration properties of membranes.

To analyze the risk of pharmaceuticals in the environment, proposed validated methodology by NMR spectroscopy will support the evaluation of the eco-toxicological hazards of PhACs during the early development process - the questions brought forward by many academic and regulatory scientists.

Aim:

Aim was to study deviation of signals of sodium azide obtained by ^{14}N NMR spectroscopy under influence of hydrated fullerene to examine binding properties of sodium azide with hydrated fullerene. In current study we propose innovative method for detection of sodium azide by ^{14}N NMR spectroscopy.

Background:

Detection and inactivation of sodium azide in the environment is global issue, due to its widespread use in many spheres of human activity, in pharmaco-chemical industry in the synthesis of pharmaceuticals, as well pesticides, direct use in agricultural sphere, as herbicide, pesticide and insecticide, wine fermentation inhibitor, in automotive industry in the content of detonators of airbags and bactericidal agent for inhibition of germ growth.

The Organization for Economic Co-operation and Development (OCDE) [17] has included sodium azide in the list of 5,235 High Production Volume Chemicals (HPV) with a production or import greater than 1,000 tons per year (McKeen, 2010).

Sodium azide (NaN_3) has inhibitory effect on heme-containing mitochondrial respiratory chain enzyme Cytochrome C Oxidase, which is cause of CNS anoxia and hypoxia in case of acute intoxication, while in case of chronic exposure to its lower doses long-term outcome is dementia.

Against NaN_3 not exists any antidote, and thus the only method of treatment remains hemodialysis affected patients. In case of chronic intoxication prevention is possible by administration of antioxidants to workers.

For prevention of sodium azide impact is important to decrease the risk of exposure, as from occupation workplace atmosphere and also from the environment.

The environmentalist and atmospheric scientists are concerned about the safety of the use of sodium azide. Despite the widespread opinion of proponents of sodium azide use in water and soil, arguing that this chemical undergoes rapid hydrolysis and degradation (Rodríguez-Kábana & Robertson, 2000, 2001). [18], their opponents (Betterton, 1999, 2003, 2010).[19] claimed that this is not exactly what it can be anticipated, since they discovered water and soil samples containing residual amounts of sodium azide.

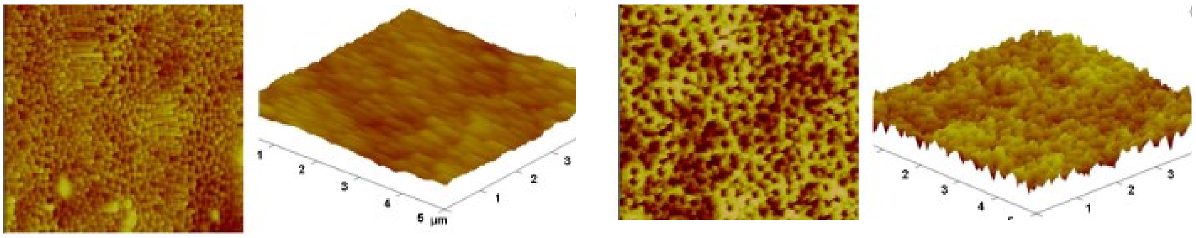
One of the most important issues is control of runoff waters and adequate membrane filtration barrier setup.

For industrial wastewater, as well municipal and hospital dialysis centers water treatment systems are using modern membrane filtration technologies.

Physicochemical modifications of membrane materials have been tried to improve performance of membrane processes for a long time. Numerous studies dealing with the surface modification of membranes have achieved by coating or grafting a functional group on the prepared membrane surface. [20].

In addition to the conventional ways of surface modification, researchers are focusing on the application of nanomaterials to modify the membrane properties thanks to recent developments of nanotechnologies. Among others, fullerene (C60) is the potential candidate expected to show an improved performance when used to modify the membrane properties. [21,22,23] Chae et al. (2009) examined the modification of ceramic microfiltration membranes coated by fullerene solution dip-coat-evaporation procedure. [24]. The dip coating procedure consisted of an initial immersion of membrane into solution for 2 seconds, drip-draining of excess solvent, followed by solvent evaporation under vacuum for several days. The surface concentration of C60 on the membranes was varied by repeating the dip-coating procedure anywhere from one to nine times. The final concentration of C60 on the membrane was determined by measuring the change in membrane weight. The surface morphology of the membrane coated with various amounts of C60 was investigated using a scanning probe microscope (SPM). The C60 nanoparticles used in this study were not chemically bound to the membrane surface.

Pic. 1. Surface morphology of the ceramic membranes: (left) anodisc 200 nm with C60 0.030mg cm^{-2} and (right) anodisc 200 nm with C60 0.058mg cm^{-2} (Source: doi:10.1016/j.memsci.2008.12.023. Courtesy Chae at el. 2009).



2. Materials and methods:

We studied binding properties between sodium azide and hydrated fullerene, without adding any catalyst, heating or microwave irradiation, under conventional conditions, to see if any interaction may occur to recommend in water filtration and pharmaceutical waste-water treatment applications, e.g. in different phases of filtration, as in membranes and carbon filters enriched by fullerenes.

The experimental part of this project is performed in the Magnetic Resonance Unit at the Center of Technology Innovation and Transfer (CACTUS) of the University of Santiago de Compostela. Experiments were conducted during 2012-2014 and obtained results analyzed.

University of Santiago de Compostela (USC) is equipped with NMR spectroscopy and propriety technology of MESTRE Labs, which is the software used worldwide.

The Magnetic Resonance Unit at the University of Santiago de Compostela provides the optimum research instrumentation required for this part of the project. The NMR facility provides three state-of-the-art high magnetic field NMR spectrometers of 500 MHz and 750 MHz.

Experimental

C60hyfn production, characterization and preparation of C60FWS

For C60FWS preparation (C60HyFn water solution), C60 fullerene samples with purity of more than 99.5% (MER Corporation, Tuscon, AZ, USA) have been used. C60FWS was produced without using of any solubilizers or chemical modification [25].

C60HyFn concentration of 8.88×10–4 M was used as stock solution for preparing C60FWS prior the experiment. This method is based on transferring of fullerene from organic solution into the aqueous phase with the help of ultrasonic treatment. To obtain C60FWS is possible with C60 concentration up to 5.5×10–3 M (~4 mg/ml).

Titration:

We performed ^{14}N NMR spectroscopy of pure sodium azide water solution and obtained satisfactory results with visualization of two peaks corresponding to three atoms of nitrogen with chemical shifts corresponding to 204.78 ppm and 56.06 ppm.[26]

We added hydrated fullerene 50 mM solution (144 mg/l) (IPAC, Ukraine, Kharkov) to sodium azide water solution with titration.

In our study we performed two series of experiments, with different concentrations of sodium azide. First, with molar concentration of 1M, and second, with 10M solution.

We performed titration by fullerene water solution with decreasing concentrations. Thus, ratios of NaN_3:C60 were <10:1 in the first set of experiment. In the first series of experiment we used standard addition method of titration. Standard is hydrated fullerene C60 and with decreasing the ratios of NaN_3:C60 which were subsequently 10:1, 1,36:1, 0,88:1 and 0,38:1.

In the second set of experiments we used higher concentration of sodium azide 10M with ratio of NaN_3:C60 which was equal to 100:1.

3. Results

The sample prepared at molar ratio NaN_3:C60 100:1 show a small change in the peak position and so does a change in the linewidth respect to the other samples explored in the titration study. Those changes could be indicative of a weak binding interaction between NaN_3 and C60. At high molar ratio 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). The mentioned effects could indicate a weak binding interaction between NaN_3 and C60.

Fig. 1 ^{14}N NMR titration study. C60 fullerene added to NaN_3 water solution. Superimposition of two spectra of sodium azide: black line is corresponding to pure sodium azide water solution and green to sodium azide titrated with fullerene water solution at lowering concentrations. At low molar ratio NaN_3:C60 (<= 10 :1) no appreciable change of ^{14}N chemical shift or linewidth occurs for two peaks of NaN_3 (Signals A and B).

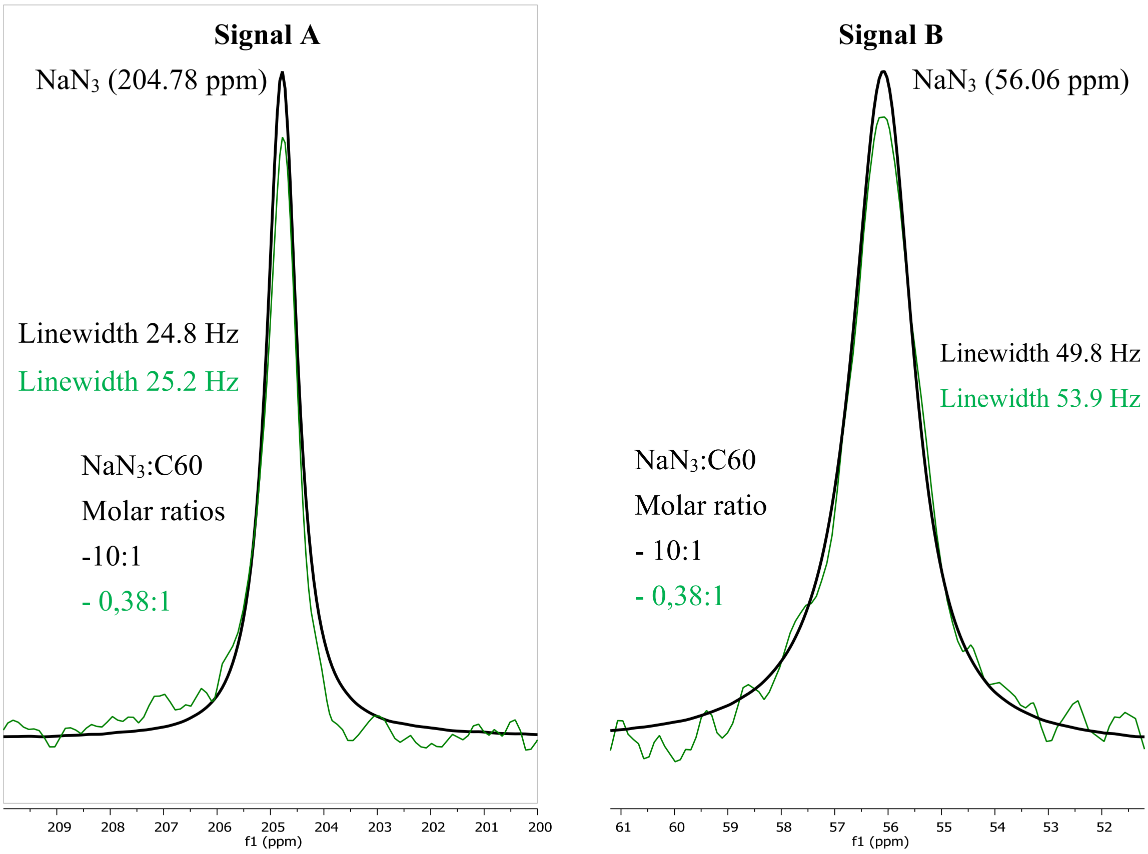
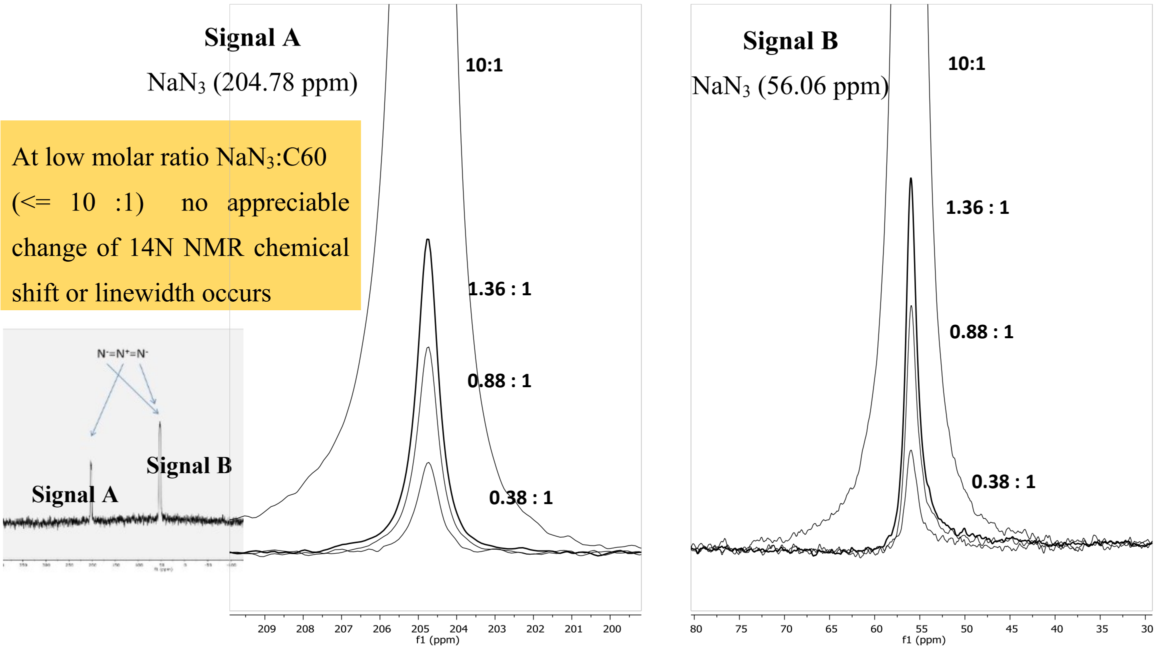


Figure 2. ^{14}N NMR superimposition of four different spectra at low molar ratios of NaN_3:C60 (<= 10:1).



At low molar ratio NaN_3:C60 (<= 10 :1) no appreciable change of 14N NMR chemical shift or linewidth occurs

Figure 3. Superimposition of two spectra at high ratio of NaN_3:C60 (>10 :1), particularly 100:1 There are some subtle changes of ^{14}N chemical shift and linewidth of both NaN_3 peaks.

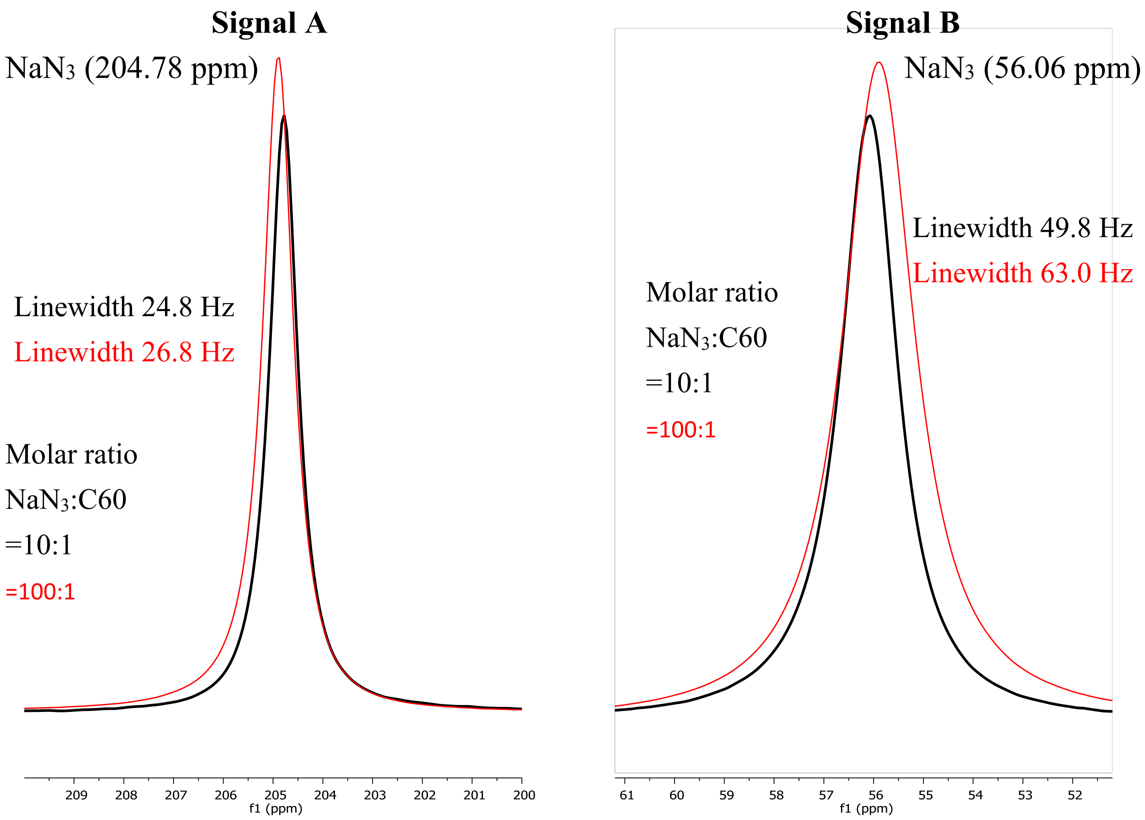
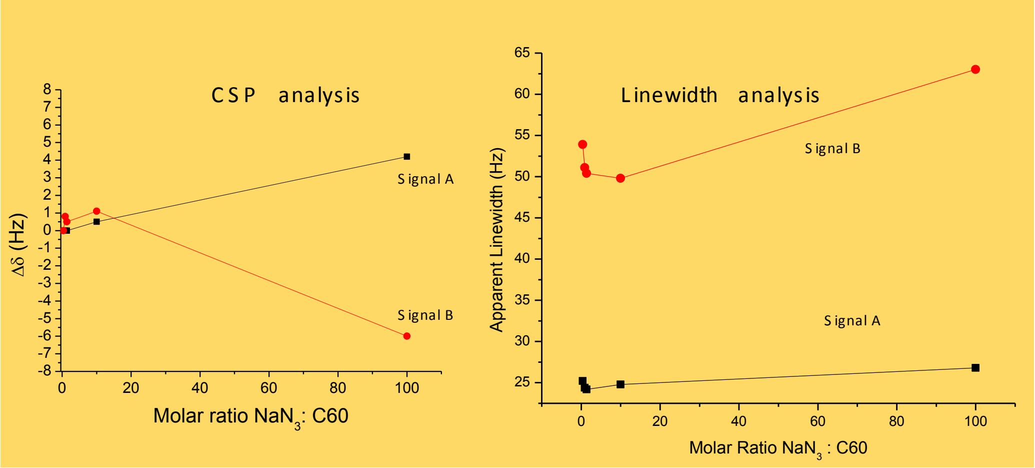


Figure 4. Chemical Shift Perturbations (CSP) and Linewidth study of ^{14}N peaks of NaN_3 at several molar ratios.



To confirm obtained results it is required extra experiment measuring the ^{14}N spectrum of a sample containing only sodium azide in water at the same concentration of 10 M without C60 to discard a false positive. There were anticipated two possible outcomes of this experiment: if the changes mentioned above does not occur in this sample, then they must be due to C60, and therefore the weak-binding is confirmed, but if the changes mentioned above do occur also in this sample, then there is likely an effect of the high concentration of NaN_3 in the sample, but we cannot say that we have detected a weak-binding between C60 and NaN_3.

The results obtained with the control sample of pure NaN_3 at 10 M demonstrated that there are changes in the chemical shift position and line-broadening related to the molar ratio 100:1 of NaN_3:C60 in the sample. These results can be interpreted as binding interaction occurring between NaN_3 and C60 molecules, from the two ^{14}N peaks of NaN3, the one that is more affected is the one that resonates at approximately 56 ppm.

Discussion:

Some solutes, such as C60 can have a dynamic interaction of binding with other solutes, such as Na N_3. They are exchanging during time between a bound state when the two molecules are packed together and unbound state in which the two molecules are independent. Chemically this is represented by equilibrium:

N_3 + C60 <====> N_3:C60
Free state Bound state

The double arrow indicates that the equilibrium moves in both direction during time, in our case the timescale of this reaction is likely below <100 ms for moving in both directions.

In the instant that the two molecules are bound their atoms are packed together and as consequence their electron clouds introduce a slight shielded/de-shielded of the external magnetic field that is felt by the nuclei of any of the two molecules. Thus, the ^{14}N NMR peaks of N_3 molecule feels on average a slightly different magnetic field when C60 is present and therefore resonate at a slightly different chemical shift than when C60 is not present. We made a titration with C60 and followed the changes of chemical shifts of ^{14}N. The fact that changes are observed by addition of C60 demonstrates that the two molecules interact and bind together dynamically.

The influence of C60 in the chemical shifts of NaN_3 is not linear with the concentration, i.e. the two molecules interact one to one proportion as indicated above, it has a quadratic dependence with the concentration.

4. Conclusion:

The results demonstrate that there are changes in the chemical shift position and line-broadening related to the molar ratio NaN_3:C60 in the sample (100:1). One of two peaks of ^{14}N, which resonates at 56 ppm and corresponds to two external nitrogen atoms of NaN_3 is more affected by influence of hydrated fullerene C60. These results can be interpreted as binding interaction occurring between NaN_3 and C60 molecules.

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Conflicts of Interest

The authors declare no conflict of interest.

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