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NMR-Based Chemosensing via p-H_2 Hyperpolarization: Application to Natural Extracts

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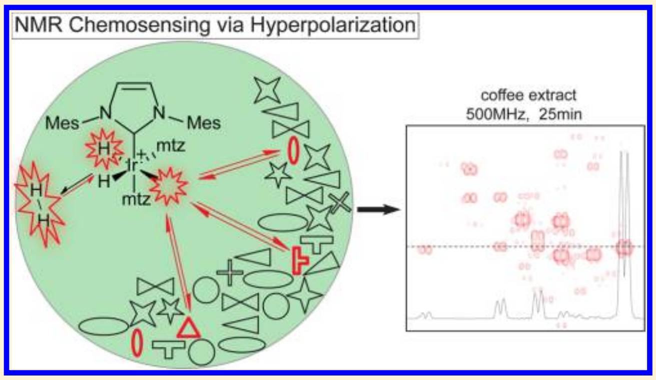
S *Supporting Information*

ABSTRACT: When dealing with trace analysis of complex mixtures, NMR suffers from both low sensitivity and signal overlap. NMR chemosensing, in which the association between an analyte and a receptor is "signaled" by an NMR response, has been proposed as a valuable analytical tool for biofluids and natural extracts. Such chemosensors offer the possibility to simultaneously detect and distinguish different analytes in solution, which makes them particularly suitable for analytical applications on complex mixtures. In this study, we have combined NMR chemosensing with nuclear spin hyperpolarization. This was realized using an iridium complex as a receptor in the presence of parahydrogen: association of the target analytes to the metal center results in approximately 1000-fold enhancement of the NMR response. This amplification allows the detection, identification, and quantification of analytes at low-micromolar concentrations, provided they can weakly associate to the iridium chemosensor. Here, our NMR chemosensing approach was applied to the quantitative determination of several flavor components in methanol extracts of ground coffee.



Nuclear Magnetic Resonance spectroscopy is widely used for identification and quantification of analytes in solution. However, when dealing with complex mixtures such as biofluids or natural extracts, several issues arise that can seriously hamper NMR chemical analysis. In the first place, NMR detection of dilute components (i.e., at low micromolar concentration) is impeded by the low sensitivity of the technique. This problem can be alleviated by *nuclear spin hyperpolarization,* i.e. transiently increasing the spin population differences, which results in large NMR signal enhancements. This has already been demonstrated in the context of mixtures analysis for hyperpolarization methods such as *dissolution dynamic nuclear polarization* (dissolution-DNP)[1−3] or *signal amplification by reversible exchange* (SABRE).[4−16] An additional hurdle is represented by NMR signal overlap. Since solutes in complex mixtures are usually not isotopically labeled, NMR studies are often limited to ^1H measurements. Unfortunately, in solutions containing hundreds of components at concentrations ranging from submicromolar to high millimolar, the modest ^1H resonances dispersion (typically ∼10 ppm) determines a formidable signal crowding. This represents a serious obstacle to the quantitative determination of any analyte, except for the most concentrated ones. Under these circumstances, the lack of specificity of NMR represents a major disadvantage in chemical analysis. This limitation has been addressed by "hyphenated" methods,[17] i.e. by coupling NMR with separation techniques, such as Liquid Chromatography, to simplify the composition and the analysis of the system under investigation. Alternatively, selective observation of the species of interest has been achieved exploiting specific interactions with *ad hoc* devised receptors, as recently demonstrated for NMR-based chemosensors.[18−22] Here we follow the latter approach and report on a chemosensing method that relies on the weak association of analytes to an iridium complex which serves as a selecting receptor. Importantly, this association can be exploited to generate highly enhanced NMR signals via parahydrogen (p-H_2) induced hyperpolarization.[23,24] Therefore, our approach allows at once to overcome NMR sensitivity limitations and to detect the analytes of interest, while removing the large background originating from the complex matrix. We have applied our NMR chemosensing experiment to quantitatively determine specific classes of flavor components in a methanol extract of roasted ground coffee.

During roasting of green coffee beans, a series of reactions take place (e.g., Maillard reactions, Strecker degradations, degradation of trigonelline, sugars, phenolic acids and lipids, etc.), producing a multitude of volatile compounds that provide an important contribution to coffee flavor.[25] These molecules are for a large part derivatives of heterocyclic compounds, e.g. furans, pyrroles, thiophenes, pyrazines, thiazoles, oxazoles, etc.

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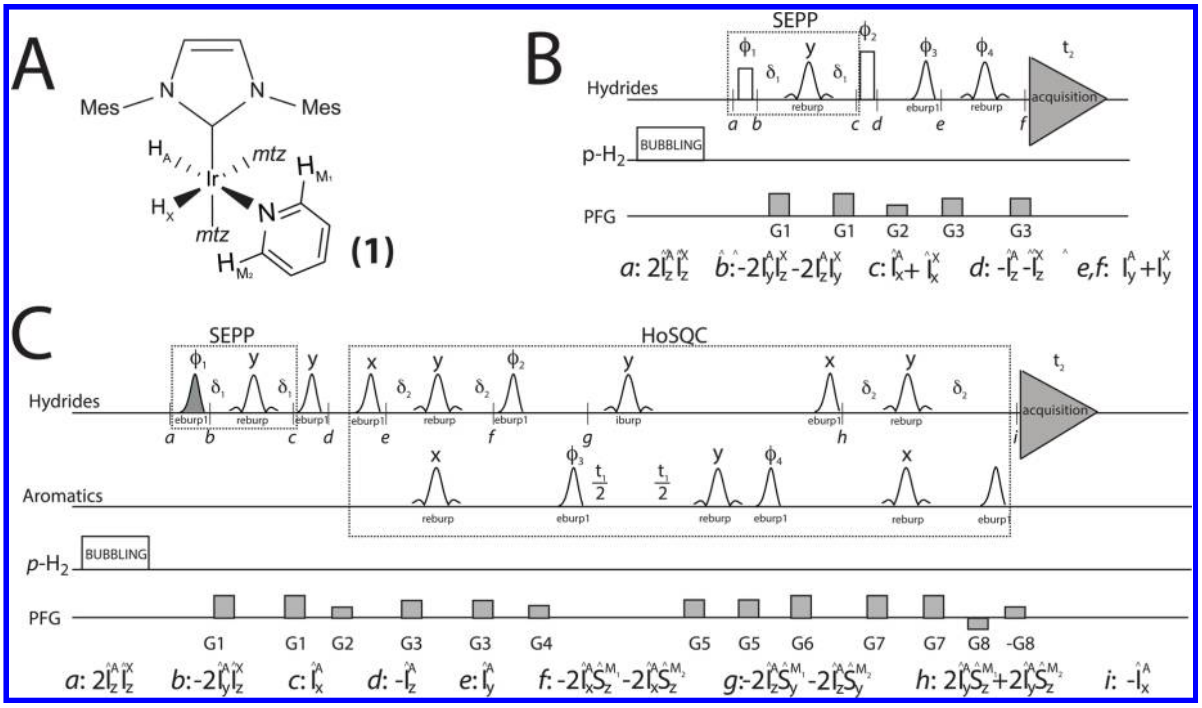
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Figure 1. (A) Schematic representation of the asymmetric chemosensor complex (**1**) for a pyridine-like analyte in the presence of a large excess of 1-methyl-1,2,3-triazole (*mtz*) as cosubstrate. Note that only hydride H_A, *trans* with respect to the analyte, displays an appreciable scalar coupling interaction with the aromatic protons H_M. (B) Selective Excitation of Polarization using PASADENA (SEPP)[28,29] pulse scheme to acquire a 1D p-H_2 enhanced NMR spectrum of the hydrides, centered at −21.729 ppm. Bubbling 5 bar of 51% enriched p-H_2 occurs for ca. 1 s at the beginning of the pulse sequence. Rectangular pulses indicate low-power (RF field ca. 2.5 kHz) 45- and 90-degree pulses, while shapes represent selective pulses with a bandwidth of 2000 Hz. Delay durations: δ_1 = 29.4 ms. Phase cycling: ϕ_1: x, −x; ϕ_2: 2(y), 2(−y); ϕ_3: 4(x), 4(y), 4(−x), 4(−y); ϕ_4: 4(y), 4(−x), 4(−y), 4(x); receiver: x,−x,−x,x,y,−y,−y,y,−x,x,x,−x, −y,y,y,−y. (C) 2D SEPP-HoSQC experiment. Bubbling 5 bar of 51% enriched p-H_2 occurs for ca. 1 s at the beginning of the pulse sequence. Band selective pulses on the hydrides (centered at −23.116 ppm) and on the aromatic protons (centered at 8.040 ppm) are used. The greyed excitation pulse is selective for the high field hydrides ("A"). Delay durations: δ_1 = 29.4 ms, δ_2 = 58.82 ms; Phase cycling: ϕ_1: x, −x; ϕ_2: 2(y), 2(−y); ϕ_3: 4(x), 4(−x); ϕ_4: 8(x), 8(−x) receiver: x,−x,−x,x,2(−x,x,x,−x),x,−x,−x,x. Quadrature detection in the indirect dimension is accomplished by States-TPPI.

Because of their low concentrations, further aggravated by signal crowding, most of these volatile species are not detectable by routine NMR methods. However, several of them (e.g., pyridine and pyrazine derivatives) weakly associate to metalorganic iridium complexes in a methanol solution. Here we demonstrate that their signals can be selectively detected by hyperpolarized NMR chemosensing techniques. Thanks to the selective amplification of the NMR chemosensor response, we could quantitatively detect target components at micromolar concentrations.

■ EXPERIMENTAL SECTION

Coffee Sample. A commercially available vacuum-packed ground coffee sample was purchased from a local grocery market in The Netherlands. This sample was declared as "regular" coffee of Arabica variety, with medium degree of roast and medium-scale grinding, designed to be normal filter coffee.

Chemicals. Complex precursor [Ir(COD) (IMes)Cl] (IMes =1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene; COD = cyclooctadiene) and cosubstrate 1-methyl-1,2,3-triazole (*mtz*) were synthesized according to published methods.[26,27] Methanol-d_4 was purchased from Cambridge Isotope Laboratories. All other chemicals were purchased from Sigma-Aldrich (pyridine, pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2,6-dimethylpyrazine, 3-hydroxypyridine). Parahydrogen (p-H_2) was produced with an in-house designed 2 L vessel embedded in a liquid nitrogen bath. Normal hydrogen (purity 5.0) was cooled down to 77 K in the presence of 100 mL of 4−8 MESH charcoal (Sigma-Aldrich). The resulting 51% p-H_2 was transported to an aluminum cylinder (Nitrous Oxides Systems, Holley Performance Products, Bowling Green, KY, USA),[28] with an adjustable output-pressure valve.

Methanol Extraction. Ground roasted coffee (1.8 g) was suspended in methanol-d_4 (5 mL) and stirred for 30 min at room temperature. After centrifugation, the supernatant was collected with a syringe, leaving behind a soggy coffee residue. The liquid was filtered using a 0.45 μm MF-Millipore MCE Membrane Filter Unit by Millex.

NMR Sample Preparation. A stock solution of 4.8 mM [Ir(COD) (IMes)Cl] complex precursor, 72 mM 1-methyl-1,2,3-triazole (*mtz*) as cosubstrate, and 100 mM ethanol as a reference was prepared in methanol-d_4 under nitrogen pressure. Prior to the NMR measurements, 120 mg of stock solution, corresponding to 1/4 of the final NMR sample volume, was transferred into a 5 mm Wilmad quick pressure valve (QPV) NMR tube. The tube was pressurized under 5 bar of H_2 to convert the complex precursor into the activated symmetric complex $[Ir(IMes)(H)_2(mtz)_3]Cl$. Complete activation required approximately 120 min. Immediately before the NMR measurements, 360 mg of coffee extract (3/4 of the total NMR sample volume) was added to the activated catalyst solution in the QPV NMR tube. Final concentrations of the stock solution components were 1.2 mM iridium complex, 18 mM *mtz*, and

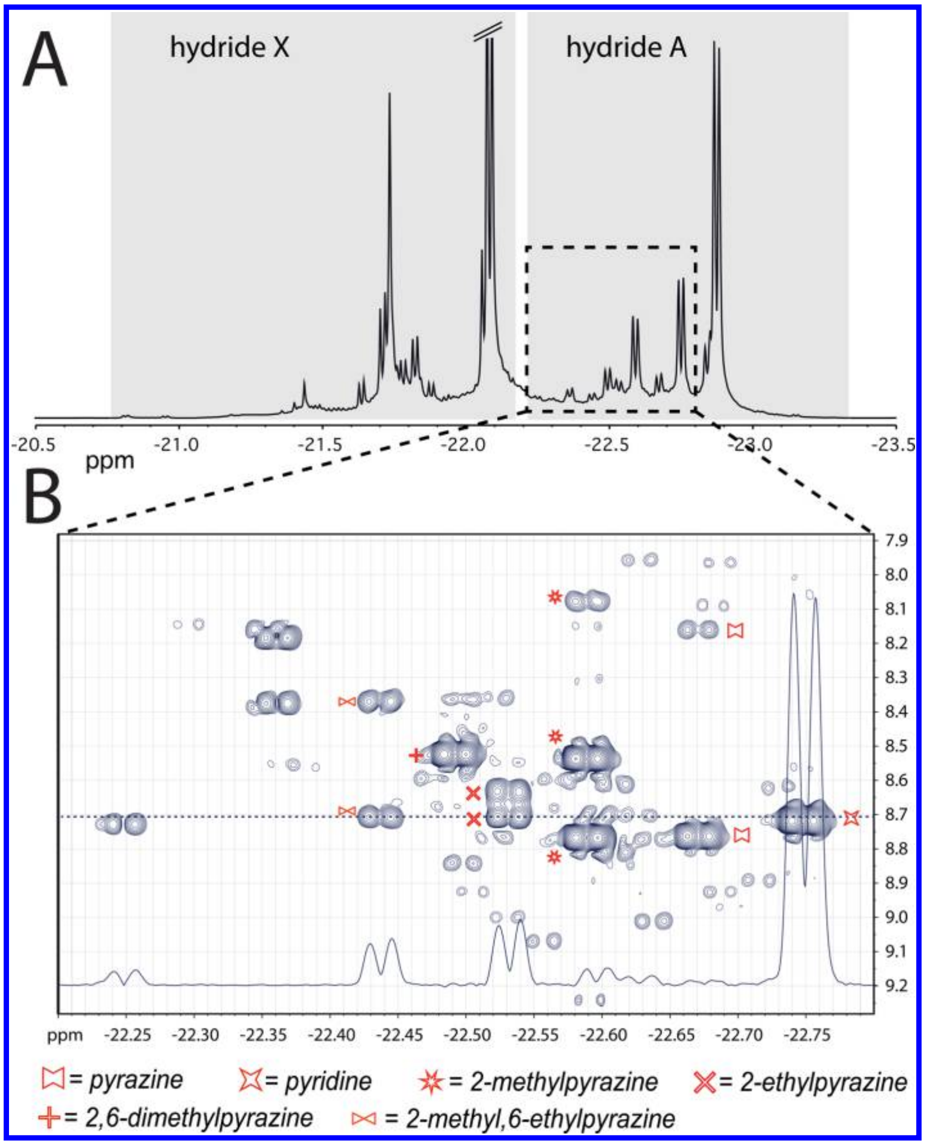

Figure 2. (A) p-H_2 enhanced NMR hydride signals of coffee extract in methanol-d_4 acquired with the pulse scheme sketched in Figure 1B. The sample contains 1.2 mM metal complex, 18 mM *mtz*, and 5 bar 51% enriched p-H_2. (B) High-field p-H_2 enhanced 2D correlation spectrum between hydrides and substrate aromatic protons in the receptor complexes (**1**), acquired on the same sample as (A) with the pulse scheme sketched in Figure 1C in ca. 20 min. Assignment of the most concentrated species is indicated. The 1D trace is shown to illustrate the signal-to-noise ratio at the dotted line in the spectrum.

25 mM ethanol, while the coffee extract components were diluted to 75%.

Standard Addition. For the standard addition experiments stock solutions (100 mM and 1 mM) of the compound under investigation were prepared using coffee extract in methanol-d_4 as a solvent. A typical standard addition series consisted of the original coffee extract and four additional samples at increasing concentration of the analyte. Typically, the highest analyte concentration in the series corresponded to ca. 2.5−4 times the original concentration in the coffee extract. All solutions were prepared by gravimetric mixing of solvents and analytes, assuming for all methanolic extracts and solutions a density equal to methanol-d_4 (0.888 g/mL).

Theory. In the following we will refer to the species that can bind to the chemosensing receptor as "analytes" or "substrates", interchangeably. We have previously shown[15] that in the presence of a dilute target analyte and a large excess of *mtz*, the activated symmetric complex $[Ir(IMes)(H)_2(mtz)_3]Cl$ is partly converted into the asymmetric complex (**1**) (see Figure 1A in the case of a pyridine derivative). Because of the chemical inequivalence of the two hydrides, the p-H_2 derived singlet state is rapidly dephased, leaving longitudinal spin order as surviving term.[29,30] The SEPP[31,32] NMR pulse scheme displayed in Figure 1B allows the conversion of such longitudinal spin order into hydrides enhanced magnetization, which can be detected as an NMR signal increased up to 3 orders of magnitude. We have recently shown that this spin-order can also be exploited to enhance the NMR signals of analyte protons in the metal complex via long-range scalar couplings.[24]

The 2D pulse scheme here employed (Figure 1C) differs from the previously presented one[24] in that a "forth-and-back" coherence transfer pathway is followed, with acquisition on the hydrides rather than on aromatic protons of the substrate. After the initial SEPP block, hydrides enhanced magnetization is transferred via homonuclear-INEPT[33] to the analyte protons for t_1 evolution and then back, following a Homonuclear Single

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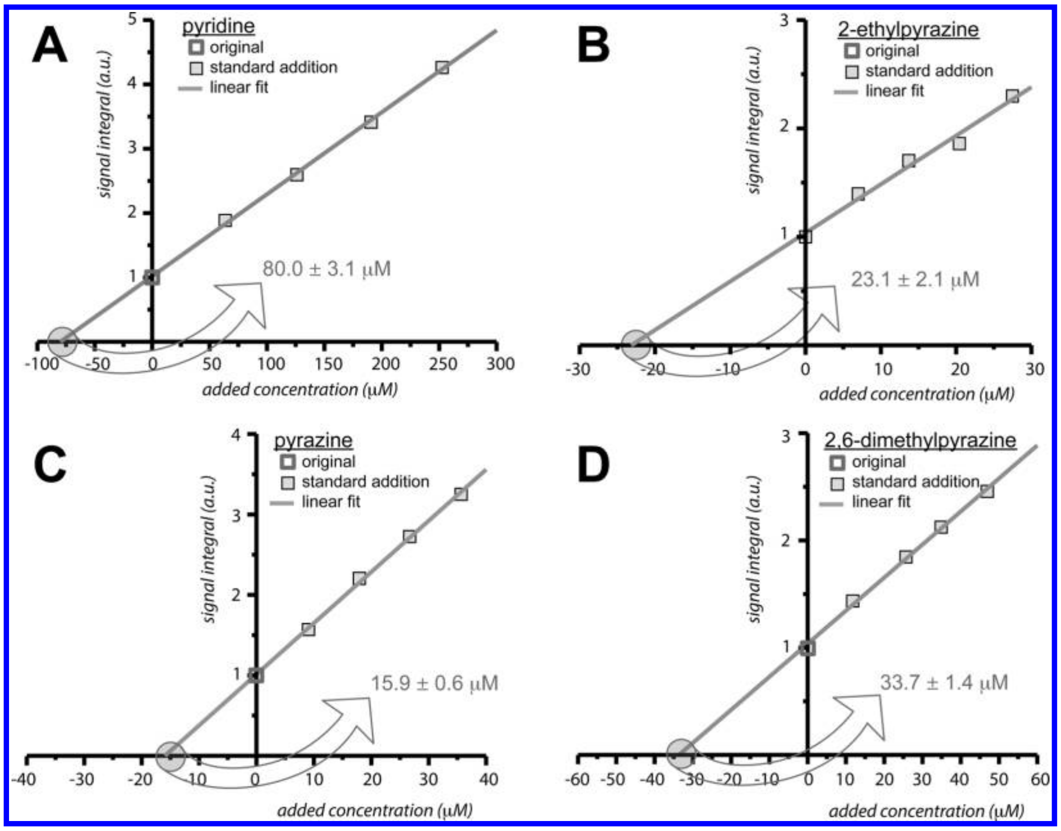

Figure 3. Standard-addition curves for *ortho* proton resonances of pyridine (A), 2-ethylpyrazine (B), pyrazine (C), and 2,6-dimethylpyrazine (D). Concentrations are estimated from the abscissa intercept (circled) of the standard-addition curves (gray lines). Experimental uncertainties were derived by error propagation.

Quantum Coherence (HoSQC) pathway. We have recently proposed a similar scheme for the determination of the long-range coupling constants between hydride and substrate protons.[34] The acquired 2D spectrum correlates the hydrides with the substrate protons in the bound form only; no NMR signal from free molecules in solution is observed with the proposed experiment. Analogously, the symmetric complex $[Ir(IMes)(H)_2(mtz)_3]Cl$ is not detected by this 2D SEPP-HoSQC experiment, due to the chemical equivalence of the two hydrides. The coherence transfer along the pulse sequence is illustrated in Figure 1C at specific time points in terms of product operator formalism[35] for a spin system consisting of two hydrides (A and X) and two *ortho* protons (M_1 and M_2); this description can be extended to more complex spin systems.

NMR. All NMR spectra were acquired at 283 or 298 K and at 499.91 MHz ^{1}H resonance frequency using a Varian UnityInova 500 spectrometer equipped with a triple-resonance HCN room temperature probe, with a shielded *z*-gradient coil. Typically, 2D data matrices consisting of $48(t_1) \times 2400(t_2)$ complex points were acquired with eight scans per increment in ca. 20 min. The 2D data sets were processed with NMRPipe[36] or iNMR,[37] using 72° shifted squared sine-bell apodization in both dimensions, prior to zero filling to $1024(t_1) \times 16384(t_2)$ complex points, and Fourier transformation.

■ RESULTS

NMR investigations of coffee conducted so far have mostly focused on species at relatively high concentrations (typically mM).[38−41] Conversely, concentrations in the methanol extract are expected between high nM and sub-mM for several flavor components. As previously discussed, spectral crowding in the coffee matrix hampers the signal identification for dilute solutes.

Detection and quantification of aromatic species at low micromolar concentration requires, therefore, an excellent suppression of undesired signals originating from more concentrated components (up to 1000-fold). Here, we have devised an NMR-based chemosensor that reveals analyte association to the receptor by NMR signals in the hydride region of the ^{1}H spectrum, generally free from background contributions.[23] For each species associating to the iridium center, the chemosensor response consists of a pair of hyperpolarized hydride lines ("A" and "X" in complex (**1**)) in the NMR spectrum. Thanks to the p-H_2 derived hydride signal enhancement (up to 1000-fold increase compared to NMR at thermal equilibrium), it is possible to lower the detection limits of this NMR chemosensor to submicromolar concentrations. The spectrum in Figure 2A, recorded in a few minutes, shows several hydrides signals well above the noise threshold. The strength of NMR chemosensing is evident when several different analytes associate to the receptor: every substrate molecule binding to the iridium center is revealed by different spectral lines, thanks to the sensitivity of chemical shifts to the structure of complex (**1**). In principle, identification (and quantification) of the species bound to the iridium complex could be performed by titrating a given compound in the coffee extract and following the signal increase of the corresponding hydride(s). However, this method proves impractical in the present case, due to the overlap of the hydride resonances, as clearly illustrated inside the boxed region of the spectrum in Figure 2A. We have previously shown[24] that high field continuous hyperpolarization via p-H_2 offers also the possibility to acquire 2D correlation spectra with enhanced sensitivity between hydrides and aromatic protons. Similarly, the 2D SEPP-HoSQC experiment employed here provides an efficient tool to separate overlapping signals from different substrates, as evidenced by the plot in Figure 2B. Note that all correlations in the 2D spectrum appear as doublets because of the J_{AX} scalar coupling between the two hydrides in complex (**1**).

Assignment of the strongest 2D correlations, reported in Figure 2B, was obtained by spiking the coffee extract. In the presence of a large excess of *mtz* cosubstrate, the 2D signal integral depends linearly on the substrate concentration.[15,24] This allows the determination of the analyte concentration in the submicromolar concentration regime by standard addition. A few representative standard addition curves are shown in Figure 3.

The value of each analytic concentration (expressed in μM) can be obtained from the abscissa intercept of the corresponding linear standard-addition curve. An uncertainty in the concentration determination of ca. 5−10% is obtained from error propagation.[16] The results of the standard addition experiments are compared in Table 1 with the concentration values (in ppm, see the Supporting Information) reported in the literature for comparable coffee samples. For most components an excellent agreement was found with the results obtained using different techniques (i.e., HPLC,[42] SIDA-GC/MS[45]).

Table 1. Analytes Concentration in Roasted Coffee Derived from Standard Addition

analyte	concn (μM)	concn (ppm)	reference concn (ppm)
3-hydroxypyridine[42]	273.7 ± 24.4	138.4 ± 14.0	140−400
pyridine[43,44]	80.0 ± 3.1	31.2 ± 1.3	49[43]− 204[44]
pyrazine[45]	15.9 ± 0.6	6.3 ± 0.3	5.6−12.6
2-methylpyrazine[45]	82.5 ± 9.2	37.8 ± 4.2	34.8−70.6
2-ethylpyrazine[45]	23. ± 2.1	11.7 ± 1.1	4.1−9.0
2,6-dimethylpyrazine[45]	33.7 ± 1.4	17.3 ± 1.0	14.3−24.6

DISCUSSION

As demonstrated by previously reported research,[18−22] NMR chemosensing can combine the high specificity of receptors with the sensitivity of NMR chemical shifts to molecular structure. This is particularly important in the analysis of complex mixtures in which several homologous species can associate to a receptor; in these cases, the response of an NMR-based chemosensor allows the identification and differentiation of the target analytes. This has been achieved also in the present work, with the quantitative determination of several substituted pyrazines and pyridines in methanol extracts of ground coffee.

So far, NMR-based chemosensing has been realized following two different approaches, i.e. either selectively detecting the signals of the analytes of interest[21,22] or measuring the variation of some NMR properties of the receptor upon association with the target.[18,20] A recent implementation of the former approach relied on the combination of different highly selective NMR tools (i.e., diffusion filters,[46] NOE and relaxation filters[47]) to reveal the weak association of primary amines to gold nanoparticles coated with receptor units, while discarding background signals.[22] The detection limit for such chemosensors, determined by the lowest measurable NMR response, is estimated to be in the submillimolar concentration range.

Alternatively, NMR-based chemosensors have been realized such that analyte binding is signaled by chemical shift changes for specific resonances of the receptor.[18,20] Being virtually free from background interferences, ^{19}F or ^{129}Xe nuclear spins offer maximum sensitivity to probe the ligand−receptor association. Furthermore, the high sensitivity of ^{19}F or ^{129}Xe chemical shifts to the local environment results in detectable changes in the NMR spectra upon analyte association to the receptor. Notably, nuclear spin hyperpolarization was used for the ^{129}Xe NMR-based chemosensor to maximize the sensitivity.[20] However, the small ^{129}Xe chemical shift changes observed upon binding of the target analytes to the receptor has so far determined a detection limit in the submillimolar range, despite the high signal-to-noise ratio of the NMR spectrum.

Here, we follow a different strategy, by detecting ^{1}H chemical shifts from both the receptor (the hydrides) and the bound analyte in a 2D correlation spectrum. In the absence of analytes, the receptor (i.e., the iridium center) is fully complexed by the cosubstrate *mtz*. In this case, the two hydrides are chemically equivalent, and no signal is obtained from the SEPP-based NMR experiments displayed in Figures 1B and 1C. Only upon association of an analyte to the receptor (by displacement of a *mtz* ligand), hyperpolarized hydride signals can be observed. Our method relies, therefore, on the detection of an NMR signal, rather than on a chemical shift change, to reveal association to the receptor. As a consequence, the large signal enhancement provided by *p*-H_2 derived hyperpolarization results in a drastic reduction of the detection limits to low-micromolar concentrations.

2D NMR spectra have been previously employed for quantitative determination of analytes in solution.[48] However, in the case of hyperpolarization NMR spectra, a quantitative data analysis requires some caution, as previously discussed:[16] a comparison of the 2D signal integrals of hyperpolarized samples can provide only a semiquantitative estimate of the relative concentrations, even in the present case with most of the analytes being structurally homologous. However, by using calibration techniques such as the standard addition method,[49] it is possible to determine absolute amounts, here of different flavor components in coffee. Our results are in very good agreement with previously reported data based on different techniques, which confirms the validity of the proposed NMR approach.

CONCLUSIONS

We have presented a new implementation of NMR-based chemosensing that allowed the detection and quantification of pyridine and pyrazine derivatives in a methanol coffee extract. Using NMR for signaling the receptor binding is particularly important for complex mixtures in which several target molecules are present. As shown in the present application, the sensitivity of chemical shifts to molecular structure allows a straightforward discrimination of the analytes bound to the receptor.

In general, the detection limit of NMR chemosensors is determined by the intrinsic (in)sensitivity of the technique. Recent implementations of NMR chemosensing for chemical analysis report such limit in the submillimolar range.[20,22] Here, interaction with the receptor, an iridium complex, in the presence of *p*-H_2, results in approximately 1000-fold amplification of the NMR response. As a consequence, a detection limit in the low-micromolar concentration range is obtained. The presented method was applied, for the first time, to a complex natural extract and resulted in the NMR detection and quantification of several aroma components in coffee. Evaluation of the method was obtained by comparison of the obtained absolute concentrations with reported values derived from different experimental techniques. In most cases an excellent agreement was found. The proposed chemosensor targets specifically analytes that associate to the iridium receptor. In almost all cases reported so far, this binding occurs via a nitrogen lone pair, which makes several nitrogenous heterocyclic compounds, such as pyridine and pyrazine derivatives, ideal targets for the proposed technique. This chemosensing approach can be extended to other targets such as nucleobases,[23,50] amino acids,[51] drugs,[52,53] and tagged oligopeptides[54] that can be hyperpolarized by weakly associating to an iridium complex in the presence of p-H_2. Therefore, it can represent a valuable tool in the quantitative determination of these compounds in complex mixtures such as biofluids or natural extracts.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b00184.

> Data concerning extraction efficiency, analytes concentration determination, and experimental details about NMR experiments (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

p-H_2	parahydrogen
mtz	1-methyl-1,2,3-triazole

■ REFERENCES

(1) Ardenkjær-Larsen, J. H.; Fridlund, B.; Gram, A.; Hansson, G.; Hansson, L.; Lerche, M. H.; Servin, R.; Thaning, M.; Golman, K. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 10158−10163.

(2) Giraudeau, P.; Shrot, Y.; Frydman, L. *J. Am. Chem. Soc.* **2009**, *131*, 13902−13903.

(3) Dumez, J.-N.; Milani, J.; Vuichoud, B.; Bornet, A.; Lalande-Martin, J.; Tea, I.; Yon, M.; Maucourt, M.; Deborde, C.; Moing, A.; Frydman, L.; Bodenhausen, G.; Jannin, S.; Giraudeau, P. *Analyst* **2015**, *140*, 5860−5863.

(4) Adams, R. W.; Aguilar, J. A.; Atkinson, K. D.; Cowley, M. J.; Elliott, P. I. P.; Duckett, S. B.; Green, G. G. R.; Khazal, I. G.; Lopez-Serrano, J.; Williamson, D. C. *Science* **2009**, *323*, 1708−1711.

(5) Adams, R. W.; Duckett, S. B.; Green, R. A.; Williamson, D. C.; Green, G. G. R. *J. Chem. Phys.* **2009**, *131*, 194505.

(6) Cowley, M. J.; Adams, R. W.; Atkinson, K. D.; Cockett, M. C. R.; Duckett, S. B.; Green, G. G.; Lohman, J. A. B.; Kerssebaum, R.; Kilgour, D.; Mewis, R. E. *J. Am. Chem. Soc.* **2011**, *133*, 6134−6137.

(7) Dücker, E. B.; Kuhn, L. T.; Münnemann, K.; Griesinger, C. *J. Magn. Reson.* **2012**, *214*, 159−165.

(8) Lloyd, L. S.; Adams, R. W.; Bernstein, M.; Coombes, S.; Duckett, S. B.; Green, G. G.; Lewis, R. J.; Mewis, R. E.; Sleigh, C. J. *J. Am. Chem. Soc.* **2012**, *134*, 12904−12907.

(9) Van Weerdenburg, B. J. A.; Glöggler, S.; Eshuis, N.; Engwerda, A. H. J.; Smits, J. M. M.; De Gelder, R.; Appelt, S.; Wymenga, S. S.; Tessari, M.; Feiters, M. C.; Blumich, B.; Rutjes, F. P. J. T. *Chem. Commun.* **2013**, *49*, 7388−7390.

(10) Theis, T.; Truong, M. L.; Coffey, A. M.; Shchepin, R. V.; Waddell, K. W.; Shi, F.; Goodson, B. M.; Warren, W. S.; Chekmenev, E. Y. *J. Am. Chem. Soc.* **2015**, *137*, 1404−1407.

(11) Pravdivtsev, A. N.; Yurkovskaya, A. V.; Vieth, H. M.; Ivanov, K. L. *Phys. Chem. Chem. Phys.* **2014**, *16*, 24672−24675.

(12) Pravdivtsev, A. N.; Yurkovskaya, A. V.; Vieth, H. M.; Ivanov, K. L. *J. Phys. Chem. B* **2015**, *119*, 13619−13629.

(13) Shchepin, R. V.; Truong, M. L.; Theis, T.; Coffey, A. M.; Shi, F.; Waddell, K. W.; Warren, W. S.; Goodson, B. M.; Chekmenev, E. Y. *J. Phys. Chem. Lett.* **2015**, *6*, 1961−1967.

(14) Daniele, V.; Legrand, F.; Berthault, P.; Dumez, J.; Huber, G. *ChemPhysChem* **2015**, *16*, 3413−3417.

(15) Eshuis, N.; Hermkens, N.; Van Weerdenburg, B. J. A.; Feiters, M. C.; Rutjes, F. P. J. T.; Wijmenga, S. S.; Tessari, M. *J. Am. Chem. Soc.* **2014**, *136*, 2695−2698.

(16) Eshuis, N.; Van Weerdenburg, B. J. A.; Feiters, M. C.; Rutjes, F. P. J. T.; Wijmenga, S. S.; Tessari, M. *Angew. Chem., Int. Ed. Engl.* **2015**, *54*, 1481−1484.

(17) Albert, K. *On-Line LC-NMR and Related Techniques*; Wiley & Sons: New York, NY, 2002.

(18) Zhao, Y.; Chen, L.; Swager, T. M. *Angew. Chem., Int. Ed. Engl.* **2016**, *55*, 917−921.

(19) Plaunt, A. J.; Clear, K. J.; Smith, B. D. *Chem. Commun.* **2014**, *50*, 10499.

(20) Garimella, P. D.; Meldrum, T.; Witus, L. S.; Smith, M.; Bajaj, V. S.; Wemmer, D. E.; Francis, M. B.; Pines, A. *J. Am. Chem. Soc.* **2014**, *136*, 164−168.

(21) Perrone, B.; Springhetti, S.; Ramadori, F.; Rastrelli, F.; Mancin, F. *J. Am. Chem. Soc.* **2013**, *135*, 11768−11771.

(22) Salvia, M.-V.; Salassa, G.; Rastrelli, F.; Mancin, F. *J. Am. Chem. Soc.* **2015**, *137*, 11399−11406.

(23) Wood, N. J.; Brannigan, J. A.; Duckett, S. B.; Heath, S. L.; Wagstaff, J. *J. Am. Chem. Soc.* **2007**, *129*, 11012−11013.

(24) Eshuis, N.; Aspers, R. L. E. G.; Van Weerdenburg, B. J. A.; Feiters, M. C.; Rutjes, F. P. J. T.; Wijmenga, S. S.; Tessari, M. *Angew. Chem., Int. Ed.* **2015**, *54*, 14527−14530.

(25) Flament, I. *Coffee Flavour Chemistry*; John Wiley & Sons, Ltd.: Chichester, 2002.

(26) Kelly, R. A., III; Clavier, H.; Giudice, S.; Scott, N. M.; Stevens, E. D.; Bordner, J.; Samardjiev, I.; Hoff, C. D.; Cavallo, L.; Nolan, S. P. *Organometallics* **2008**, *27*, 202−210.

(27) Seefeld, M. B.; Heerding, D. A.; Peace, S.; Yamashita, D.; McNulty, K. C. Inhibitors of AKT activity, WO2008/098104 A1, August 14, 2008 (Smithkline Beecham Corporation).

(28) Feng, B.; Coffey, A. M.; Colon, R. D.; Chekmenev, E. Y.; Waddell, K. W. *J. Magn. Reson.* **2012**, *214*, 258−262.

(29) Bowers, C. R.; Weitekamp, D. P. *Phys. Rev. Lett.* **1986**, *57*, 2645−2648.

(30) Torres, O.; Procacci, B.; Halse, M. E.; Adams, R. W.; Blazina, D.; Duckett, S. B.; Eguillor, B.; Green, R. A.; Perutz, R. N.; Williamson, D. C. *J. Am. Chem. Soc.* **2014**, *136*, 10124−10131.

(31) Sengstschmid, H.; Freeman, R.; Barkemeyer, J.; Bargon, J. *J. Magn. Reson., Ser. A* **1996**, *120*, 249−257.

(32) Barkemeyer, J.; Bargon, J.; Sengstschmid, H.; Freeman, R. *J. Magn. Reson., Ser. A* **1996**, *120*, 129−132.

(33) Miao, X.; Freeman, R. *J. Magn. Reson., Ser. A* **1995**, *117*, 128−131.

(34) Eshuis, N.; Aspers, R. L. E. G.; Van Weerdenburg, B. J. A.; Feiters, M. C.; Rutjes, F. P. J. T.; Wijmenga, S. S.; Tessari, M. *J. Magn. Reson.* **2016**, *265*, 59−66.

(35) Sorensen, O. W.; Eich, G. W.; Levitt, M. H.; Bodenhausen, G.; Ernst, R. R. *Prog. Nucl. Magn. Reson. Spectrosc.* **1984**, *16*, 163−192.

(36) Delaglio, F.; Grzesiek, S.; Vuister, G. W.; Zhu, G.; Pfeifer, J.; Bax, A. *J. Biomol. NMR* **1995**, *6*, 277−293.

(37) Balacco, G.; Marino, C. 2005. http://www.inmr.net/ (accessed Feb 1, 2016).

(38) Bosco, M.; Toffanin, R.; De Palo, D.; Zatti, L.; Segre, A. *J. Sci. Food Agric.* **1999**, *79*, 869.

(39) Ku Madidah, K. Y.; Zaibunnisa, A. H.; Norashikin, S.; Rozita, O.; Misnawi, J. *APCBEE Proc.* **2013**, *20*, 1623.

(40) Wei, F.; Furihata, K.; Koda, M.; Hu, F.; Miyakawa, T.; Tanokura, M. *J. Agric. Food Chem.* **2012**, *60*, 1005.

(41) Ciampa, A.; Renzi, G.; Taglienti, A.; Sequi, P.; Valentini, M. *J. Food Qual.* **2010**, *33*, 199.

(42) Kurakane, S.; Igarashi, K. *Food Sci. Technol. Res.* **2006**, *12*, 148−151.

(43) Hughes, E. B.; Smith, R. F. *J. Soc. Chem. Ind., London* **1946**, *65*, 284−286.

(44) Stofberg, J.; Stoffelsma, J. *Perfum. Flavor.* **1981**, *5*, 19−35.

(45) Pickard, S.; Becker, I.; Merz, K.-H.; Richling, E. *J. Agric. Food Chem.* **2013**, *61*, 6274−6281.

(46) Chen, A.; Shapiro, M. J. *J. Am. Chem. Soc.* **1998**, *120*, 10258−10259.

(47) Rastrelli, F.; Jha, S.; Mancin, F. *J. Am. Chem. Soc.* **2009**, *131*, 14222−14223.

(48) Giraudeau, P. *Magn. Reson. Chem.* **2014**, *52*, 259−272.

(49) Martineau, E.; Tea, I.; Akoka, S.; Giraudeau, P. *NMR Biomed.* **2012**, *25*, 985−992.

(50) Hövener, J.-B.; Schwaderlapp, N.; Lickert, T.; Duckett, S. B.; Mewis, R. E.; Highton, L. A. R.; Kenny, S. M.; Green, G. G. R.; Leibfritz, D.; Korvink, J. G.; Hennig, J.; Von Elverfeldt, D. *Nat. Commun.* **2013**, *4*, 2946−2950.

(51) Glöggler, S.; Muller, R.; Colell, J.; Emondts, M.; Dabrowski, M.; Blümich, B.; Appelt, S. *Phys. Chem. Chem. Phys.* **2011**, *13*, 13759−13764.

(52) Zeng, H.; Xu, J.; Gillen, J.; McMahon, M. T.; Artemov, D.; Tyburn, J.-M.; Lohman, J. A. B.; Mewis, R. E.; Atkinson, K. D.; Green, G. G. R.; Duckett, S. B.; Van Zijl, P. C. M. *J. Magn. Reson.* **2013**, *237*, 73−78.

(53) Glöggler, S.; Emondts, M.; Colell, J.; Müller, R.; Blümich, B.; Appelt, S. *Analyst* **2011**, *136*, 1566−1568.

(54) Ratajczyk, T.; Gutmann, T.; Bernatowicz, P.; Buntkowsky, G.; Frydel, J.; Fedorczyk, B. *Chem. - Eur. J.* **2015**, *21*, 12616−12619.