

Chemosensors & ^{19}F Benchtop NMR Spectroscopy: Quantification of Caffeine in Coffee

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NMRReady

Introduction

The detection and/or quantification of an analyte is paramount in many applications, ranging from food quality control, biological chemistry to environmental monitoring.^[1] The analytes could be alkaloids in beverages, bioactive marker molecules in cells, or toxic substances in river water, all of which are present at low concentrations. As 'real-world' samples are usually complex mixtures, time-consuming pre-treatments, such as extraction and separation aimed at removing the interferences from the matrix are often needed to ensure reliable data. Therefore, rapid and precise detection methods without the need of separation are of great demand.

Chemical sensors or chemosensors are molecular constructs (**receptors**) that are sensitive to small molecules or metal ions (**analytes**) giving a measurable change in their molecular properties. The most commonly used properties are electrochemical, absorbance, emission and very recently nuclear magnetic resonances (NMR).

NMR spectroscopy is an informative technique that is used to characterize a species by identifying the chemical and/or steric environment of its nuclei in a magnetic field. This technique is ideal for analytes in pure form; however, its use in the analysis of complex mixtures is hampered by the overlap of signals from different mixture constituents.^[2] To overcome this obstacle, which has even more of an affect in low-field NMR,^[3] we turn to the use of chemosensors.

The molecular design of a chemosensor capable of producing a detectable signal in the NMR time scale is not trivial. The system must have: (1) a sterically constrained environment to allow size discrimination; and (2) sufficient binding force that a static complex is generated on the NMR time scale. Furthermore, the signal must be easy to quantify in a fully resolved spectrum. Fluorine-19 is an excellent nuclide to produce high-intensity, well-resolved signals in NMR because the chemical shifts range between +250 and -300 ppm and the resonances are very sharp, which minimizes signal overlap.^[4]

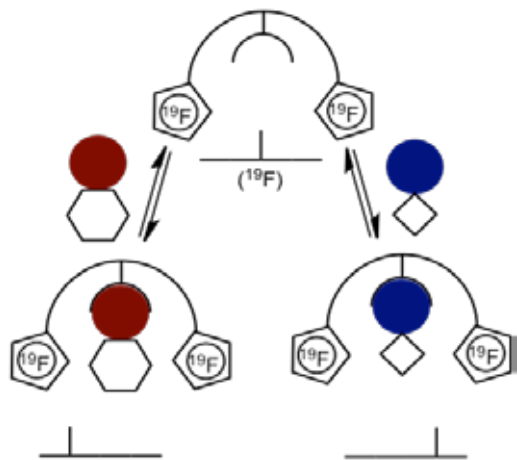
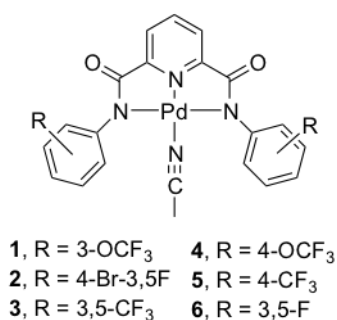


Figure 1. Schematic representation of chemosensor monitoring the change in ^{19}F chemical shift

Recently, Swager and coworkers reported a new chemosensing platform utilizing delicately designed ^{19}F -labeled receptors.^[5] The ^{19}F probes are located in proximity to the bound analyte and produce a unique ^{19}F NMR 'fingerprint' for each analyte, thereby allowing an unambiguous identification in complex mixtures. In this application note, the advantages offered by the molecular design of the Swager group is highlighted to detect and quantify the caffeine content in coffee without any sample preparation!



Scheme 1. Palladium pincer complexes with fluorine probes.

Quantification of Caffeine Level in Coffee

Caffeine is a chemical found in various beverages (e.g., coffee, tea, sodas and energy drinks). It displays unique physiological functions and often improves mental alertness. However, caffeine has no nutritional value and a high dose of caffeine may cause uncomfortable, potentially lethal side effects. A rapid and reliable method to quantify caffeine content is extremely desirable. However, coffee is difficult to analyze owing to the fact that it is an extremely complicated crude mixture, the primary constituents of which are water, carbohydrates, fibers, proteins, free amino acids, lipids, minerals, organic acids, chlorogenic acid, trigonelline, and caffeine. The direct quantification of caffeine in coffee without pre-treatment is thus a challenging task for traditional analytical methods.

Figure 2 illustrates the quantification of the caffeine level in Starbucks coffee using the NMReady without pre-treatment. Coffee (300 μL) was mixed with a mixture of palladium complex and 4-nitrobenzotrifluoride (internal standard) in CD_3OD (300 μL). Upon complexation with caffeine, a characteristic ^{19}F NMR signal at -63.5 ppm was produced and assigned as the palladium complex bound with caffeine. The big resonance at -62.7 ppm corresponds to the excess complex 1 added to the mixture.

As NMR resonances all have the same response factor, the concentration of caffeine is determined directly by integrating the ^{19}F resonances from the caffeine-bound complex and the internal standard (see procedure). The benchtop NMReady (60 MHz) gives a caffeine concentration of 610 mg/L, which is the same that was obtained with a 400 MHz high-field NMR spectrometer (c.f., 612 mg/mL).^[6]

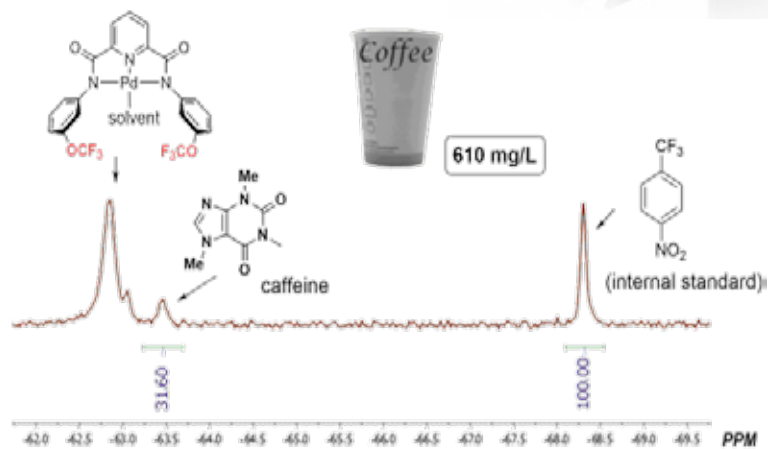
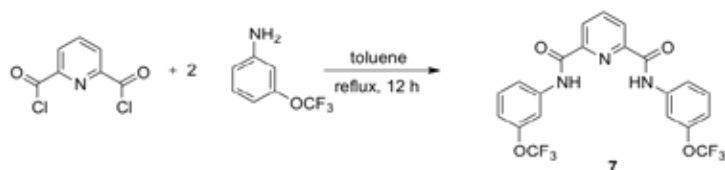


Figure 2. ^{19}F spectrum of a mixture of palladium complex, internal standard and coffee.

Procedure^[6]

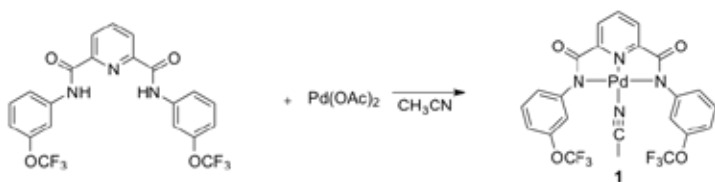
Synthesis of complex 1

A solution of 2,6-pyridinedicarbonyl dichloride (500 mg, 2.45 mmol, 1.0 equiv) and 3-(trifluoromethoxy)aniline (868 mg, 4.9 mmol, 2.0 equiv) was refluxed for 12 h in toluene (30 mL) under argon. At room temperature the precipitate was filtered off and washed with toluene (20 mL) and hexanes (20 mL) and then dried under air to give the product as a white solid in 88% yield (1.050 g, 2.16 mmol).^[6]



Scheme 2. Synthesis of ligand 7

Ligand 7 (200 mg, 0.44 mmol, 1.0 equiv) was suspended in an acetonitrile solution (10 mL) of Pd(OAc)₂ (103 mg, 0.46 mmol, 1.05 equiv). The resulting mixture was stirred at 35 °C for 12 h, and filtered through a 0.02 μM syringe filter. The filtrate was concentrated to give the crude product, which was transferred to a filtering funnel and washed extensively with water and hexanes. The yellow powder was dried under air to give complex 2 in 87% yield (264 mg, 0.38 mmol).^[6]



Scheme 3. Synthesis of complex 1

Protocol for determining the caffeine content:

Coffee (300 μL) was mixed with a mixture of palladium complex (4–6 mg) and 4-nitrobenzotrifluoride (1.14 mg) in CD₃OD (300 μL). Then the ¹⁹F NMR spectrum (256 scans) was acquired on an NMReady spectrometer. The caffeine content was determined from rearranging equation 1 to get equation 2:

$$(mmol A)/(mmol B) = ((I A/N A)/(I B/N B)) \quad [1]$$

$$mgA = \frac{I A}{N A} \frac{N B}{I B} \frac{mg B}{M B} M A \quad [2]$$

where:

mg = milligrams

I = relative integral

N = the numbers of fluorines contributing to the respective signals

M = molecular weight

Using the integrals obtained from the acquired fluorine spectrum in figure 2, the number of fluorines giving rise to each resonance, and the molecular weight of each compound, we get the following:

$$mg \text{ caffeine} = \frac{31.60}{6} \frac{3}{100.00} \frac{1.14 \text{ mg}}{191.11 \text{ g/mol}} 194.19 \text{ g/mol}$$

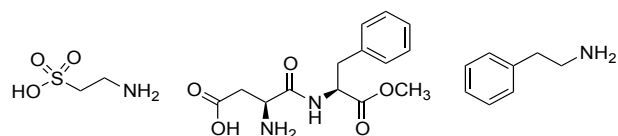
$$mg \text{ caffeine} = 0.183$$

Since we got 0.183 mg of caffeine in the initial 0.3 mL, the final concentration is 610 mg/mL.*

Experiment Modifications

In this experiment we only detected the amount of caffeine in coffee, but the experiment can be easily modified depending on the laboratory size and the main target of the experiment. The instructor can prepare several stock solutions with different amount of caffeine and give them to the students for quantification.

Another nitrogen containing compounds such as taurine, aspartame, and phenylethylamine can also be quantified with the complexes shown in scheme 1. For more details check reference 6.



Scheme 4. Potential N-complexes for identification

* We can calculate the moles of caffeine present using the integration given by the caffeine-bound complex because the numbers of moles are equal.

Conclusions

In this experiment we have used our NMReady spectrometer to quickly quantify the caffeine content in regular coffee without any sample preparation. This is a great experiment to show students that NMR spectroscopy is not only the best technique for structural elucidation, but it can also be used for quality control and for quantification of components.

In combination with ^{19}F NMR chemosensory method, NMReady spectrometer provides the same accuracy in determining the caffeine level in coffee as a high-field NMR spectrometer. This experiment also shows that the use of NMR spectroscopy is no longer hampered by the space, power, and liquid nitrogen supply that are required to maintain a high-field instrument.

Acknowledgement

Yanchuan Zhao, Lily Chen, and Timothy M. Swager at Massachusetts Institute of Technology performed the experimental procedure and part of the writing in this application note. The ^{19}F NMR chemosensing technique described in this note is based on Reference 1 and was supported by a National Institutes of Health (NIGMS) grant GM095843.

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For additional ideas of how to incorporate the NMReady-60 family of spectrometers into undergraduate teaching, please see:

Unknown Identification by ^1H NMR Spectroscopy

Synthesis & Stereoselectivity Determination of Ethyl Cinnamate via Wittig Rxn

Synthesis of Biodiesel

Aldol Condensation

available at:

www.nalysis.com/experiments.html