



High Resolution Magic Angle Spinning

Sample Preparation



HRMAS-Rotor with Insert



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HRMAS-Rotor without Insert: difficult to shim

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B) <u>Sample Preparation: Practical Experience</u>

- 2) Non liquid, viscous samples and Semisolids":
- a) The use of inserts

-The inserts have to be fitted with the supplied gauge. This procedure creates a well defined and reproducible volume. The thin sidewalls of the spacer should not be damaged.

-The black sample packing tool, delivered with the HRMASprobe, has a long and a short end. The short end is 3 mm wide and is used to press in the spacer. The volumes mentioned in this presentation are related to this preparation.





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Sample Preparation

a) How to open the Rotor:

The safest way to remove a cap from the rotor:

- use a cooling spray to cool down the KEL-F cap (5 sec)

- the cap shrinks and can be removed by hand, using a tissue

If the spacer is used:

- remove the sealing-srew
- use the extraction screw to remove the spacer
- cool down the rotor around the spacer
- the spacer can easily be removed
- sometimes the sample is removed along with the spacer!



HRMAS-Rotor with Insert:

Insert and remove the spacer with the extraction screw Do not use force when inserting (~ 1 mm)

The correct position is obtained using the 3 mm gauge



Sample Preparation

a) Cleaning of HR-MAS Rotors and related parts

- always use protecting gloves

Same procedure for cleaning for all parts:

- semi solid samples should be cleaned mechanically with a spatula
- washing with H2O or acetone
- washing of spacer, insert, sealing screw and extraction screw
- cleaning in the ultrasonic bath with solvent (acetone) ~3min
- dry all parts with paper
- dry all parts 20 min in the oven at 65 °C



Sample Preparation

b) Standard Test Samples in HRMAS Rotor:

1) Humptest (3%) sample: why check the weight?

- Rotors with air bubbles can not be shimmed to specifications.

- Solvent can evaporate after some time. Prepare fresh sample or check balance (total mass should be noted after preparation).

Use only caps without any damage.
 Check this with a magnifying glass!

- Without insert, fill inner hole of cap too (64 ul version)
- 50 ul Rotor: 41 43 mg of humptest solution (d=0.79 g/cm³)



Sample Preparation

b) Standard Test Samples in HRMAS Rotor:

- 2) Proton Sensitivity Test:
- 2 mMol sucrose in D2O, stabilized with NaN₃
- For easy reproducibility use HRMAS Rotor without insert.
- The 50 ul rotor is totally filled with 52 55 mg sample
- Check content with balance,
- weigh the total mass for further check

Aquisition parameters at 4.5 kHz spinrate: ns= 8; aq= 2.7 sec; d1= 3 sec; ds= 2; zgpr; lb= 1.0

USA: test with ns= 1, sino real from anomeric proton

If the D2O is good enough, the experiment can be done without water suppression (pulprog = zg).



Sample Preparation

b) Standard Test Samples in HRMAS Rotor:

3) Carbon Sensitivity Test ASTM:

- Use HRMAS rotor without insert
- Fill 50 ul rotor totally with ~50 mg (d= 0.939 g/cm³)
- Check content with balance, weigh the total mass for further use.
- Before using the same rotor once than more, weigh the total mass.



Sample Preparation: Practical Experience

c) Non liquid, viscose samples and "Semisolids":

1) Biological cell membranes and protein samples:

- add 5 10% D2O before centrifugation of cells
- transfer pellet into rotor
- sometimes a centrifuge helps to bring the pellet to the bottom of the rotor
- make up to a total volume of about 15 ul with the surplus solution from the centrifugation.
- adjust insert with 3 mm gauge and close with the screw
- -<u>after</u> fixing the screw, remove surplus solution with clean tissue.
- weigh the absolute sample amount using a balance



Sample Preparation: Practical Experience

c) Non liquid, viscous samples and "Semisolids":

- 2) Polymers, resins for SPS and other swelling samples:
 - check swelling factor in the specific solvent
 - calculate appropriate sample amount for available rotor volumes
 - add solvent in excess

Typical values for resins used in combinatorial chemistry: up to 2.5 mg sample (dry!) swells in rotor with insert to ~12 ul
8 - 12 mg sample (dry) swells without insert to ~50 ul (~65 ul)



Sample Preparation: Practical Experience

c) Non liquid, viscous samples and "Semisolids":

- 2) Polymers, resins for SPS and other swelling samples:
 - transfer <u>dry</u> resin sample to rotor and weigh out
 - add deuterated solvent using a capillary or a syringe with a thin needle and watch swelling procedure (lense)
 - an extra needle can also be used to stir the sample
 - this procedure allows bubble free swelling inside the rotor
 - weigh added solvent with balance
 - up to 2.5 mg use 12 ul rotor preparation method
 - up to 12 mg use 50 (or 65) ul rotor preparation method



c) <u>Sample Preparation: Practical Experience</u>

- 3) Food Samples:
 - wash sample with D2O
 - optimise sample for appropriate rotor volume
 - up to ~12 mg: use rotor with spacer
 - up to ~64 mg: use HRMAS rotor (12ul) without spacer
 - best sensitivity with 50 ul Rotor (with spacer)
 - samples like plant leaves can be rolled like a cigar, cut to the exact length and placed into the rotor.
 - remember: lipids from fingers show strong NMR-signals.
 - fatty samples should be prepared with spacer





c) <u>Sample Preparation: Practical Experience</u>

- 4) Medical tissue samples:
 - in general can be handled like food samples
 - sometimes it is not feasible to cut the sample into smaller pieces, because a histological examination is required after the NMR-experiments. In this case you can modify the length of the insert to fix the sample in the center of the rotor.

 d) The sample preparation is an important condition for good NMR results.
 The NMR experiments are Standard Automation Experiments with very typical 'HR'-values for powerlevels and pulselength.



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Stator Position:

Manual Insert: only in Insert / Eject position !

HR MAS probes with sample insert/eject capability

Magic Angle Position: -never insert a sample in this position -switch to MA may damage the probe -use MAS-Automation





Insert- and Eject position

MAS stator in magic angle position





stator3.tif



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HR-MAS Rotor System









Tools in the Lab:



A magnifying glass makes handling more easy!





Tips and Tricks:

An Eppendorf (100 ul) vial can be used as funnel too.

Static electricity can be overcome by washing the sample in with the solvent

Just cut the bottom







Tools in the Lab:

- The level of filling of the rotor can be checked using a balance
- Plastic weighing boats with a hole can be used as rotor holder.







Tools in the Lab:

Useful tools for HR-MAS sample preparation.











Insert position for rotors, rotors are stapled on top of each other, up to 40 possible with extension tube

reservoir for rotors ejected after measurement

eject tube [^]





Top loading MAS/HR-MAS





HZ 05538	"HR-MAS Rotor 12 ul" with spacer for smallest volume complete 4 mm rotor + TEFLON spacer + 3 KEL-F caps
	Rotor volume without spacer ~65 ul.
HZ 05537	same as HZ 05538 but with KEL-F spacer, only for special application.
H 5202	extraction screw for spacer
H 6304	KEL-F cap for 4 mm rotor - set of 3
HZ 3909	TEFLON spacer (insert) for 12 ul volume with HZ 05538
HZ 04800	sealing KEL-F screw for all inserts 2 ∗ 2 mm
HZ 05754	cap removing tool





Order No. HRMAS Rotor Description

New April 2000:

HZ 07213"HR-MAS Rotor 50 ul" with spacer for best sensitivity
complete rotor with Teflon spacer, caps, sealing screw
Rotor volume without spacer ~90 ul.HZ 07123Teflon spacer (insert) for 50 ul with HZ 07213

HZ 04800 sealing srcew 2 * 2 mm KEL-F for all inserts

New April 2001:

H 9759 Test Set for HR-MAS Specifications: KBr, Hump and Sensitivity 1 HR-MAS-Rotors 50 ul filled with 3% humptest 1 HR-MAS-Rotors 50 ul filled with 2 mMol sucrose in D20 Tools and Manual: HR-MAS Rotor Preparation

