**HYGROSCOPIC TENDENCIES OF SUBSTANCES USED AS CALIBRANTS FOR QUANTITATIVE NMR SPECTROSCOPY†**

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**KEYWORDS**

Nuclear magnetic resonance (NMR) spectroscopy, quantitative NMR, qNMR, standards, calibrants, hygroscopicity, water content, coulometric Karl Fischer titration.

**ABSTRACT**

Atmospheric moisture can contaminate calibrants for quantitative nuclear magnetic resonance (qNMR) spectroscopy and cause systematic errors in qNMR measurements. Therefore, coulometric Karl-Fischer (CKF) titration was used to evaluate the hygroscopic tendencies of several organic compounds that are commonly used as calibrants for qNMR spectroscopy: benzoic acid, dimethyl sulfone, 1,3,5-trimethoxybenzene, acetanilide, dimethyl terephthalate, and 1,2,4,5-tetramethylbenzene. Samples were placed in a sealed humidity chamber at 100 % relative humidity (RH) and a temperature of 295.4 ± 0.9 K. Over the course of months, portions of each sample were analyzed by CKF titration. All the compounds except dimethyl sulfone were resistant to changes in water content and thus are good choices for qNMR experiments. In contrast, dimethyl sulfone absorbed about 25 mass % of water over 5 weeks at 100 % RH; such behavior could compromise qNMR experiments under certain conditions.

**INTRODUCTION**

Quantitative nuclear magnetic resonance (qNMR) spectroscopy is used to determine the mass fraction of analytes in a sample material.1-3 For an internally calibrated qNMR experiment, a sample and a calibrant are weighed into the same container and then dissolved in a solvent. The mass fraction purity of a specific analyte (*w*x) in the sample material is determined by comparison of integrated peak intensities from the calibrant (*I*cal) and the analyte (*I*x) as shown in equation 1. Here, *w*cal is the mass fraction purity of the calibrant, *N* is the number of nuclei giving rise to each spectral peak, *M* is the relative molecular mass of the analyte or calibrant, and *m* is the mass of sample or calibrant.1-3

$w\_{x}= \frac{I\_{x}}{I\_{cal}} ∙ \frac{N\_{cal}}{N\_{x}} ∙ \frac{M\_{x}}{M\_{cal}} ∙ \frac{m\_{cal}}{m\_{x}} ∙w\_{cal}$ (equation 1)

Substances used as qNMR calibrants1,4-9 need to have a well-known purity that does not change over time.8 In this regard, contamination of qNMR calibrants by atmospheric moisture is of special concern for two reasons.10 First, sample preparation for qNMR experiments is universally done in air (although the humidity of the air is sometimes controlled11). Indeed, the accuracy of the weighing procedure for sample preparation12 would be compromised by static electricity in the low-humidity environment of an inert atmosphere glovebox. Second, the presence of water in deuterated NMR solvents obscures water contamination of the qNMR calibrant. Therefore, it is essential to understand the hygroscopic tendencies of substances that are used as qNMR calibrants. This issue is often discussed,1,3-7,13 but careful experimental verification of non-hygroscopicity is rare.1 It has been suggested that substances should be excluded as calibrants if the weighed value of the calibrant increases (or decreases) by more than 0.02 mg over 10 min of exposure to air.5,6 This is a useful rule if relatively large quantities (≥10 mg) of calibrant are used to prepare the qNMR sample;12 however, it requires an additional experimental step and does not account for contamination of the qNMR calibrant over time with repeated openings of the source container. This study identifies substances that can be used as qNMR calibrants in humid environments without additional control experiments.

**EXPERIMENTAL SECTION**

The benzoic acid used in this study was NIST Standard Reference Material 39j (99.9996 mol %). All other materials were purchased from commercial suppliers and used as received. Two grades of acetanilide from the same supplier were tested. The supplier’s purity specifications for these materials are shown in Table 1. These materials were tested in two groups, with a sample from the same container of 1,3,5-trimethoxybenzene included in each group.

**Table 1.** The supplier’s purity specification and the test group for each sample compound.

|  |  |  |
| --- | --- | --- |
| **Compound name** | **Supplier’s purity specification** | **Test group** |
| benzoic acid \* | 99.9996 % | 1 |
| dimethyl sulfone | 98 % | 1 |
| 1,3,5-trimethoxybenzene † | ≥99 % | 1 |
| 1,2,4,5-tetramethylbenzene | >98 % | 1 |
| acetanilide | 99 % | 2 |
| acetanilide (sublimed) | ≥99.9 % | 2 |
| 1,3,5-trimethoxybenzene † | ≥99 % | 2 |
| dimethyl terephthalate | ≥99 % | 2 |
| \* NIST Standard Reference Material 39j |
| † Same container of material used for duplicate tests |

For the hygroscopicity tests, approximately 5 g of each compound was placed in an individual aluminum pan and spread to a thickness of about 2 mm. These samples were placed in a humidity chamber that was constructed from a plastic desiccator with water in place of desiccant. During the hygroscopicity test, the humidity chamber was sealed in order to maintain 100 % relative humidity (RH). The average temperature in the laboratory where the hygroscopicity tests occurred was 295.4 ± 0.9 K.

Over time, portions of each sample were analyzed for water content by CKF titration14-18 with an ethanol-based KF reagent. A minimum of three replicate titrations was done for each sample at each time point. Samples from the humidity chamber experienced less than 5 minutes of exposure to laboratory air (which had a RH that ranged from 10 % to 50 %) before introduction into the KF titration vessel. Approximately 0.25 g of sample was weighed into a custom aluminum pan, the septum cap on the titration vessel was removed, the solid sample was quickly dumped into the titration vessel, and the septum was replaced. The titrated mass of sample was determined by weighing the aluminum pan before and after the sample introduction procedure. The titration was initiated after the sample had completely dissolved in the KF reagent. For most of the samples, a 1 min delay for dissolution was sufficient. However, for 1,2,4,5-tetramethylbenzene a 3 min delay was needed, and for dimethyl terephthalate a 10 min delay and a higher reagent temperature (313 K instead of room temperature) were necessary.

The titration procedure described above briefly exposes the inside of the titration vessel to atmospheric moisture. To correct for this effect, “blank” titrations were performed in which no sample compound was added (i.e., the aluminum pan was empty). The average mass of water titrated in these blank experiments was then subtracted from the mass of titrated water for each sample titration. This correction was relatively small (≤7 µg of water), but the scatter in replicate blank titrations was a significant source of uncertainty.

At each time point, at least two titrations of a commercial water-concentration standard were done. The water-concentration standard was packaged in single-use glass ampoules and consisted of *o*-xylene with a water concentration of 102 ± 4 µg/g (at k = 2). CKF titration is a primary method that does not require calibration; however, titrations of the water-concentration standard verified proper instrument performance and aided in the assessment of measurement uncertainty. Since the water-concentration standard was a liquid, these titrations followed a different protocol (syringe injection through the septum cap on the titration vessel).18

The combined standard uncertainty in the water concentration was calculated by quadrature addition of the following quantities: (1) the standard deviation in replicate sample titrations, (2) the uncertainty in the blank subtraction as calculated from the standard deviation in replicate blank titrations, and (3) the average offset of the titrated value from the known concentration of the commercial water standard. The uncertainty in sample mass was insignificant.

**RESULTS AND DISCUSSION**

In this work, several compounds that are used as qNMR calibrants were exposed to a RH of 100 % for up 476 days. The compounds tested were benzoic acid, dimethyl sulfone, 1,3,5-trimethoxybenzene, two samples of acetanilide (differing in initial purity), dimethyl terephthalate, and 1,2,4,5-tetramethylbenzene. These compounds were chosen because they are sold commercially as qNMR calibrants (or are commercially available at purities ≥99.9 % and have a history of use as qNMR calibrants). Changes in the water content of each sample were monitored by CKF titration, which is especially useful for the determination of low water concentrations.13-18

The compounds that performed best in these tests were benzoic acid and the higher purity (sublimed) sample of acetanilide, Table 2 (graphs of the data in Table 2 are available in the Supporting Information). Both samples started with very low water content, and no significant change in water content was observed during the tests. High-purity benzoic acid has been reported previously to be non-hygroscopic at 86 % RH.1 No significant change was observed in the water content of dimethyl terephthalate or 1,2,4,5-tetramethylbenzene, but the results for these two compounds come with caveats. The uncertainty in water concentration of dimethyl terephthalate was relatively high due to larger scatter in replicate sample titrations (likely due the large drift correction that was necessitated by the 10 min titration delay); hence, a change in water content of up to 95 mg/kg could have been obscured by the measurement uncertainty. For 1,2,4,5-tetramethylbenzene, noticeable sublimation of the test sample was observed between time points; this behavior might have influenced the results of the hygroscopicity test and, in any case, such behavior is undesirable in a calibrant.

**Table 2.** Water content (with combined standard uncertainty) as a function of storage time at 100 % relative humidity (RH). Nominally negative water concentrations can be observed due to the blank subtraction procedure, but negative results were always less than the measurement uncertainty.

|  |  |  |
| --- | --- | --- |
|   | **Days at** |  |
| **Compound name** | **100% RH** | **water (mg/kg)** |
| benzoic acid \* | 0 | 7 ± 22 |
|  | 2 | 12 ± 24 |
|  | 7 | 33 ± 17 |
|  | 35 | 29 ± 9 |
|  | 77 | 16 ± 6 |
| dimethyl sulfone | 0 | 824 ± 28 |
|  | 2 | 14692 ± 3073 |
|  | 7 | 74531 ± 1351 |
|  | 35 | 249620 ± 20347 |
|  | 77 | NA ‡ |
| 1,3,5-trimethoxybenzene † | 0 | 518 ± 27 |
|  | 2 | 498 ± 23 |
|  | 7 | 572 ± 16 |
|  | 35 | 660 ± 24 |
|  | 77 | 698 ± 13 |
| 1,2,4,5-tetramethylbenzene | 0 | 106 ± 42 |
|  | 2 | 149 ± 35 |
|  | 7 | 116 ± 17 |
|  | 35 | 135 ± 22 |
|   | 77 | 111 ± 35 |
| acetanilide | 0 | 1 ± 9 |
| (99 % purity) | 8 | 213 ± 9 |
|  | 31 | 383 ± 8 |
|  | 64 | 494 ± 12 |
|  | 476 | 772 ± 9 |
| acetanilide | 0 | −1 ± 9 |
| (sublimed, 99.9 % purity) | 8 | 0 ± 4 |
|  | 31 | 0 ± 6 |
|  | 64 | 2 ± 2 |
|  | 476 | 4 ± 8 |
| 1,3,5-trimethoxybenzene † | 0 | 358 ± 13 |
|  | 8 | 415 ± 6 |
|  | 31 | 481 ± 8 |
|  | 64 | 557 ± 4 |
|  | 476 | 1028 ± 12 |
| dimethyl terephthalate | 0 | −35 ± 76 |
|  | 8 | −2 ± 48 |
|  | 31 | 81 ± 55 |
|  | 64 | 28 ± 95 |
|  | 476 | 81 ± 84 |
| \* NIST Standard Reference Material 39j |
| † Same container of material used for duplicate tests |
| ‡ Liquid and solid phases present; no measurement made |

Both 1,3,5-trimethoxybenzene and the lower-purity sample of acetanilide took up a small quantity of water during the tests. The water content of those samples increased by about 670 mg/kg or 770 mg/kg, respectively, over the course of 476 days (Table 2, test group 2). Because purity and crystallinity are known to affect hygroscopicity,19 it is not surprising that the lower purity sample of acetanilide performed less well. Considering this, we cannot rule out that a purer sample of 1,3,5-trimethoxybenzene would have performed better. Nevertheless, even after more than a year at 100 % RH, the mass % purity of these two samples changed by <0.1 %.

Both test groups included a sample of 1,3,5-trimethoxybenzene from the same container. In both trials the water content of the sample increased by about 200 mg/kg over the first ~2 months. However, it is notable that the initial water concentration of the two samples was found to be different by 160 mg/kg. The two trials were started several months apart, during which time the source container had been opened repeatedly. While the magnitude of the change would be negligible for most work, it illustrates the problem of shifting purity with storage.

Dimethyl sulfone exhibited, by far, the highest hygroscopicity of the compounds tested. Over the course of 35 days at 100 % RH, the water content of the dimethyl sulfone sample increased by almost 2.5 × 105 mg/kg, at which time the sample had begun to form a liquid phase and appeared as a slush. This is an important finding because dimethyl sulfone is considered to be one of the best substances to use as a qNMR calibrant.4,13 However, the situation is not as bad as it may first appear. One can calculate that the mass % of water in dimethyl sulfone increased by <0.01 % for every 10 minutes at 100 % RH. Therefore, the hygroscopicity of dimethyl sulfone is only a concern after repeated openings of the source container in high humidity environments. Additionally, at lower RH, dimethyl sulfone is not hygroscopic. When a separate test sample of dimethyl sulfone was exposed to laboratory air (with a RH that varied from 10 % to 50 %) for 77 days, the water content *decreased* slightly from an initial value of 824 ± 28 mg/kg to a final value of 456 ± 10 mg/kg. This result may seem counterintuitive, but it is normal for a substance to be non-hygroscopic below a characteristic RH.19 In fact, the cutoff values of RH for hygroscopic behavior are sometimes used in classification schemes for hygroscopicity.19 In short, dimethyl sulfone can be used without concern at ≤50 % RH.

**CONCLUSION**

High-purity samples of acetanilide and benzoic acid performed the best in the hygroscopicity tests and are therefore recommended as qNMR calibrants in high humidity environments. Both compounds completely resisted water uptake over long periods of storage at 100% RH. On the other hand, dimethyl sulfone should be avoided as a qNMR calibrant in high humidity environments unless its water content is verified with additional measurements.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website.

Graphs of the data in Table 2 (PDF).

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**Notes**

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**ACKNOWLEDGMENTS**

We thank Dr. Tara J. Fortin at NIST for providing laboratory temperature and relative humidity data for this work.

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TOC Graphic

