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Journal of Magnetic Resonance 159 (2002) 158–160
JMR Journal of Magnetic Resonance
www.academicpress.com

Device for aeration and mixing of cell and organelle suspensions during NMR experiments

Roger M. Bourne*,[1] and Philip W. Kuchel

School of Molecular and Microbial Biosciences, University of Sydney, Sydney, Australia

Received 20 May 2002; revised 1 September 2002

Abstract

A device for aeration and mixing of cell or organelle suspensions in a vertical bore NMR magnet is described. Multiple external sensors (e.g., ion-selective electrodes) may be immersed in the suspension within the bore of the magnet. The sensors are positioned to avoid noise due to contact with gas bubbles and proximity to the probe head. The required sample volume is minimised. The modular design of components permits the use of the device in magnets of various internal dimensions, or with probe heads of different sample tube diameter, by modification of the simpler components of the assembly.

Keywords: Cells; Organelles; Suspensions; Ion-selective electrodes

1. Introduction

We describe here a number of design modifications which significantly improve the versatility and functionality of previously described apparatus for aeration and mixing of cell or organelle suspensions in a vertical bore NMR magnet in the presence of external sensors (e.g., ion-selective electrodes) within the bore of the magnet. The principle of operation is based on that of devices described previously [1–5]. The design improvements include: inclusion of up to four sensors or probes; positioning of sensors to avoid noise due to contact with gas bubbles and proximity to the probe head; minimisation of required sample volume; and modular design of components to permit the use of the device in magnets of various internal dimensions, or with probe heads of different sample tube diameter.

2. Description of individual parts

The apparatus is illustrated in Fig. 1. The design shown is for a 10-mm diameter sample tube and 50-mm vertical bore magnet with Bruker probe head.

2.1. Sample tube

The sample tube is a standard 10-mm thin-wall round bottom NMR tube cut to appropriate length for the particular probe head.

2.2. Draft tube

The draft tube separates the descending and ascending liquid streams in the sensitive region of the probe head. It is a short section of 5-mm thin-wall NMR sample tube. A capillary containing a chemical shift or concentration reference may be fixed inside with a small amount of silicone adhesive.

2.3. Bottom tube

The bottom tube is made from Delrin that is more dimensionally stable in aqueous solutions than Teflon. It holds the draft tube by a press-tight fit and is itself pressed into the base of the upper barrel. Three channels

* Corresponding author. Fax: +61-2-9351-4105.
E-mail address: rbourne@imrr.usyd.edu.au (R.M. Bourne).
[1] Present address: Institute for Magnetic Resonance Research, Blackburn Bldg (D06), University of Sydney, Sydney 2006, Australia.

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PII: S1090-7807(02)00103-9

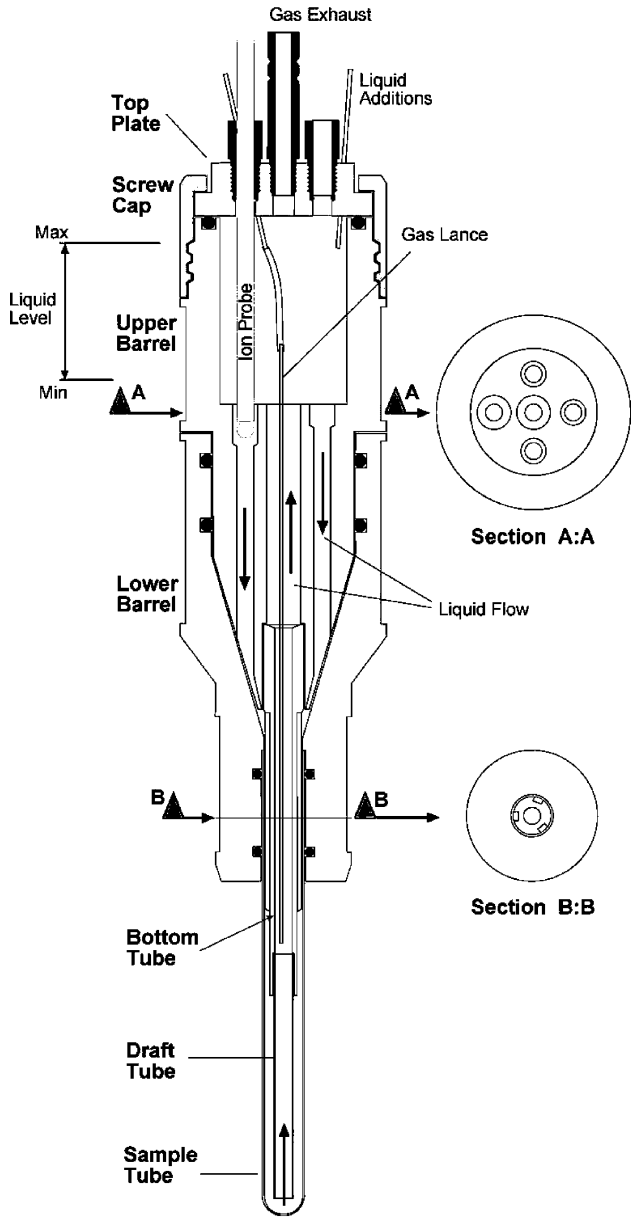

Fig. 1. Diagram of mixer apparatus. The device illustrated has a 10-mm sample tube and fits a 50-mm bore magnet with Bruker probe head. Arrows indicate the direction of liquid recirculation. Only one external ion probe is shown.

are milled into the sides of the bottom tube to conduct the flow of descending liquid. The bottom tube is a close fit within the sample tube to minimise the volume of sample suspension outside the sensitive region of the probe head. Gas is bubbled into the lower part of the bottom tube at a point about 1 cm above the top of the probe head receiver coils. This enhances mass transfer between the gas and liquid phases by maximising contact time between the gas and suspension, and improves recirculation by maximising the hydrostatic density difference between the ascending (inner) and descending (outer) sample volumes.

2.4. Lower barrel

The lower barrel is machined from clear acrylic and is identical in external dimensions to the usual NMR sample tube holder/spinner. A pair of O-rings retain the sample tube in the base of the barrel. A pair of O-rings retains the lower barrel in the upper barrel. The use of clear acrylic (polished after machining) permits visual inspection of the sample prior to insertion in the magnet.

2.5. Upper barrel

The upper barrel is machined from clear acrylic and forms the main reservoir for the sample. Wells in the body of the barrel allow the immersion of the probes in the descending flow of suspension where they are free from noise due to contact with gas bubbles. The dimensions of the upper barrel are primarily dependent on the type of experiments to be performed rather than magnet and probe head geometry. A single upper barrel could be used with different lower barrels for experiments in different magnets and/or different probe heads. Adequate head-space should be provided below the top plate for the collapse of any foam which may form or sample will be lost through the exhaust tube. Although not shown, a port on the side of the upper barrel is useful for flushing out the upper part of the apparatus between experiments, without the need to remove the top plate.

2.6. Top plate

The top plate is machined from Delrin and contains the mounting points for external probes, entry points for gas and liquid additions, and gas exhaust. The gas exhaust and the silicone-rubber hose attached to it form the main mechanical support for insertion and removal of the apparatus from the magnet. Ion-probes inserted through the top plate are clamped by brass hollow screws and O-rings (O-rings alone would be adequate for most applications). The gas supply and liquid additions penetrate the top plate through stainless steel capillaries (heavy gauge stainless steel needles). If flexible ion-selective electrodes are used then they will be more easily inserted into their respective wells if their tips are each at slightly different levels.

2.7. Screw cap

The screw cap clamps the top plate onto the upper barrel to make an air- and liquid-tight seal that prevents accidental overflow into the magnet bore. The cap was machined from a Schott bottle screw cap which required a slight reduction in diameter.

Acknowledgments

We are grateful to Andrew Townsend and Ross Taylor for their expert technical advice and assistance in design and fabrication of this apparatus. We thank Drs. Bob Chapman and Bill Bubb for expert NMR input and Bill Lowe for expert technical assistance.

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