

# An *in Situ* Drying Method for Preparing NMR Samples with Extremely Low Water Content

Martin Goetz

Fachbereich Chemie, Martin-Luther-Universität Halle-Wittenberg, Kurt-Mothes-Straße 2, D-06120 Halle/Saale, Germany

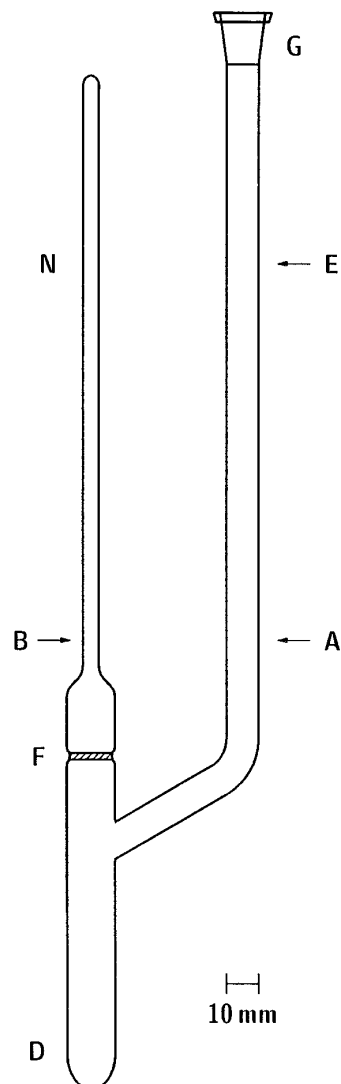
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**A very simple closed apparatus and procedure are described which allow *in situ* drying of NMR samples by using molecular sieves and the freeze-pump-thaw technique. Limitations of the method are discussed. With acetonitrile, a residual water content of  $3 \times 10^{-4}$  M could be reached.** © 1998 Academic Press

For certain NMR investigations, it is mandatory to use samples with an extremely low water content. Examples from our own laboratory include time-resolved CIDNP experiments involving photogenerated radical ion pairs (1) and dynamic NMR studies of proton transfer (2) in aprotic solvents. In the former case, residual water protonates the radical anions, which opens up new decay pathways and may thus reduce their stability so much as to render the measurements impossible; in the latter case, water provides an additional transfer pathway (3), which can lead to grossly wrong kinetic results.

Many organic solvents, such as dimethyl sulfoxide, are highly hygroscopic. It has been our experience that surprising effort is needed to prepare NMR samples with a consistently low water content in these cases, and that failures to achieve this are not infrequent even if solvents and solutes are carefully predried and sample preparation is carried out with syringe techniques under inert gas, or even in a glove box. Condensing the predried solvent into the NMR tube that is part of a closed vacuum system (4) yields better results but is rather complicated. In this article, a much simpler apparatus and technique are described, by which an extremely low water concentration can be reached. Yet the procedure does not demand much experimental skill, nor is it time consuming.

The method has the following three characteristics: First, drying is accomplished *in situ*, i.e., *after* preparation of the solutions, so during their preparation no special caution must be observed. Second (and a prerequisite for the former), a nonchemical drying agent, molecular sieve, is used, which does not react with most solutes and is easily removed. Third, drying takes place in a sealed apparatus, which includes the NMR tube, so further uptake of moisture is impossible.



The drawing shows the apparatus, which is made from glass. A standard NMR tube (N) is attached to the main body of the vessel by the usual glass-blowing techniques. The tube is joined at point B. To remove the water film on the inner

surfaces the assembly is dried overnight at 150 to 170°C before use. Immediately after taking it out of the oven, the bottom of part D is loaded with a few (3 to 5) beads of freshly activated molecular sieve (of a pore size suitable for the solvent to be dried, 3 Å in most cases (5)), and the vessel is closed with a stopper. When very high demands are being made, it is advantageous to connect it to a vacuum line with the greaseless 14/20 glass joint G, evacuate it to high vacuum and heat it gently with a flame, especially the region D, where the molecular sieve is contained, and that of the glass frit F. As soon as the vessel has cooled to room temperature, the solution is put into part D with a long-needled syringe. In a slightly more complicated version of the apparatus, which has an extension with a stopcock added at point E, the filling in of the molecular sieve and of the solution can also be performed under inert dry gas. However, it has been our experience that this is unnecessary if these operations are carried out reasonably quickly, so we normally use the simpler variety shown in the drawing.

With the vessel connected to a vacuum manifold, several (3 to 5) freeze–pump–thaw cycles are performed. The movement of the solution during the thawing greatly increases the drying speed; besides, it is known that with molecular sieves the obtainable degree of drying is better at lower temperatures (6). Also, at least partial evacuation is required anyhow for the later sealing of the vessel. Two additional benefits of the freeze–pump–thaw step not pertinent to the water content are, first, that the stability of samples that are susceptible to oxidation is enhanced considerably, which is especially important in photo-CIDNP measurements, and second, that the linewidths are reduced because  $T_1$  is lengthened, which is advantageous for dynamic NMR experiments. With the solution frozen, the vessel is sealed with a flame at point A. The solution is left to stand over the molecular sieve for a few hours more, overnight, or longer to complete the process to the desired degree of drying (see below). The apparatus is then turned upside-down, and the solution is filtered through a G4 glass frit F into the NMR tube. Filtration instead of decanting is necessary because molecular sieves contain fine dust particles that are detrimental to resolution (7). To increase the filtration rate, part D can be warmed gently. Cooling down the NMR tube for this purpose is less advantageous because the solvent tends to evaporate in the glass frit, so the latter might become clogged by the solute. Finally, the NMR tube is sealed with a flame at point B.

As the drying does not need attendance, except possibly a small stirring of the vessel from time to time, the only time-consuming part of the procedure is the freeze–pump–thaw step. This, however, also takes only a few minutes per sample because the vacuum manifold allows preparation of several samples in parallel.

The reported *in situ* drying method is of course only feasible because molecular sieves are physical drying agents. Nevertheless, their advantages for the described purpose are not paid for by a loss of efficiency. On the contrary, for most organic

solvents, molecular sieves are either the best drying agents available or only marginally lower in performance than the best (chemical) drying agents (5, 6, 8). The only slight weakness of the method is that an exact predetermination of reactant concentrations in the samples is impossible: On the one hand, the reactants are to some extent adsorbed on the surface of the molecular sieve (adsorption in the pores, which would lead to a removal of the substrates from the solution, is impossible for all but the smallest organic molecules). On the other hand, volatile reactants and solvent are lost to some degree during the freeze–pump–thaw procedure. However, we found that the deviations between expected and actual solute concentrations rarely exceed 30%.

The efficiency of drying and its dependence on the time the solution is allowed to stand over the molecular sieve in the sealed apparatus were monitored by NMR. Test samples for this purpose contained solvent only. The ratios of the integrals of solvent and water in the  $^1\text{H}$  NMR spectra were used for evaluation. This assaying procedure can of course only be applied to nonexchanging, that is, in particular, nonhydroxylic solvents; otherwise, Karl Fischer methods must be used. To obtain absolute values for the water content, the degree of deuteration of the solvent was first determined independently, without the described drying procedure, from samples containing precisely known amounts of calibration substances. Water was taken to be present as HDO only. This assumption limits the precision of the absolute values: If a major part of the water signal were due to  $\text{H}_2\text{O}$  stemming from the uptake of atmospheric moisture, the evaluation would yield a water concentration that is too high, up to a factor of 2 at most.  $\text{D}_2\text{O}$  present would pass unnoticed, suggesting an overoptimistically low water content. However, *relative* drying efficiencies would not be affected in these two cases.

The following results were obtained with acetonitrile; similar findings are expected for other solvents. The water concentration before drying is typically about  $5 \times 10^{-2}$  M. Because only a few beads of the molecular sieve are used and these are not uniform, exact reproducibility is not to be expected and the numbers given are typical values. We found that immediately after the freeze–pump–thaw step, the water content is decreased by a factor of about 20. Allowing the solution to stand overnight over the molecular sieve leads to a further reduction to about one-half. After a week, the water content has dropped down by another factor of about 2. Longer drying times have no noticeable effect. According to our measurements, the limiting water content that can be reached is about  $3 \times 10^{-4}$  M. This is in good accordance with values published for the drying efficiency of molecular sieve in the undeuterated solvent (6), so the preceding assumptions regarding the determination of the concentrations are probably not far wrong.

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