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Measurement of the Temperature of the Resting Rotor in Analytical Ultracentrifugation

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Abstract

Accurate measurements of rotor temperature are critical for the interpretation of hydro dynamic parameters in analytical ultracentrifugation. We have recently developed methods for a more accurate determination of the temperature of a spinning rotor utilizing iButton® temperature loggers. Here we report that the temperature measured with the iButton on the counterbalance of a resting rotor, following thermal equilibration under high vacuum, closely corresponded to the temperature of the spinning rotor with a precision better than 0.2 °C. This strategy offers an inexpensive and straightforward approach to monitor the accuracy of the temperature calibration and determine corrective temperature offsets.

Keywords

sedimentation velocity; hydrodynamics; temperature calibration

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The measurement of the sedimentation coefficient, reporting on macromolecular size and shape, is one of the traditional applications of analytical ultracentrifugation [1]. The enhanced hydrodynamic resolution afforded by modern sedimentation velocity data analysis and direct boundary modeling [2] has now enabled their more precise determination, and advances in the structure-based prediction of the translational hydrodynamic friction [3–5] have greatly improved the interpretation of their absolute values: For example, in the context of small-angle scattering data, sedimentation coefficients can provide hydrodynamic constraints in the model building of the gross macromolecular shape [6]. Similarly, absolute values of the sedimentation coefficient have proven highly useful in the identification of anomalous protein hydration [7], and for corroboration of solution structures from molecular docking and nuclear magnetic resonance [8–10].

In recent studies on the accuracy of analytical ultracentrifugation data [11,12] we have occasionally encountered large temperature calibration errors that translate to systematic errors of ~7% in the sample viscosity [12], severely limiting the interpretation of the sedimentation coefficient. This error is significantly higher than the statistical precision of ~0.1 % in replicate determinations of sedimentation coefficients carried out side-by-side in the same rotor and instrument [12,13]. Therefore, we have developed a method for the external calibration of rotor temperature [12] using a miniaturized temperature-logging integrated circuit, the Maxim Thermochron iButton®. We have experimentally validated the iButton temperature measurements in reference experiments in ten instruments, using bovine serum albumin monomer as an *s*-value standard [11,12].

Initially, the iButton was placed in custom-fabricated holders fitting into standard cell housings used for analytical ultracentrifugation [12]. This allowed for temperature measurements of the spinning rotor at speeds up to ~3,000 rpm, a value limited by the mechanical stability of the iButton battery and integrated circuit in the gravitational field. In this first study, we observed highly reproducible instrument-dependent deviations between the temperature reported on the ultracentrifuge console, as measured by the instrument radiometer, and the values measured by the iButton. More recently, we have developed a modified rotor handle that can hold the iButton in the center of rotation, allowing for temperature measurements at even the highest rotor speeds currently permitted in analytical rotors [14]. In such a manner, the rotor temperature can be monitored independently throughout the actual sedimentation experiment, including the cooling from adiabatic stretching of the rotor during acceleration [14].

Interestingly, we found that the iButton temperatures were highly consistent and independent of iButton placement [14]. Furthermore, we found that the difference between the apparent temperature reported by the instrument and the iButton temperature to be independent of rotor speed, and, when tested in a single instrument, largely independent of the temperature set-point [14]. Finally, when using the evacuated and temperature equilibrated rotor chamber as a convenient device to compare calibration accuracy of different iButtons, we fortuitously observed that the temperature measured on top of the resting rotor was, within the precision of the iButtons, identical to the temperature of the spinning rotor [14]. This coincidence was unexpected, due to the potentially different heat flows and temperature regulation in the resting or spinning rotor.

In the present work, we systematically investigated whether an iButton placed on a resting rotor, in an evacuated and temperature equilibrated rotor chamber, can be reliably used to measure instrumental deviations in temperature calibration.

Temperature measurements were carried out using Maxim Thermochron iButtons (DS1922L, Maxim Integrated Products, San Jose, CA, USA) [12,14], with 11-bit resolution corresponding to 0.0625 °C increments. iButtons were either calibrated with a reference thermometer [12], or purchased with a calibration provided by the vendor (Thermodata Corporation, Marblehead, MA, USA) [14]. iButton temperature logs were retrieved using a USB connector attached to an office computer and temperature equilibration was verified from the time-course of readings. The equilibrium temperature was obtained from the constant-temperature readings recorded over at least 30 min at equilibrium.

Temperature measurements of the resting rotor were carried out in Optima XL-A or ProteomeLab XL-I analytical ultracentrifuges (Beckman Coulter, Indianapolis, IN, USA). An An-50 Ti rotor equipped with a counterbalance in one rotor hole was placed in the rotor chamber such that the radiometer was maximally covered by the metal of the rotor. The monochromator arm was not installed. The iButton was programmed to log temperature in 1 or 2 min intervals and loosely placed at the center on top of the counterbalance, with the sensor side down (i.e. the wider, flat surface up) (Figure 1). The rotor chamber was closed, the vacuum button on the console pressed, and the temperature set-point adjusted to 20.0 °C. After a rough vacuum was established, a run speed of 0 rpm was entered, and the 'Start' button pressed to engage the diffusion pump, when present. After 3 – 4 hours, the rotor chamber was opened, the iButton was retrieved, and the temperature data were saved.

For comparison, temperature measurements were also carried out in the spinning rotor. iButtons were placed in custom-fabricated holders fitting into standard cell housings used for analytical ultracentrifugation, as described in [12]. The rotor speed was set to 1,000 rpm, and a run of 3 – 4 hours was conducted with a nominal temperature set-point of 20.0 °C in the presence of the monochromator arm. Additionally, in some instruments, iButtons were placed in rotors with custom-modified handles, as described in [14], and 3 – 4 hour runs were conducted at 50,000 rpm, with the monochromator arm installed.

Table 1 shows a comparison of the measured equilibrium temperatures in thirteen different instruments with the iButtons placed on the counterbalance in a resting rotor, in a rotor hole adapter spinning at 1,000 rpm, and where possible, in the rotor handle spinning at 50,000 rpm. For these experiments we chose the An-50 Ti 8-hole rotor in order to minimize variability arising from the placement of the rotor within the field of view of the radiometer. However, similar results were obtained when using the An-60 Ti 4-hole rotor (data not shown). It was important to note the console temperature and verify that it reached the set-point of exactly 20.0 °C. Different lengths of time were required for temperature equilibration, depending on the instrument and iButton position. However, in all cases, we verified that thermal equilibration occurred using the time-course of logged temperature readings.

In most of the instruments studied, significant discrepancies between the temperature set-point and measured temperature are apparent, as reported [12]. Furthermore, in some cases the temperature values are different from those published previously for the same instruments [12,14]. Usually, these changes are associated with instrument repair or maintenance. In one case the repair was to adjust the temperature calibration; however, this is usually not necessary as long as the real experimental temperature is known. Typically the instrument performance was highly stable, with drifts of 0.2 °C or less over several months (data not shown).

In all thirteen instruments, the iButton temperature recorded in the different locations at different rotor speeds was highly consistent. The average deviation of 0.05 °C (amounting to ~1 bit of the iButton), and the maximum deviation of 0.18 °C, compares well with the estimated iButton accuracy of 0.1 – 0.2 °C, considering their resolution, variability, and calibration errors [12,14].

The agreement between the temperatures measured on top of the counterbalance of the resting rotor and that of the spinning rotor was unexpected, and suggests that the temperature control works rather well, except for apparent offsets and occasional adventitious changes in the calibration point of the temperature sensor. Modern analytical ultracentrifuges employ a radiometer for measuring the rotor temperature based on the thermal equilibration of the rotor temperature with a blackened metal disk via black-body infrared radiation, which in turn is interrogated by thermocouples with a large heat sink as reference point, as described by Waugh & Yphantis [15]. In an experiment where we isolated the radiometer from the rotor by way of an aluminum cover, the acceleration of the rotor to 50,000 rpm from a pre-equilibrated state did not lead to any significant changes in the measured temperature by the radiometer (data not shown), suggesting that there are no significant heat flows impinging on the radiometer and heat sink during rotor acceleration and run. At the same time, in this experiment the rotor temperature monitored with an iButton in the rotor handle showed the typical adiabatic cooling, but with slower than usual recovery, suggesting that this is a major cause of heat flow during the run.

Interestingly, in some instruments we measured a resting rotor temperature ~ 0.2 °C above that of the spinning rotor when the monochromator arm (both with and without the laser attachment for interference optics) was present during the resting-rotor experiment. Since it is connected to the heat sink, the monochromator arm can exhibit temperatures different from that of the rotor (data not shown) and thus act as a radiative heat source. For this reason, the monochromator arm was absent in the final measurements of the resting-rotor temperature, so as to achieve a better estimate of the spinning rotor temperature. In addition, to prevent atmospheric interference with the reading of the radiometer, high vacuum was established in the rotor chamber by means of the secondary diffusion or turbomolecular pump. Engagement of the diffusion pump was ensured by starting a 'Run' at zero rpm.

The most important conclusion of this approach and our previous work on temperature control [12,14] is the necessity for periodic and independent monitoring of temperature accuracy, such that the appropriate temperature-dependent density and viscosity corrections can be applied. While the real-time measurement with iButtons in the rotor handle is still the

optimal approach when carrying out hydrodynamic experiments [14], the observations described in the present communication show that good temperature data can be obtained using commercial, inexpensive iButtons without any custom fabrication or rotor modification. These results open up this approach for routine use in analytical ultracentrifugation laboratories.

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References

1. Svedberg T, Nichols JB. Determination of size and distribution of size of particle by centrifugal methods. *J Am Chem Soc.* 1923; 45:2910–2917.
2. Schuck P. Analytical ultracentrifugation as a tool for studying protein interactions. *Biophys Rev.* 2013; 5:159–171. [PubMed: 23682298]
3. Aragon SR. Recent advances in macro molecular hydrodynamic modeling. *Methods.* 2011; 54:101–14. [PubMed: 21073955]
4. Harding SE, Longman E, Carrasco B, Ortega A, García de la Torre J. Studying antibody conformations by ultracentrifugation and hydrodynamic modeling. *Methods Mol Biol.* 2003; 248:93–113. [PubMed: 14970492]
5. Ortega A, Amorós D, García de la Torre J. Prediction of hydrodynamic and other solution properties of rigid proteins from atomic- and residue-level models. *Biophys J.* 2011; 101:892–8. [PubMed: 21843480]
6. Perkins SJ, Nan R, Li K, Khan S, Abe Y. Analytical ultracentrifugation combined with X-ray and neutron scattering: Experiment and modelling. *Methods.* 2011; 54:181–99. [PubMed: 21256219]
7. Zhao H, Chen Y, Rezabkova L, Wu Z, Wistow GJ, Schuck P. Solution properties of γ -crystallins: Hydration of fish and mammal γ -crystallins. *Protein Sci.* 2014; 23:88–99. [PubMed: 24282025]
8. Venditti V, Ghirlando R, Clore GM. Structural Basis for Enzyme I Inhibition by α -Ketoglutarate. *ACS Chem Biol.* 2013; 8:1232–40. [PubMed: 23506042]
9. Takayama Y, Schwieters CD, Grishaev A, Ghirlando R, Clore GM. Combined use of residual dipolar couplings and solution X-ray scattering to rapidly probe rigid-body conformational transitions in a non-phosphorylatable active-site mutant of the 128 kDa enzyme I dimer. *J Am Chem Soc.* 2011; 133:424–7. [PubMed: 21162528]
10. Schwieters CD, Suh JY, Grishaev A, Ghirlando R, Takayama Y, Clore GM. Solution structure of the 128 kDa enzyme I dimer from *Escherichia coli* and its 146 kDa complex with HPr using residual dipolar couplings and small- and wide-angle X-ray scattering. *J Am Chem Soc.* 2010; 132:13026–45. [PubMed: 20731394]
11. Zhao H, Ghirlando R, Piszczek G, Curth U, Brautigam CA, Schuck P. Recorded Scan Times Can Limit the Accuracy of Sedimentation Coefficients in Analytical Ultracentrifugation. *Anal Biochem.* 2013; 437:104–108. [PubMed: 23458356]
12. Ghirlando R, Balbo A, Piszczek G, Brown PH, Lewis MS, Brautigam CA, et al. Improving the thermal, radial, and temporal accuracy of the analytical ultracentrifuge through external references. *Anal Biochem.* 2013; 440:81–95. [PubMed: 23711724]
13. Errington N, Rowe AJ. Probing conformation and conformational change in proteins is optimally undertaken in relative mode. *Eur Biophys J.* 2003; 32:511–517. [PubMed: 12830332]
14. Zhao H, Balbo A, Metger H, Clary R, Ghirlando R, Schuck P. Improved Measurement of the Rotor Temperature in Analytical Ultracentrifugation. *Anal Biochem.* 2014; 451:69–75. [PubMed: 24530285]

15. Waugh DF, Yphantis DA. Rotor Temperature Measurement and Control in the Ultracentrifuge. *Rev Sci Instrum.* 1952; 23:609–614.

Figure.

Configuration of an 8-hole rotor positioned in the rotor chamber, with counterbalance and iButton for the measurement of the temperature of a resting rotor. Note the absence of the monochromator arm in the experiment. The inset shows a closeup from which the orientation of the iButton can be discerned.

Table 1

Temperatures measured in different instruments and at different speeds of the 8-hole rotor

Instrument	resting rotor^(a) (°C)	rotor hole^(b) 1,000 rpm (°C)	rotor handle^(c) 50,000 rpm (°C)	max difference (°C)
Momo	20.29	20.29	20.35	0.06
Winnie	20.26	n/a	20.20	0.06
Annie	19.89	19.95	n/a	0.06
Ivy	19.89	19.89	n/a	0
Howie	19.89	19.89	19.89	0
Anne	20.01	19.95	19.95	0.06
Clotho	19.60	19.60	n/a	0
Larry	19.91	19.91	19.91	0
Shemp	21.28	21.28	21.22	0.06
Moe	19.10	n/a	18.92	0.18
Ted	20.91	20.97	20.91	0.06
Curly	19.66	19.66	19.66	0
Joe	19.35	19.35	19.23	0.12

^(a) iButton placed on counterbalance of resting rotor (Figure 1), in evacuated and thermally equilibrated rotor chamber, without monochromator;

^(b) iButton placed in custom cell assembly adaptor thermally equilibrated while spinning at 1,000 rpm [12];

^(c) iButton placed in custom rotor handle thermally equilibrated while spinning at 50,000 rpm [14].