Title

Understanding temperatures and pressures during short radiochemical reactions

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Abstract

Automated radiosynthesizers are critical for the reliable, routine production of PET tracers. To perform reactions in these systems, the temperature of the reactor heater is controlled, and the liquid temperature within the reaction vessel is presumed to closely follow. In reality, the liquid temperature can lag by several minutes and generally does not reach the heater temperature. Furthermore, because different synthesizers have different heating mechanisms and geometries, discrepancies are certain to exist between the actual temperatures experienced by the reaction mixture on different synthesizers. For dissimilar reactors, this can necessitate re-optimization of conditions when adapting a synthesis from one system to another, especially for the short-duration reactions common in radiochemistry. Herein, we study the relationship between the temperatures of the reactor heater and reaction liquid for various solvents using the ELIXYS radiosynthesizer as a representative example of a vial-based system. Our aims are to quantitatively illustrate this discrepancy to the community and provide data necessary to enable efficient translation of protocols between other radiosynthesizers and the ELIXYS.

Keywords

Radiosynthesis, reaction temperature, reaction time, radiosynthesizer characterization, standardization, protocol sharing

Background

The preparation of positron emission tomography (PET) tracers has evolved over the years to include increasing automation (Alexoff, 2003; Keng et al., 2012; Krasikova, 2007). For automation to be successful, however, thorough characterization of the reagent delivery, reaction, solution transfer, and other mechanisms of the radiosynthesizer needs to be performed and understood (Sachinidis et al., 2010). Only then is one able to specify appropriate parameters for the automated radiosynthesis protocol such that the system consistently and efficiently produces the desired product from a given input of reagents.

An especially critical parameter in determining the reaction rates and yields of both the desired product and the formation of side-products is the reaction temperature. Consistent with normal practice in the field of organic synthesis, radiochemists typically report the temperature of the reactor heater (e.g., metal block, oil bath, etc.) along with the time during which the reaction vessel (e.g., glass vial) is heated at the given temperature. Though it would be desirable to directly monitor and control the temperature of the vessel contents, the combination of a harsh chemical environment, high pressure, and a small reaction vessel makes it very challenging to accurately and routinely monitor the liquid temperature. (Though IR sensors can be placed outside the reaction vessel, circumventing many of these issues, the readout may not be accurate, representing a mixture of the temperature of the reaction vessel contents and the temperature of the glass vessel itself.) Thus, in automated radiosynthesizers, there is a system-specific discrepancy between the monitored reactor heater temperature and the actual liquid temperature that should be considered when developing synthesis protocols. The actual liquid temperature is furthermore related to the vapor pressure that is developed when performing sealed reactions; it is critical to

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understand this relationship, especially when developing protocols involving reactions under superheated conditions (i.e., above the solvent's normal boiling point), so that pressure limitations of the synthesizer are not exceeded.

During relatively lengthy reaction times (e.g., >15-30 min for milliliter-scale volumes), the temperature of the reaction vessel contents is likely to equilibrate, reaching a stable value for much of the reaction time. However, as the reaction time decreases, this equilibrium may not be established, or may only be established for a relatively small fraction of the total heating time, with the rest of the time occurring during temperature ramping (i.e., heating up or cooling down). This problem is further intensified at higher reaction temperatures, which increase the equilibrium temperature and the required liquid heating time.

Performing reactions primarily during a temperature ramping phase can make it difficult to reproduce reaction conditions precisely, particularly when trying to adapt reaction conditions from one type of radiosynthesizer to another. Engineering design differences between synthesizers can significantly impact the time it takes the liquid temperature to ramp up toward the reactor heater setpoint, the liquid temperature reached at equilibrium, and the time it takes the liquid to cool back down to a certain temperature. Differences in the heating mechanism (e.g., immersion in an oil bath, resistively or thermoelectrically heating a metal "jacket" around the vessel, inductive heating, or applying microwaves) have a major impact on these parameters. Even among different synthesizers based on jacket heating (i.e., the most common approach), there can be significant differences. For example, the heating rate depends on the power output of the heater, the thermal mass of the heater and associated systems, the thickness and thermal conductivity of the reaction vessel, the effectiveness of the thermal interface between the heater and the vessel, etc. The equilibrium temperature depends on the power output of the heater and the degree of thermal insulation of the reaction vessel from the rest of the environment. The cooling time depends on the heat removal capacity of the cooling system (e.g., exposure to ambient air, exposure to forced air, or recirculating liquid coolant) as well as the same geometric and mechanical factors that affect heating rate.

With all of these potential variations, it follows that to achieve the same liquid temperature profile for a reaction on different synthesizers, it may be necessary to use different reactor heater setpoints, reaction durations, and cooling times. This could be responsible in part for the significant variation that is sometimes seen in reported protocol parameters (especially for short-duration or high-temperature heating steps) for producing the same tracer on different systems. As an example, **Table 1** illustrates the conditions used for the 3-step radiosynthesis (i.e., [^{18}F]fluorination, deprotection, and *N*-hydroxysuccinimide (NHS) ester formation) of *N*-succinimidyl-4- ^{18}F fluorobenzoate (^{18}F SFB), a commonly used prosthetic group for radiolabeling peptides and proteins with [^{18}F]fluoride, across different platforms. Though the reagent types and amounts vary slightly, the fundamental chemistry is the same for each reaction step, yet there is large variation in parameters among these publications. Inconsistency among radiosynthesizers, coupled with a lack of data or understanding of the reactor heating characteristics, could explain, in part, the difficulty and high cost of adopting new tracers at production facilities, which in turn creates a bottleneck in the investigation and validation of novel PET tracers. This is a particularly pressing issue in the radiopharmaceutical field and must be overcome to accelerate the expansion of PET and diversification of available tracers.

To increase awareness in the community, we illustrate the above heating-related issues (i.e., discrepancy between actual and measured temperatures, temperature ramping time, and vapor pressure limitations) via an extensive characterization of the relationship between the temperatures of the reactor heater (T_{heater}) and liquid within the vessel (T_{liquid}) for a representative vial-based radiosynthesizer (ELIXYS, Sofie Biosciences). This system was chosen for its ability to be readily modified to enable simultaneous monitoring of both temperatures, while also supporting a particularly wide range of operating temperatures and pressures (Lazari et al., 2013) compared to other systems. We characterized the T_{liquid} profiles over time for common reaction solvents to determine heating times, cooling times, and maximum liquid temperatures at equilibrium (T_{max}) for different reactor heater temperature setpoints (T_{set}). Furthermore, we examined the effect of solutes using mock fluorination solutions for [^{18}F]SFB, 2-deoxy-2- ^{18}F fluoro-D-glucose ([^{18}F]FDG), and 2'-deoxy-2'- ^{18}F fluoro- β -D-arabinofuranosylcytosine (D-[^{18}F]FAC) previously performed on the ELIXYS (Lazari et al., 2014). Finally, for highly volatile solvents, we

investigated the operating temperature limitations arising from the high vapor pressure generated at elevated T_{set} values. In addition to highlighting the importance of the heating issues, we believe the presented data and analyses could aid in efficiently translating synthesis protocols between ELIXYS and other thoroughly characterized systems to facilitate protocol sharing without the need for extensive re-optimization.

Material and Methods

Materials

Potassium carbonate (K_2CO_3), 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix K_{222}), and anhydrous ($\geq 99\%$) acetonitrile (MeCN), dimethyl sulfoxide (DMSO), *N,N*-dimethylformamide (DMF), 1,2-dichloroethane (DCE), chloroform (CHCl_3), dichloromethane (DCM), methanol (MeOH), and toluene were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and used as received. Mannose triflate, 2-*O*-(trifluoromethylsulfonyl)-1,3,5-tri-*O*-benzoyl- α -D-ribofuranose (D-sugar), and 4-(ethoxycarbonyl)-*N,N,N*-trimethylbenzenaminium triflate (SFB precursor) were purchased from ABX (Advanced Biochemical Compounds, Germany). All water was deionized to 18 M Ω and passed through a 0.1 μm filter before use.

Liquid temperature monitoring

All temperature profile data was collected using an ELIXYS fully-automated radiosynthesizer (Sofie Biosciences, Inc.; Culver City, CA, USA) using cassettes modified to accommodate a thermocouple immersed within the contents of the reaction vessel for *in situ* temperature monitoring during heating. As shown in **Figure 1**, a 7"-long, K-type, Teflon®-coated, hypodermic needle thermocouple (HN-7-K-TEF, J-KEM Scientific; Saint Louis, MO, USA) was inserted through the polytetrafluoroethylene (PTFE)-coated silicone gasket (Specialty Silicone Products, Inc., Ballston Spa, NY, USA, and Cannon Gasket, Upland, CA, USA) where the dip-tube would normally be located. The thermocouple was held in place with a standard 1/4"-28 fluidic fitting with 1/16" ID ferrule (IDEX Health and Science; Oak Harbor, WA, USA). The thermocouple reading is sensitive to the temperature only for the 2 mm of its length closest to the tip. For each experiment, a new piece of the PTFE-coated silicone gasket material (cut to 2.2 x 2.7 cm) was prepared and affixed to the bottom of the cassette prior to insertion of the thermocouple. Instead of the usual two holes punched in this part of the gasket, only a single hole was punched, using a 23G blunt tip needle, to ensure a pressure-tight seal around the thermocouple.

For each experiment, a clean, dry 5 mL glass V-vial (W986259NG, Wheaton; Millville, NJ, USA) was pre-weighed and 1.0 mL of the given solvent was added to the vial. The vial was then weighed again to determine an exact starting mass and volume. The filled vial (reaction vessel), was placed into one of the three vessel heaters. Using commands built into the debugging mode of the ELIXYS, the heater and vessel were aligned with the thermocouple and pneumatically raised such that the top of the vessel sealed against the gasket. The vertical position of the thermocouple was adjusted such that the tip was submerged and positioned 5 mm above the bottom of the vessel. Starting near room temperature (~ 23 - 28°C), each experiment consisted of the following temperature cycle: The reactor heater was rapidly heated until T_{heater} reached the desired T_{set} , T_{heater} was held at T_{set} for a heating duration (t_{reaction}) of 30 min, and then the heater was rapidly cooled (i.e., by means of the built-in recirculating liquid coolant system) until T_{liquid} dropped to 35°C . Stirring of the liquid was not implemented at any point during data collection as inconsistent T_{liquid} fluctuations were observed when stirring was performed, presumably due to mechanical disturbance of the thermocouple by the stir bar. Upon completion of the experiment, the vial was weighed again to determine the remaining mass and volume of solvent to compute the amount lost during the experiment. Greater than 10% loss during the 30 min of heating was considered to be a failure in correctly sealing the vessel.

The above procedures were repeated for each solvent with T_{set} values of 40°C , 60°C , 80°C , 120°C , 140°C , 160°C , and 180°C . 185°C is the maximum operating temperature of the ELIXYS synthesizer without special modification. Solvents studied include MeCN, H_2O , DMSO, DMF, DCE, CHCl_3 , DCM, MeOH, and toluene. Each profile was collected in triplicate for each T_{set} for the first 5 (commonly-used)

reaction solvents, and one time for the last 4 (occasionally-used) solvents. Additionally, individual T_{liquid} profiles for MeCN and DMSO were also collected using 0.5, 2.0, 3.0, and 4.0 mL volumes at each of the above T_{set} values.

Temperatures were electronically collected every 0.5 s using temperature recording functions built into a custom debugging mode of the ELIXYS system. The reaction vessel in the ELIXYS is heated by contact with a metal jacket consisting of three spring-loaded segments, each containing a 100 W cartridge heater and thermocouple for individual temperature control. During operation, all three feedback circuits are set to the same T_{set} , but independent control provides superior temperature uniformity around the vial. T_{heater} was taken as the average of the three individually-controlled heating elements, and T_{liquid} was taken from the reading of the hypodermic thermocouple. A representative overlay of both T_{heater} and T_{liquid} profiles over time is shown in **Figure 2A**.

Analysis of T_{liquid} profiles

Temperature vs. time profiles were analyzed in Excel (Microsoft; Redmond, WA, USA). The following parameters were extracted from each T_{liquid} profile (**Figure 2B**). T_{max} was determined by obtaining the average temperature reached by the liquid during equilibrium (i.e., the plateau region between 10 and 25 min of each profile). To determine a characteristic heating time, a straight line was extended from the inflection point during the ramping phase until it intersected with the horizontal line at T_{max} . The time necessary to reach this intersection point after heater activation (i.e., time $t = 0$) was defined as t_{heat} . The time for the liquid to cool down to 35°C, $t_{\text{cool-35}}$, was defined as the duration between the time the heater was deactivated (and cooling system activated) and the time at which T_{liquid} reached 35°C during the cooling phase. A shorter cooling time, i.e., the time necessary to cool the solvent below its normal boiling point ($t_{\text{cool-bp}}$), was similarly calculated by taking the time between heater deactivation and T_{liquid} reaching the normal boiling point of the solvent. Cooling to at least the boiling point or lower is essential to avoid solvent “bumping” (i.e. violently bubbling/splashing) when unsealing the reactor. For temperature profiles that were repeated multiple times, T_{max} , t_{heat} , $t_{\text{cool-35}}$ and $t_{\text{cool-bp}}$ values were computed as an average of the individually determined values and uncertainty was estimated using the standard deviation. Using linear regression, a “calibration” equation relating T_{set} and T_{max} was also determined for each solvent.

To determine whether the presence of solutes significantly altered the T_{liquid} profile, mock solutions mimicking the fluorination reaction conditions to produce the ^{18}F -fluorinated intermediates of [^{18}F]SFB, [^{18}F]FDG, and D-[^{18}F]FAC were studied in parallel with the respective pure solvents under the same heating conditions. To prepare the mock [^{18}F]FDG and D-[^{18}F]FAC reaction solutions, 1 mg of K_2CO_3 and 10 mg of K_{222} were dissolved in 0.3 mL of water and 0.5 mL of MeCN and added to the reaction vessel. The contents was evaporated at 110°C to dryness and cooled to room temperature. To this, 20 mg of mannose triflate (for [^{18}F]FDG) or 10 mg of D-sugar (for D-[^{18}F]FAC) in 1 mL of MeCN was added to the reaction vessel, the solution was mixed for 20 s. Mock [^{18}F]FDG and D-[^{18}F]FAC T_{liquid} profiling experiments were performed for 30 min at 130°C and 160°C, respectively. A T_{liquid} profile was also obtained for a 3 min reaction of the mock [^{18}F]FDG solution as a model short-duration reaction, which was performed in Lazari et al. (2014). To prepare the [^{18}F]SFB mock reaction solution, 4.25 mg of K_2CO_3 and 22 mg of K_{222} were dissolved in 0.3 mL of water and 0.5 mL of MeCN and added to the reaction vessel. The contents was evaporated at 110°C to dryness and cooled to room temperature. To this, 5 mg of the SFB precursor in 1 mL of DMSO was added. Profiling experiments were conducted for 30 min at 110°C.

Determination of maximum T_{set} for each solvent

Some of the solvents tested exhibited significant solvent loss (i.e., leaking) when heated at the highest temperatures, suggesting that the vapor pressure being generated exceeded the maximum operating conditions of the ELIXYS synthesizer. We performed experiments to determine the maximum T_{set} that could be used for these solvents. Reaction vessels were prepared, weighed, and filled by the same procedure as the temperature profiling experiments. The vessel was then sealed against an unmodified gasket, (i.e., without pre-punching or thermocouple insertion) and heated for 15 min without monitoring of T_{liquid} . Seal integrity was determined by calculating the solvent loss after heating and judged on a pass/fail basis, where loss < 10% was considered a pass and loss $\geq$ 10% was considered a failure. For each

solvent, experiments were repeated at progressively increasing T_{set} values starting from the highest previously observed success, and the lowest T_{set} that consistently resulted in significant leakage was determined. Using this T_{set} , the corresponding T_{max} was calculated using the calibration equation and the theoretical vapor pressure at this T_{max} was calculated using the Clausius-Clapeyron equation to determine the equivalent pressure limitation of the synthesizer for each solvent (Haynes, 2013; Jenkins, 2008).

Results

T_{liquid} profiles

An example of a temperature profile containing both the reactor heater temperature, T_{heater} , and the liquid temperature, T_{liquid} , is displayed in **Figure 2**. When the heaters are activated at time $t = 0$, T_{heater} rapidly ramps up to the setpoint, T_{set} , remains stable for t_{reaction} (30 min), and then rapidly drops down to room temperature (i.e., the temperature of the coolant) when the heaters are deactivated and cooling system activated. T_{liquid} initially ramps up quickly after a small delay, but then the temperature rise slows down (near $t = t_{\text{heat}}$), taking considerable time to reach the equilibrium temperature, T_{max} . During cooling, T_{liquid} ramps down quickly after a small delay, but the rate of cooling slows as the temperature approaches ambient conditions. Despite the lack of active stirring during measurements, convective heat transfer and refluxing of the solutions at elevated temperatures likely efficiently homogenized the temperature of the liquid within the vessel. If stirring were possible, we expect it would have very little effect on the results reported here.

For each 30 min heating experiment, solvent loss was found to be low to negligible (i.e., below 10%, or 0.1 mL for a 1.0 mL starting volume) for all solvents and T_{set} values with the exception of CHCl_3 at 180°C, MeOH at 160°C and 180°C, and DCM at 160°C and 180°C. Further studies were performed for these solvents to determine the T_{set} of failure (see below). Note that if any leakage occurs during heating, occasional “dips” in T_{liquid} are often observed (example in **Supplementary Figure 1**). No such fluctuations were observed for the experiments with solvent loss < 10%. An example of the T_{liquid} profiles for 1.0 mL of MeCN heated at various T_{set} values can be seen in **Figure 3**. T_{liquid} profiles for the other solvents can be found in **Supplementary Figure 2**.

Maximum equilibrium liquid temperature, T_{max}

In general, the T_{liquid} equilibrated at some maximum value, T_{max} , below T_{set} . The T_{max} values are tabulated for 1.0 mL volumes of the different solvents in **Table 2**. For the range of tested temperatures, T_{max} for each solvent was found to vary linearly with T_{set} . **Table 3** summarizes the results of the linear regression analysis and provides linear equations that allow prediction of T_{max} for a given T_{set} (or vice versa). Data for other solvent volumes is given in **Supplementary Table 1**. The solvent volume appears to have only a very minor effect on T_{max} .

T_{max} values obtained for the mock reaction solutions simulating [^{18}F]fluorination of [^{18}F]SFB, [^{18}F]FDG, and D-[^{18}F]FAC are listed in **Table 4** along with the pure solvent T_{max} values for comparison. The presence of solutes seems to have minimal effect on the equilibrium behavior (i.e., T_{max}) in the range tested. Similar independence on solute content was observed for the non-equilibrium conditions as a result of short-duration (3 min) heating time (at $T_{\text{set}} = 130^\circ\text{C}$), where $T_{\text{max}} = 123 \pm 2^\circ\text{C}$ ($n = 3$) for the solution and $T_{\text{max}} = 125 \pm 2^\circ\text{C}$ ($n = 3$) for MeCN alone. (Note that, in this non-equilibrated experiment, T_{max} was taken as the maximum T_{liquid} temperature reached before cooling began). A comparison of the full T_{liquid} profiles for the 3-min heated MeCN and mock [^{18}F]FDG solution is shown in **Figure 4**.

Heating and cooling times

The heating time, t_{heat} , and cooling times, $t_{\text{cool-35}}$ and $t_{\text{cool-bp}}$, for the 1.0 mL solvent volumes are listed in **Tables 5, 6, and 7**, respectively. In general, heating and cooling times were longer for higher T_{set} values. The t_{heat} values were found to be relatively independent of solvent, whereas both $t_{\text{cool-35}}$ and $t_{\text{cool-bp}}$ exhibited small but significant variations among solvents. Comparing the heating and cooling times of the mock reaction solutions with pure solvents (**Supplementary Table 2**) revealed the solutions to require

slightly longer heating and cooling times. Heating and cooling times for different volumes of MeCN and DMSO are compared in **Supplementary Table 3**.

Maximum temperatures and pressures

The solvents MeCN, H₂O, DMSO, DMF, DCE, and toluene all exhibited low loss when heated for 30 min at each T_{set} including 180°C. For the remaining solvents, the operating limits were investigated with finer granularity. For CHCl₃, MeOH, and DCM, the onset of leaking was observed to occur at T_{set} values of 179°C, 161°C, and 146°C, respectively. (Note that since leakage generally occurs relatively early, only 15 min heating was needed for these experiments.) **Figure 5** summarizes the theoretical vapor pressures of all tested solvents at T_{liquid} values ranging 0 – 180°C. The specific failure points for CHCl₃ (212 psi), MeOH (217 psi), and DCM (194 psi) are marked on the graph after conversion of T_{set} at failure to T_{max} at failure using the calibration equations (**Table 3**). The minimum of the corresponding pressures (i.e., 194 psi) can be used as a general estimate of the pressure limit on sealed reactions in the ELIXYS system.

Discussion

Equilibrium temperature

During heating, energy is transferred from the heater to the vessel walls and then to the vessel contents. Though heat enters the liquid only from the vessel walls, convection or stirring within the vessel rapidly homogenizes the temperature throughout the liquid. The rate of heat transport depends on the temperature difference between the heater and the vessel; thus, the rate of temperature increase of the vessel contents is initially rapid and then slows down as the contents of the vessel approach the setpoint, T_{set} . Since the reaction vessel cannot be completely isolated from the surrounding environment during heating, heat losses invariably occur, resulting in the equilibrium temperature of the reaction vessel contents, T_{max} , to always be somewhat lower than T_{set} . The magnitude of this discrepancy in ELIXYS is shown in **Tables 2** and **4**. The ELIXYS employs a similar thermal heating mechanism and vessel geometry to those found in other automated radiosynthesizers, particularly those used in routine clinical production of PET tracers; therefore, we expect most radiosynthesizers to exhibit a similar discrepancy. Additionally, a direct comparison of oil bath heating vs. ELIXYS heating reported in Lazari et al. (2013) revealed comparable T_{liquid} heating profiles, indicating that the same phenomenon can be expected with manual radiochemistry setups. Note that since it is the heater temperature that is monitored by nearly all radiosynthesizers, it may appear to the operator that the reaction is being performed at this exact temperature, but as demonstrated here, the reaction solution never quite reaches this temperature, even at equilibrium.

The discrepancy between T_{set} and T_{max} increases in magnitude for higher T_{set} values. At $T_{\text{set}} = 180^\circ\text{C}$, the T_{max} values ranged from 3 – 8% below T_{set} , depending on the solvent. Though this discrepancy has not been extensively studied in the literature for vial-based systems, one study by Bejot et al. (2011), using a batch-type microfluidic reactor, reported a similar discrepancy between T_{heater} and T_{liquid} of 6 – 8%. Additionally, we observed that there was a linear correlation between the T_{set} and T_{max} values for each solvent (**Table 3**). For the mock reaction solutions, the T_{max} value was found to be similar to the T_{max} value of the pure solvent (within one standard deviation), suggesting that T_{max} for solutions can be approximated using data from the pure solvents (**Table 3**) for radiochemical reactions performed on the ELIXYS radiosynthesizer at any T_{set} between 40 – 180°C. More importantly, if the relationship between these temperatures is known for a different type of synthesizer, it becomes straightforward to translate synthesis conditions between the two systems: T_{max} can be predicted by using the data from one system, and then using the data from the second system, an adjusted T_{set} value to be used on the second system can be determined.

Heating times

The temperature ramping time during heating, t_{heat} , was characterized as a function of T_{set} as well as solvent type and solvent volume. As shown in **Table 5**, the T_{liquid} heating time indeed requires up to just over 2 min, depending on T_{set} . Despite the different heat capacities of the solvents, heating times were

found to depend very little on the type of solvent. Compared to pure solvents, the mock [^{18}F]fluorination reaction solutions seem to require slightly more heating time (< 0.5 min) (**Supplementary Table 2**). The reported times include the time necessary to ramp the temperature of the heater itself, T_{heater} , to the given T_{set} . In the worst case, this ramp time is just over 1 min (**Table 5**). The effect of this is that during the first ~ 1 min of heating, the temperature differential between the heater and the reaction vessel (and thus the rate of heat energy transfer) is not maximized. Systems involving movement of the vessel to a pre-heated oil bath, as, for example, in some manual synthesizers, would likely exhibit slightly faster ramping times, with all other conditions being equal. As noted for the equilibrium temperature, since it is the heater temperature that is monitored by nearly all radiosynthesizers, it may appear to the operator that heating of the reaction solution to T_{set} is completed in ~ 1 min, but as demonstrated here, the liquid requires substantially longer ramping time to equilibrate.

The ramping time is expected to be on the order of minutes in other radiosynthesizers with conventional heating as well, and may be faster or slower depending on the power of the heating system and other geometrical factors. Unfortunately, this parameter is not generally specified for commercial systems. The ELIXYS system employs 300 W of heating power per reactor, which is believed to be higher than most. Exotic types of heaters, such as microwave (Kimura et al., 2014) and pre-heated flow-microreactors (Pascali et al., n.d.) can provide significantly faster ramping but are not in widespread use. If temperature ramping data are available for systems with differing heating mechanisms than the ELIXYS, comparison of heating rates could help in translating protocols between these systems and the ELIXYS. For example, when adapting a synthesis from a relatively quick-heating system to a slower-heating system, it may be necessary to increase the time the heater remains at the reaction temperature to account for the longer time needed to reach equilibrium.

It should be noted that the definition of t_{heat} is a characteristic time and not the actual time to reach thermal equilibrium. A detailed look revealed the T_{liquid} to be on average $> 80\%$ of the T_{max} at time = t_{heat} for all solvents and T_{set} values (see **Supplementary Table 4**). Qualitatively, t_{heat} coincides with the transition between rapid heating and slowing of heating as T_{max} is approached. In general, 1 – 2 min of additional heating time beyond time = t_{heat} was needed to reach T_{max} .

The ramp time becomes most relevant in short reaction steps, where the ramp time is a significant fraction of the reaction time. One can be certain that reactions performed with < 2 min duration occur primarily, if not entirely, during the temperature ramping phase. An example of this is the 3 min mock [^{18}F]FDG [^{18}F]fluorination at $T_{\text{set}} = 130^\circ\text{C}$ (**Figure 4**), in which T_{liquid} was nearing, but had not reached, equilibrium. Ultimately, any reaction that is dominated by time spent during ramping is likely to be difficult to reproduce on other systems, and should be avoided if one desires the synthesis protocol to be usable on a variety of automated systems.

Cooling times

After the heating step (e.g., reaction) is complete, the reaction vessel contents must be cooled to quench the reaction and to reduce the vapor pressure within the headspace. If the vessel is unsealed under pressurized conditions, there is a significant risk that the liquid contents can spill out (or “bump”) due to the sudden pressure change. Because nearly all radiosynthesizers show a readout of the temperature of the heater and not the liquid contents, it can be difficult to determine how long the vessel should be cooled. In ELIXYS, for example, the heater cools down to ambient conditions extremely rapidly due to the recirculating liquid coolant, but additional time is needed to cool the contents.

The cooling times of the reactors contents down to 35°C (**Table 6**) were observed to vary from under 1 min at the lowest setpoints to just over 3 min for some solvents. The mock [^{18}F]fluorination reaction solutions required additional cooling time (**Supplementary Table 2**). Unlike heating times which were approximately independent of solvent, cooling times to 35°C ($t_{\text{cool-35}}$) appeared to depend more significantly on the type of solvent. From shortest to longest cooling times, the ordering of the commonly used solvents was DCE, DMF, (MeCN and H_2O), and DMSO. The $t_{\text{cool-35}}$ did not appear to be directly correlated with the specific heat capacities of the solvents: likely the cooling time depends on a combination of physical properties (**Supplementary Table 5**). For simplicity, we recommend using a fixed

cooling time of 3.5 min for all cooling steps in the ELIXYS system, as this was the highest observed time across all solvents and T_{set} values.

In protocols with a large number of heating steps (reactions or evaporations), the cooling time can become a significant part of the overall synthesis time. In fact, due to the efficient cooling mechanism in ELIXYS (pumping room temperature liquid coolant through each reactor heater), the cooling times described here are expected to be shorter than that for other systems (e.g., which achieve cooling by simply removing the vessel from the heating jacket or by blowing compressed air over the external surface of the vessel). To reduce synthesis time, especially if there are many cooling steps, it may be desirable to fine-tune the cooling time based on the particular solvent and T_{set} , rather than just using a fixed value. To further reduce the time needed for cooling, one can consider cooling to just below the normal boiling point, $t_{\text{cool-bp}}$, (**Table 7**) rather than 35°C. This would require substantially less time, yet still greatly reduce the chance of bumping. In general, these cooling times were less than 1 min, except for DCM, which required up to 1.7 min at the highest achievable T_{set} . The ordering of solvents from shortest to longest cooling times to the boiling point ($t_{\text{cool-bp}}$) was DMSO, DMF, H₂O, DCE, and MeCN, which is different from the $t_{\text{cool-35}}$ ordering. As might be expected, the $t_{\text{cool-bp}}$ values are inversely correlated to the normal boiling points of the solvents (**Supplementary Table 5**).

Tabulation of cooling data for other synthesizers with different cooling mechanisms could not only help users to determine optimal cooling parameters but could help translate protocols between these systems and ELIXYS. For example, when adapting a synthesis from a relatively slow-cooling system to a faster-cooling system, it might be necessary to increase the time the heater remains at the reaction temperature to account for the ‘delayed’ onset of cooling in the slower system.

Effect of reaction volume

The solution volume is another variable that could influence the temperature profile. Larger volumes in a given reaction vessel increase the heat capacity and thus require more heat energy to achieve the same temperature change. On the other hand, larger volumes also increase the liquid surface area in contact with the reaction vessel wall, thus increasing the rate of heat energy transfer. Typical vial-based radiochemical reaction volumes vary from ~0.2 – 2.0 mL; therefore we chose 1.0 mL to perform most of our measurements. To specifically study the effect of volume, we performed a few preliminary tests with MeCN and DMSO with volumes of 0.5, 1.0, 2.0, 3.0, and 4.0 mL and observed T_{max} to be relatively independent of volume, exhibiting only a standard deviation of $\pm 2^\circ\text{C}$ across the whole volume range (**Supplementary Table 1**). Heating and cooling times across the volumes for a given T_{set} and solvent exhibited standard deviations of no more than ± 0.4 min (**Supplementary Table 3**). This suggests that the data tabulated in this paper represents a good approximation of heating parameters even for volumes smaller or larger than 1.0 mL.

Seal integrity testing

Certain solvents, when heated to high temperatures, exhibited significant volume loss, presumably due to the leakage of vapor that resulted from exceeding the pressure limitation of the ELIXYS synthesizer. In some cases, deformation or even destruction of gaskets in the vicinity of the thermocouple was found. To ensure that the thermocouple, which would not normally be present during routine use of the ELIXYS, was not limiting performance, several experiments were performed without the thermocouple installed (i.e., without any holes in the gasket). Indeed, slightly increased T_{set} values could be used without failure. The failure points are shown in **Figure 5**, which revealed the current pressure limitation in the ELIXYS to be ~ 200 psi. Though most automated synthesizer manufacturers do not disclose the pressure limitations of their reactors, this pressure is far above the operating range of small-volume, chemically-resistant solenoid valves likely used in other vial-based radiosynthesizers to seal the reactor during heating. For many years, the maximum pressure rating of these valves was in the ~30-50 psi range, but some modern valves can reach ~70-100 psi operating limit. In addition to the lower operating pressure, systems with tubing and valves connected to the reaction vessel during heating could also suffer from a redistribution of solvent from the vessel into the tubing (Amarasekera et al., 2013; Paolillo et al., 2009).

It should be noted that certain non-vial-based microfluidic systems, such as the Advion NanoTek® and a prototype microfluidic device developed by Siemens, have reported pressure ratings of up to 400 psi (Ungersboeck et al., 2011) and 300 psi (Lebedev et al., 2012), respectively. Increase of the operating pressure range of the ELIXYS system up to these levels could in principle be possible by increasing the pneumatic sealing pressure of the reaction vessel against the gasket or utilizing other gasket materials but has not yet been demonstrated.

Operating radiosynthesizers and valves above their pressure limits can lead to leaks that cause dramatic drops in reaction temperature and/or substantial loss of reaction solvent (**Supplementary Figure 1**), or that can even damage the synthesizer. Given the importance of operating within the working range, we suggest that synthesizer manufacturers share data about the pressure limitations of their systems. Data about vapor pressure as a function of temperature is readily available for many solvents, but the lack of information about the pressure limitations of the synthesizer makes it impossible for users to know the practical operating temperature limit when using a particular solvent. Typically, radiochemists find out about such limitations only after observing unexpectedly low yields and/or solvent loss.

Conclusion

In this study, we have measured equilibrium temperatures and heating and cooling times of a variety of solvents and solutions during fully-sealed heating for a wide range of reactor heater setpoints using the vial-based automated radiosynthesizer, ELIXYS. These data provide a means to thoughtfully develop and optimize probe syntheses on the ELIXYS system. Additionally, these data can be expected to give a rough idea of the magnitude of these measures of performance in other systems with heating mechanism similar to the ELIXYS.

We observed that the equilibrium temperature reached within the vessel can be significantly less than the reactor heater setpoint, and that significant differences exist among different solvents, especially as setpoints increase. In addition to pure solvents, we investigated the heating of mock [¹⁸F]fluorination solutions and observed very little effect on the maximum equilibrium temperature compared to pure solvents. We also observed that, despite the efficient thermal management of the vessel and capabilities for very rapid heating and cooling on the ELIXYS, temperature changes within the liquid lagged behind the reactor heater by up to several minutes. Due to this effect, it is likely that reaction times of 1-2 min will never reach equilibrium, and thus will be difficult to replicate by others in the field unless they are using exactly the same synthesizer. Finally, for highly volatile solvents, we recognize that sealed heating is not possible at some setpoints due to the high vapor pressures that are generated, leading to loss of the reaction vessel seal integrity (or leading to valve failure in other systems). We determined the maximum operating temperature for different solvents and noted that the failure point occurred close to ~200 psi, thus indicating the pressure limit of the ELIXYS radiosynthesizer. Though this pressure data is not typically available from manufacturers of other systems, we believe it would be immensely useful to radiochemists in determining the acceptable working temperature range for different solvents without having to learn the hard way.

Of course, temperature consistency during reactions is just one of many factors necessary to ensure reproducibility across various systems. One must also pay attention to efficiency of reagent addition, solution transfer, evaporation, and purification steps. However, because these processes are largely observable by eye or with simple radioactivity measurements, and because the desired performance of each operation is clear, they can be optimized during a very small number of “cold” and/or “hot” runs on the new synthesizer. In reaction steps, the actual temperature is generally not observable, and the expected performance of the reaction is not always reported, especially for intermediate reactions, and thus there may be no baseline for comparison. The characterization of thermal performance of different synthesizers is aimed at eliminating this guesswork in translating reaction conditions from one system to another.

We encourage the community to perform characterizations of other synthesizers to facilitate adaptation of synthesis protocols from one system to another (e.g., by the process proposed in **Figure 6**), and thus enable more widespread sharing of published automated protocols among various systems with reduced

need for re-optimization. Ultimately, this could help significantly increase the availability of diverse novel tracers to both research scientists and clinicians.

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Tables

[¹⁸ F]fluorination	Deprotection	NHS ester formation	Decay-corrected RCY, synthesis time	Synthesizer	Reference
MeCN 90°C 10 min	MeCN/H ₂ O 100°C 5 min	MeCN 90°C 2 min	34–39% 68 min	GE TRACERlab FX _{FDG} (modified)	(Mäding et al., 2005)
DMSO 115°C 15 min	H ₂ O 115°C 9 min	MeCN 115°C 5 min	46±5% (<i>n</i> =4) 78 min	Siemens CPCU (modified)	(Marik and Sutcliffe, 2007)
MeCN 90°C 10 min	MeCN 120°C 3 min	MeCN 90°C 5 min	44±5% (<i>n</i> =10) 60 min	Manual apparatus	(Tang et al., 2008)
MeCN 90°C 10 min	MeCN 120°C 1 min	MeCN 90°C 5 min	43–60% ^a 85 min	GE TRACERlab FX _{F-N} (modified)	(Tang et al., 2010)
DMSO 110°C 15 min	MeCN 110°C 10 min	MeCN 110°C 5 min	69±8% (<i>n</i> =6) 78 min	Sofie Biosciences ELIXYS	(Lazari et al., 2014)

^aDecay-corrected RCY calculated from reported not-decay-corrected RCY and overall synthesis time.

Table 1. Reaction parameters for various [¹⁸F]SFB synthesis protocols from the literature. Radiochemical yields (RCY) and reaction times reported as mean ± standard deviation for the 3-step protection of [¹⁸F]SFB.

T _{set} (°C)	T _{max} (°C)								
	MeCN	H ₂ O	DMSO	DMF	DCE	CHCl ₃	DCM	MeOH	Toluene
40	41 ± 0	41 ± 1	40 ± 1	40 ± 0	41 ± 0	41	41	41	41
60	58 ± 1	60 ± 0	59 ± 1	59 ± 1	60 ± 0	59	60	61	61
80	77 ± 1	78 ± 1	78 ± 0	78 ± 0	79 ± 0	80	78	79	79
120	115 ± 1	114 ± 0	113 ± 1	118 ± 1	117 ± 1	118	117	116	118
140	133 ± 2	131 ± 1	132 ± 1	135 ± 1	136 ± 1	135	132	133	138
160	152 ± 2	148 ± 1	150 ± 2	156 ± 0	153 ± 3	155	a	a	155
180	170 ± 4	164 ± 1	168 ± 1	172 ± 1	173 ± 1	a	a	a	171

^aT_{max} was not achieved due to reactor seal failure due to excess pressure generation within reactor.

Table 2. Maximum achieved equilibrium temperature (T_{max}) derived from temperature profiles of various solvents heated to different reactor heater temperature setpoints (T_{set}). For MeCN, H₂O, DMSO, DMF, and DCE, the data are presented as mean ± standard deviation (*n* = 3). For the remaining solvents, each T_{max} is determined from a single temperature profile (*n* = 1).

Solvent	$T_{\max} = m \times T_{\text{set}} + b$					Range of $\Delta T_{\max}$ for T_{set} of 40 – 180°C (°C)	$\Delta T_{\max}/T_{\max}$ @ $T_{\text{set}} = 180^\circ\text{C}$ (%)
	m	Δm^a	b	Δb^a	R^2		
MeCN	0.93	0.02	3.0	1.9	0.9988	2 – 3	2%
H ₂ O	0.88	0.01	7.4	1.3	0.9993	1 – 2	1%
DMSO	0.91	0.01	4.1	1.2	0.9995	1 – 2	1%
DMF	0.95	0.01	2.5	1.5	0.9993	2 – 3	2%
DCE	0.94	0.01	3.9	1.6	0.9992	2 – 3	2%
CHCl ₃	0.95	0.02	2.9	2.0	0.9998	2 – 4	2%
DCM	0.92	0.05	4.6	4.4	0.9993	5 – 9	5%
MeOH	0.92	0.02	5.0	2.4	0.9998	3 – 5	3%
Toluene	0.94	0.03	4.0	3.9	0.9991	4 – 7	4%

^a Δm and Δb represent the $\pm$ 95% confidence interval (CI₉₅) for the slope and intercept of the equation, respectively.

Table 3. Linear calibration equations relating $T_{\max}$ to T_{set} for each solvent, $T_{\max} = m \times T_{\text{set}} + b$. Goodness of fit and confidence intervals from the regression are also listed. To determine the uncertainty $\Delta T_{\max}$ ($\pm$ 95% confidence interval) in $T_{\max}$, the following equation was used: $\Delta T_{\max} = \sqrt{(\Delta m \times T_{\text{set}})^2 + \Delta b^2}$. This value grows for increasing T_{set} , and we show the range of $\Delta T_{\max}$ across the whole interval from 40–180°C. We also show the maximum relative uncertainty $\Delta T_{\max}/T_{\max}$ in this range, which occurs at $T_{\text{set}} = 180^\circ\text{C}$.

T_{set} (°C)	Measured $T_{\max}$ (°C)			
	MeCN		DMSO	
	Solvent only	Reaction solution	Solvent only	Reaction solution
110	--	--	108 $\pm$ 1	108 $\pm$ 1 (mock [¹⁸ F]SFB)
130	127 $\pm$ 1	126 $\pm$ 1 (mock [¹⁸ F]FDG)	--	--
160	155 $\pm$ 1	153 $\pm$ 2 (mock D-[¹⁸ F]FAC)	--	--

Table 4. Effect of solutes on $T_{\max}$ values. $T_{\max}$ values were derived from temperature profiles of the solvent alone versus mock reaction solutions based on the same solvent. Values are reported as mean $\pm$ standard deviation ($n = 3$).

T _{set} (°C)	Heater ramp time to reach T _{set} (min)	t _{heat} (min)								
		MeCN	H ₂ O	DMSO	DMF	DCE	CHCl ₃	DCM	MeOH	Toluene
40	0.13 ± 0.03 (n=19)	1.0 ± 0.1	1.2 ± 0.2	1.6 ± 0.3	1.4 ± 0.1	1.1 ± 0.1	0.9	0.8	0.8	0.9
60	0.26 ± 0.02 (n=19)	1.3 ± 0.3	1.4 ± 0.2	1.8 ± 0.1	1.6 ± 0.2	1.3 ± 0.1	1.4	1.2	1.1	1.2
80	0.39 ± 0.02 (n=19)	1.3 ± 0.1	1.6 ± 0.3	1.7 ± 0.0	1.6 ± 0.1	1.4 ± 0.0	1.1	1.4	1.3	1.3
120	0.69 ± 0.03 (n=19)	1.6 ± 0.1	1.8 ± 0.1	2.1 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.4	1.6	1.5	1.4
140	0.90 ± 0.03 (n=19)	1.7 ± 0.0	1.9 ± 0.3	2.1 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.6	2.0	1.8	1.5
160	1.1 ± 0.0 (n=17)	1.9 ± 0.1	2.2 ± 0.1	2.3 ± 0.2	2.0 ± 0.1	1.9 ± 0.2	1.7	a	a	1.6
180	1.3 ± 0.1 (n=16)	2.0 ± 0.1	2.3 ± 0.4	2.4 ± 0.2	2.3 ± 0.1	2.1 ± 0.1	a	a	a	1.9

^aAccurate values could not be determined due to significant loss of volume during data collection.

Table 5. Heating times (t_{heat}) derived from temperature profiles of solvents at different reactor heater temperature setpoints (T_{set}). For MeCN, H₂O, DMSO, DMF, and DCE, the data are presented as mean ± standard deviation (n = 3). For the remaining solvents, the data is from a single temperature profile at each T_{set} (n = 1). For reference, the time necessary for the reactor heater to ramp up to T_{set} is also listed as the mean across all solvents ± standard deviation. (This time is included in the t_{heat} values.)

T _{set} (°C)	t _{cool-35} (min)								
	MeCN	H ₂ O	DMSO	DMF	DCE	CHCl ₃	DCM	MeOH	Toluene
40	0.62 ± 0.04	0.68 ± 0.06	0.81 ± 0.09	0.58 ± 0.04	0.63 ± 0.00	0.64	0.49	0.58	0.58
60	1.3 ± 0.1	1.3 ± 0.2	1.5 ± 0.1	1.3 ± 0.2	1.3 ± 0.1	1.4	1.1	0.99	1.1
80	1.8 ± 0.0	1.9 ± 0.1	1.9 ± 0.1	1.6 ± 0.0	1.6 ± 0.0	1.3	1.7	1.4	1.4
120	2.5 ± 0.4	2.3 ± 0.2	2.9 ± 0.5	1.9 ± 0.4	1.9 ± 0.3	1.9	1.6	1.5	1.8
140	2.8 ± 0.3	2.6 ± 0.5	2.9 ± 0.2	2.6 ± 0.2	2.3 ± 0.5	2.0	2.1	1.8	1.8
160	2.8 ± 0.1	2.8 ± 0.3	3.1 ± 0.3	2.4 ± 0.5	2.3 ± 0.8	1.8	a	a	2.2
180	3.2 ± 0.3	3.2 ± 0.3	3.5 ± 0.3	3.0 ± 0.1	2.4 ± 0.1	a	a	a	2.5

^aAccurate values could not be calculated due to significant loss of volume during data collection.

Table 6. Cooling times to 35°C (t_{cool-35}) after 30 min heating derived from temperature profiles of various solvents and reactor heater temperature setpoints (T_{set}). For MeCN, H₂O, DMSO, DMF, and DCE, the data are presented as mean ± standard deviation (n = 3). For the remaining solvents, the data is from a single temperature profile at each T_{set} (n = 1).

T _{set} (°C)	t _{cool-bp} (min)								
	MeCN	H ₂ O	DMSO	DMF	DCE	CHCl ₃	DCM	MeOH	Toluene
40	--	--	--	--	--	--	--	--	--
60	--	--	--	--	--	--	0.85	--	--
80	--	--	--	--	--	0.49	1.3	0.46	--
120	0.59 ± 0.11	0.37 ± 0.02	--	--	0.58 ± 0.03	0.81	1.4	0.71	0.27
140	0.77 ± 0.06	0.51 ± 0.02	--	--	0.72 ± 0.07	0.92	1.7	0.82	0.36
160	0.86 ± 0.05	0.63 ± 0.02	--	0.18 ± 0.03	0.73 ± 0.20	0.93	a	a	0.49
180	1.1 ± 0.1	0.75 ± 0.03	--	0.34 ± 0.02	0.90 ± 0.02	a	a	a	0.60

^aAccurate values could not be calculated due to significant loss of volume during data collection.

Table 7. Cooling times to the normal boiling points (t_{cool-bp}) after 30 min heating derived from temperature profiles of various solvents and reactor heater temperature setpoints (T_{set}). For MeCN, H₂O, DMSO, DMF, and DCE, the data are presented as mean ± standard deviation (*n* = 3). For the remaining solvents, the data is from a single temperature profile at each T_{set} (*n* = 1).

Figure 1. Schematic of sealed reactor during temperature profile data collection. The top of the reaction vessel is pushed up against a PTFE-coated silicone rubber gasket through which a thermocouple extends. The thermocouple is held in place by a threaded fluidic fitting installed on the cassette and is positioned in the solvent such that the temperature sensitive tip is fully submerged but not touching the reaction vessel.

Figure 2. Example of T_{heater} and T_{liquid} profiles over time for MeCN heated to a setpoint of 160°C for 30 min. Heating began with T_{heater} and T_{liquid} at room temperature. Once the reactor heater temperature reached the setpoint, it was maintained for 30 min (t_{reaction}). Subsequently, the heater was deactivated and cooling system activated to cool the vessel until the contents reached 35°C . Definitions of relevant temperature and time parameters are depicted for the (A) T_{heater} and (B) T_{liquid} profiles.

Figure 3. Liquid temperature (T_{liquid}) profiles over time for 1.0 mL of MeCN for various reactor heater temperature setpoints (T_{set}).

Figure 4. Comparison of the T_{liquid} profile for 1.0 mL of mock [^{18}F]FDG reaction solution and 1.0 mL of pure MeCN for a heating time of 3 min at $T_{\text{set}} = 130^{\circ}\text{C}$. The profile clearly shows that T_{liquid} was being ramped during the entire duration of heating and never reached equilibrium. (The horizontal line at $T = 126^{\circ}\text{C}$ represents the average T_{max} achieved for 30 min heating.)

Figure 5. Theoretical vapor pressure generated inside a sealed reactor as a function of liquid temperature for different solvents. Solenoid valves commonly used in automated synthesizers typically withstand pressures up to 30, 50, or 100 psi before failing. ELIXYS maintains ~ 200 psi of pressure within the reactor before solvent is lost to vapor leakage. Liquid temperatures at which sealing failure occurs in ELIXYS for the highly volatile solvents DCM, MeOH, and CHCl_3 are displayed with their corresponding vapor pressures. In other systems, failures would be observed at lower temperatures and for a greater number of the solvents.

Figure 6. Proposed procedure for translating the heating steps of a synthesis protocol from synthesizer A to synthesizer B. The translation requires that a thermal characterization (as presented in this paper for ELIXYS) is available for both synthesizers. First, parameters relating to heating steps are identified in the protocol for synthesizer A. Next, using the thermal characterization for synthesizer A (for the appropriate setpoint, solvent, and solvent volume), the parameters are converted to “universal” values. The “universal” reaction time is the reported reaction time minus any included ramping time (e.g., $t_{\text{universal}} = t_{\text{reaction}}^{\text{A}} - t_{\text{heat}}^{\text{A}} + (\text{time for heater to reach } T_{\text{set}}^{\text{A}})$). The “universal” reaction temperature is the actual liquid temperature at equilibrium (i.e., $T_{\text{universal}} = T_{\text{max}}^{\text{A}}$, obtained by converting the reported $T_{\text{set}}^{\text{A}}$ value). Finally, using the thermal characterization for synthesizer B (for the appropriate setpoint, solvent, and solvent volume), the “universal” parameters are converted to parameters for synthesizer B. The new reaction setpoint is obtained by recognizing that $T_{\text{max}}^{\text{B}} = T_{\text{universal}}$ and converting to the corresponding setpoint $T_{\text{set}}^{\text{B}}$. The new reaction time is the “universal” value plus any ramping time that is included in the heating control mechanism of synthesizer B (e.g., $t_{\text{reaction}}^{\text{B}} = t_{\text{reaction}}^{\text{universal}} + t_{\text{heat}}^{\text{B}} - (\text{time for heater to reach } T_{\text{set}}^{\text{B}})$). Finally, the cooling time is selected as the time needed to reach the desired endpoint (i.e. $t_{\text{cool-35}}^{\text{B}}$ or $t_{\text{cool-bp}}^{\text{B}}$); it is unrelated to the cooling time used for synthesizer A.