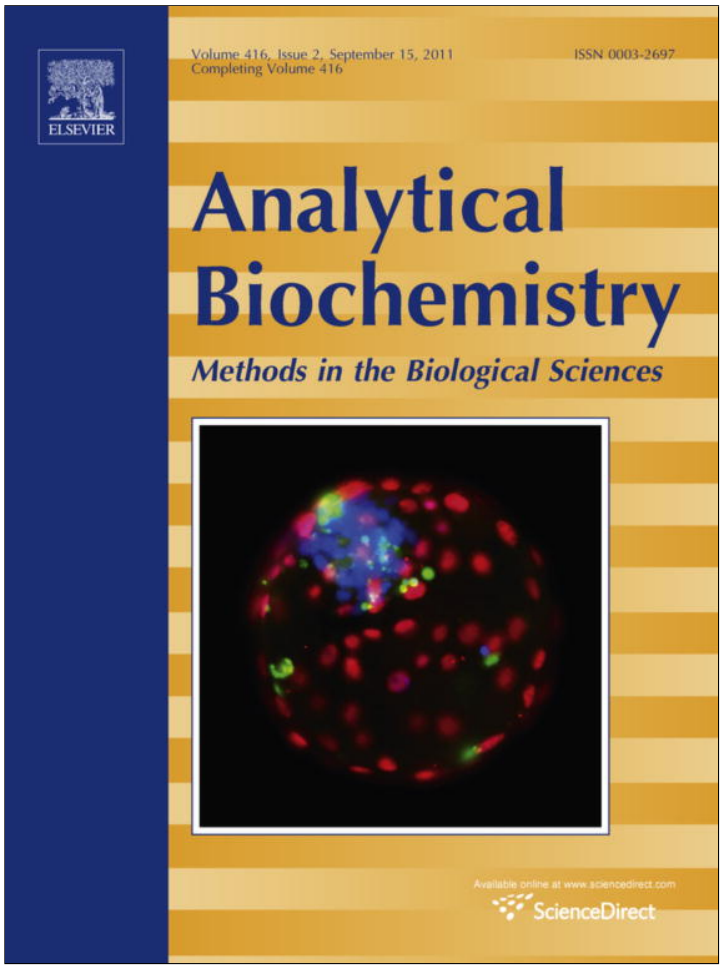

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Notes & Tips

An inexpensive high-throughput nuclear magnetic resonance tube cleaning apparatus

Bo Zhang [a], James Hodgson [b], Walter Hancock [a], Robert Powers [a,*]

[a] Department of Chemistry, University of Nebraska–Lincoln, Lincoln, NE 68588, USA
[b] Department of Chemistry, Kansas State University, Manhattan, KS 66506, USA

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ABSTRACT

Large-scale nuclear magnetic resonance (NMR) tube cleaning is currently a bottleneck in high-throughput NMR ligand affinity screens. Expensive alternatives include discarding the NMR tubes after a single use (~US $2–$8/tube), using commercial NMR tube cleaners (~$15,000), and abandoning NMR tubes for flow probe technology (~$75,000). Instead, we describe a relatively inexpensive (~$400) and easily constructed apparatus that can clean 180 NMR tubes per hour while using a modest amount of solvent. The application of this apparatus significantly shortens the time to recycle NMR tubes while avoiding cross-contamination and tube damage.

High-throughput screening (HTS)[1] is widely used in the biotechnology and pharmaceutical industries [1–3] with an expanding interest in academia [4,5]. HTS is an efficient approach for the experimental testing of large sample sets for the discovery of new drugs. Multiple reviews on HTS automation have discussed robotic systems employed to transport samples, add reagents, mix samples, and/or detect signals [6–8]. These steps are clearly essential components to a successful screen and are understandably a focal point of any assay design. Conversely, there is a complete lack of reports discussing the automation of recycling devices. For the majority of high-throughput screens, a reliance on disposable labware (e.g., assay plates, pipette tips) simplifies the execution of the assay while being reasonably cost-effective.

Fragment-based nuclear magnetic resonance (NMR) screens complement traditional HTS and have evolved to become an important and common component of drug discovery [9,10]. Fragment-based screens use a chemical library of low-molecular-weight ligands (⩽200–300 Da) [11] that correspond to fragments of known drugs or have drug-like characteristics [12]. NMR is used to identify binders, identify the ligand binding site, and assist in "growing" the fragments to improve binding affinity. There are several advantages to fragment-based NMR screens over HTS that include maximizing ligand efficiency, improving coverage of chemical space, higher hit rates and higher quality of leads, direct observation of biologically relevant interactions, and a universal assay design. Conversely, NMR ligand affinity screens are typically limited to assaying only hundreds to thousands of compounds. Another practical disadvantage is the hundreds of NMR tubes that quickly accumulate and require cleaning.

High-quality NMR tubes are generally used for ligand affinity screens and because of cost (~US $2–$8/tube) are typically not limited to a single use. A standard NMR tube cleaner (Sigma–Aldrich, St. Louis, MO, USA) can handle from one to five tubes. The NMR tube sits inverted on a glass adapter for washing, which is very fragile because it has a small diameter (<5 mm) and long length (8 in.). Thus, the routine cleaning of thousands of NMR tubes rapidly results in broken NMR tube cleaners at a cost of approximately $90–$420 per cleaner. Besides manually cleaning, there are commercial devices available such as the Bruker BioSpin Autoclean System (Billerica, MA, USA), which is capable of cleaning 60 tubes per hour but generates a significant amount of waste and has a cost of approximately $15,000. Alternatively, NMR tubes can be completely discarded from the NMR screen by using a flow probe [13]. The NMR probe contains a fixed cell with active volumes ranging from 30 to 120 μl. The NMR samples are sequentially transferred to the probe using a robotic liquid handling system. After data acquisition, the sample is either transferred to waste or collected. Challenges with flow probes may include cross-contamination between samples, clogging of the system by solid particulates that may also result in lost samples, and problems with properly positioning the sample in the probe. There is also the initial cost of approximately $75,000 for a flow probe system.

The need for a cost-effective and efficient means of cleaning thousands of NMR tubes is a routine challenge currently faced by NMR screening groups. To solve this problem, an inexpensive (~US $400) and highly effective apparatus for cleaning NMR tubes has been designed and successfully employed in our laboratory. It is extremely efficient compared with existing tube cleaners, with

* Corresponding author. Address: Department of Chemistry, 722 Hamilton Hall, University of Nebraska–Lincoln, Lincoln, NE 68588, USA. Fax: +1 402 472 9402.
E-mail address: rpowers3@unl.edu (R. Powers).

[1] *Abbreviations used:* HTS, high-throughput screening; NMR, nuclear magnetic resonance.

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180 tubes being cleaned in an hour. Obviously, the use of large amounts of solvent increases the cost of cleaning and diminishes the value of recycling NMR tubes. There are also safety and health concerns. As a result, the design of the NMR tube cleaner apparatus and the development of the protocol focused on minimizing solvent use. Only 200–300 ml of solvent is used to clean the 180 NMR tubes. The amount of solvent waste will increase proportionally if multiple solvents are used. Similarly, an apparatus that routinely cracks, chips, or scratches the NMR tubes defeats the cost-effectiveness of recycling. Of greater concern is the possibility of unnoticed tube damage that may result in a broken tube in an NMR probe. Unlike the manual cleaning of a large batch of NMR tubes where damage is common, we have not observed any damage to the hundreds of NMR tubes cleaned using our apparatus. This is primarily a result of minimal handling of the NMR tubes during cleaning and the fact that the tubes are simply placed inverted into a glass vessel.

Diagrams and pictures of our NMR tube cleaning apparatus are shown in Fig. 1. The apparatus can be easily constructed in any laboratory with access to a glass blower. The cleaner consists of three major components: (i) a specially designed glass valve assembly (switch); (ii) a 750-ml Labconco vacuum bottle, cap, and adaptor; and (iii) a standard pump system (not shown). The NMR tubes are simply placed inverted into the 750-ml Labconco vacuum bottle. The cap is placed onto the vacuum bottle, which is then partially filled with solvent. Our glass switch is attached to the cap, and the apparatus is connected to a vacuum pump. A flexibly attached glass tube extends from the glass switch to the base of the vacuum bottle. A pressure differential is used to push solvent into and out of the NMR tubes or to completely remove the solvent from the apparatus. This occurs by manipulating the two Teflon valves on our glass switch while the apparatus is attached to a vacuum pump. Basically, with the left valve closed, slowly and briefly opening the T-valve to the vacuum position evacuates the air from the Labconco vacuum bottle and places it under a slight vacuum without removing the solvent. Turning the T-valve to the vent position opens the vacuum bottle to atmosphere, breaking the vacuum and forcing solvent into the NMR tubes. Switching the T-valve back to the vacuum position places the NMR tubes back under vacuum and removes the solvent from the NMR tubes. Repeating the process two or three times effectively washes the NMR tubes with solvent. Switching the T-valve to the vent position and opening the left valve rapidly removes the solvent from the NMR tube cleaner into the filter flask. The process can be repeated with other solvents.

The general protocol for cleaning NMR tubes (Fig. 2) corresponds to the following steps: (i) empty the NMR tubes that need

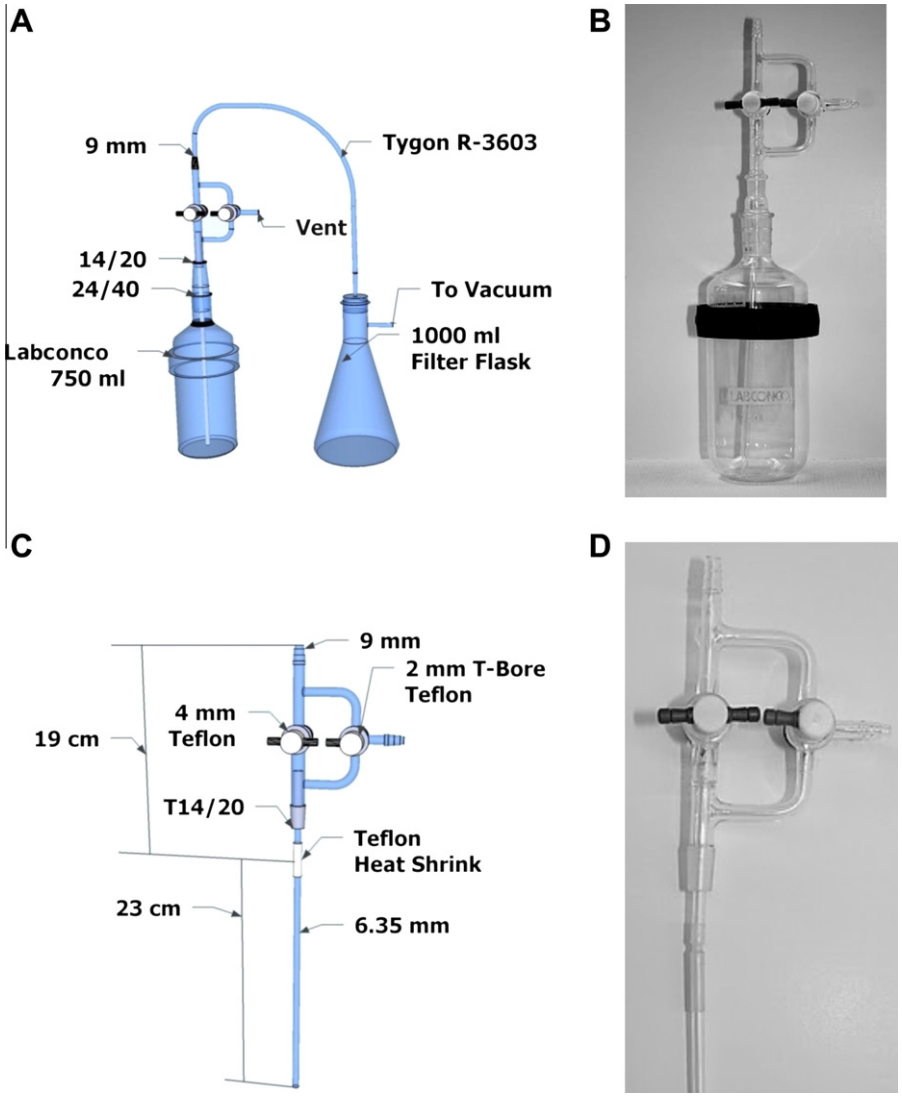

Fig.1. (A and B) Schematic drawing (A) and picture (B) of our NMR tube cleaning apparatus. (C and D) Schematic drawing (C) and picture (D) of an expanded view of the glass switch. Important features and dimensions are labeled.

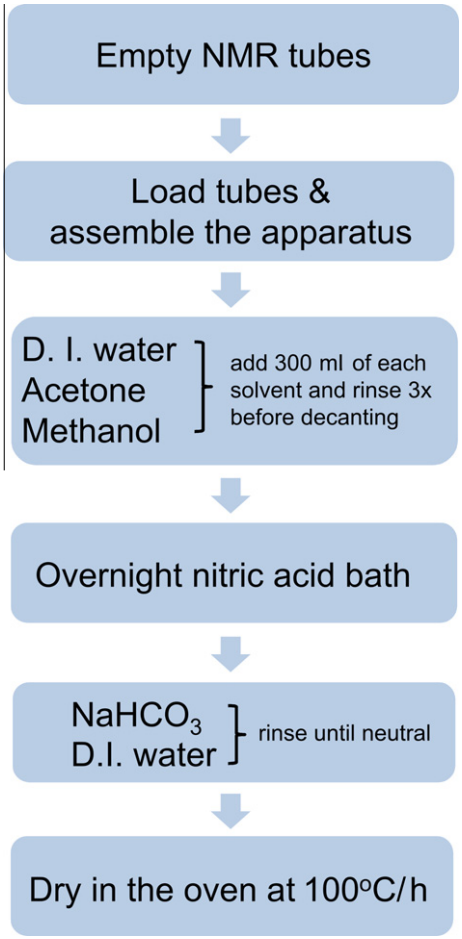

Fig.2. Flow diagram of the NMR tube cleaning process. D.I. water, deionized water.

to be cleaned and remove any labels; (ii) load tubes into the NMR tube cleaner along with solvent; (iii) install the valve assembly and hook up the filter flask and vacuum system; (iv) rinse multiple times with solvents of choice; and (v) remove tubes and dry in an oven at 100 °C for 1 h. For heavily soiled NMR tubes that contain solid films or stains, a simple solvent wash might not be sufficient. Instead, the NMR tubes are typically soaked overnight in a concentrated (<70%) nitric acid bath. The tubes are then quickly rinsed with tap water before placing in the NMR tube washer. The wash solvent is a saturated sodium bicarbonate solution in deionized water. Various solvents can be used in the NMR tube cleaner depending on need and the chemical composition of the original NMR samples.

For a typical fragment-based NMR screen, deionized water is the first wash solvent because it will effectively remove a majority of the aqueous-based samples. A second wash using a polar organic solvent such as methanol, tetrahydrofuran, or dichloromethane may also be used to remove any compound residue and promote tube drying. If necessary, a third and final wash with a nonpolar solvent such as chloroform could be employed. To ensure the effectiveness of our NMR tube cleaner, there are a few general rules that need to be followed. It is important to avoid having the wash solvent sit in the NMR tubes for an extended period of time. The rapid insertion and removal of the wash solvent provides efficient agitation that assists in the cleaning process. Conversely, prolonged exposure to a wash solvent may result in compound precipitation and the formation of a difficult-to-remove film. For a similar reason, it is highly undesirable to allow an NMR sample to evaporate to dryness. Effectively, NMR tubes should be cleaned immediately after use to obtain optimal performance from our NMR tube cleaner.

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References

[1] G. Wu, S.K. Doberstein, HTS technologies in biopharmaceutical discovery, Drug Discov. Today 11 (2006) 718–724.
[2] D.L. Kale, S.C. Chaturvedi, D. Patil, R.B. Kakde, High throughput screening (HTS) technique in drug discovery, Pharma Rev. 6 (2008) 46–51.
[3] L.M. Mayr, P. Fuerst, The future of high-throughput screening, J. Biomol. Screen. 13 (2008) 443–448.
[4] J.A. Frearson, I.T. Collie, HTS and hit finding in academia—from chemical genomics to drug discovery, Drug Discov. Today 14 (2009) 1150–1158.
[5] R.L. Stein, High-throughput screening in academia: the Harvard experience, J. Biomol. Screen. 8 (2003) 615–619.
[6] L. Zhang, M.N. Banks, J.G. Houston, Critical components in high-throughput screening: challenges and new trends, Drugs Pharm. Sci. 196 (2009) 6–20.
[7] S. Hamilton, Introduction to screening automation, Methods Mol. Biol. 190 (2002) 169–193.
[8] R.F. Trinka, F.E. Leichtfried, Robotics and automation, Drugs Pharm. Sci. 114 (2001) 493–523.
[9] P.J. Hajduk, J. Greer, A decade of fragment-based drug design: strategic advances and lessons learned, Nat. Rev. Drug Discov. 6 (2007) 211–219.
[10] R. Powers, Advances in nuclear magnetic resonance for drug discovery, Expert Opin. Drug Discov. 4 (2009) 1077–1098.
[11] P.J. Hajduk, W.R.J.D. Galloway, D.R. Spring, Drug discovery: a question of library design, Nature 470 (2011) 42–43.
[12] C.A. Lipinski, Lead- and drug-like compounds: the rule-of-five revolution, Drug Discov. Today Technol. 1 (2004) 337–341.
[13] R.L. Haner, W. Llanos, L. Mueller, Small volume flow probe for automated direct-injection NMR analysis: design and performance, J. Magn. Reson. 143 (2000) 69–78.