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• The NanoTek Microfluidic Synthesis System is a modular microfluidic chemistry system with the ability to combine both microscale and macroscale process steps. Modular components give the user maximum flexibility for both discovery and clinical applications.

Application Note

Determining the Heating Profile and Improved Reliability Using a Vacuum System with the NanoTek® LF Concentrator 2 and a 5-ml Reaction Vial for Vial-based FLT production

Introduction

In this application note, we evaluate heating solutions using a 5-ml reaction vial and incorporating a vacuum system for the drying of fluoride to optimize the production of FLT.

Equipment

NanoTek LF System
Version 1.4.0 Control Software

Karl Fischer Coulometer System:
Mettler Toledo DL 32

RadioTLC System:
Bioscan AR-2000

Reagents
3-N-Boc-5′-O-dimethoxytrityl-3′-O-nosylthymidine (ABX)
Acetonitrile, Anhydrous (Acros)
Water, HPLC Grade
FLT Standard (Sigma)

Results and Discussion

The concentrator is available with two reactor vial sizes, 3 ml and 5 ml as well as a silicone and PEEK cap.

A. 5-ml vial, silicone cap, and concentrator 2
The standard program method used with the 3-ml vial can result in incomplete drying of the fluoride prior to the addition of the precursor solution. A possible solution to this is to use the 5-ml vial, but this requires determination of the vial's heating profile as it has a larger mass of glass.

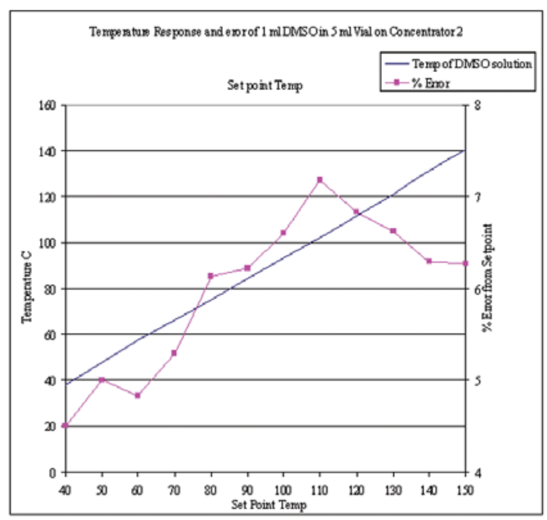
B. Temperature response of 1-ml DMSO solution in 5-ml vial
To a 5-ml vial was added 1 ml of DMSO as a standard volume. A thermometer was inserted into the vial and clamped to prevent its touching the inner vial walls while measurements were being taken. Using the digital love control unit, the temperature was raised in 10 °C increments starting at 40 °C until 150 °C was reached, allowing each step to equilibrate for three minutes prior to recording the actual solvent temperature.

The results indicate that the system possesses a lag time to reach the next higher temperature set point and the greatest % error in temperature was at 110 °C, but the largest magnitude error was at 150 °C. See Table 1 and Figure 1 below.

Table 1. Temperature Curve for 1-ml DMSO in 5-ml Vial

Set Temp	Temp of DMSO solution	% Error
40	38.2	4.50
50	47.5	5.00
60	57.1	4.83
70	66.3	5.29
80	75.1	6.13
90	84.4	6.22
100	93.4	6.60
110	102.1	7.18
120	111.8	6.83
130	121.4	6.62
140	131.2	6.29
150	140.6	6.27

Figure 1. Temperature Response of 5-ml Concentrator

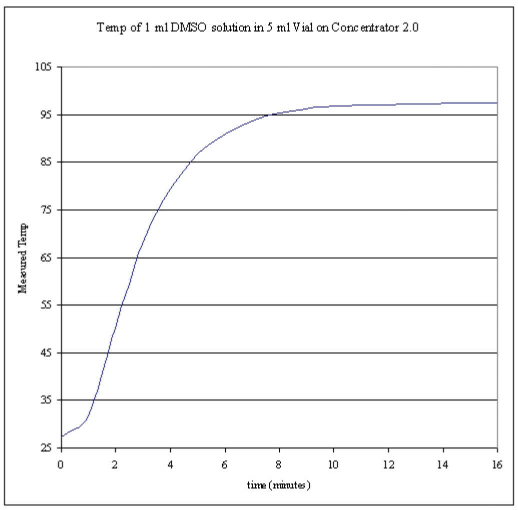


The next test was performed to determine the time for the 5-ml vial to reach a temperature close to the desired 100 °C. The 100 °C was chosen as the set point and temperature measurements were made at 1-minute intervals for 10 minutes then a final measurement was made at 16 minutes. Experiment results are shown in Table 2 and Figure 2.

Table 2. Temperature Profile for 5-ml Vial

Time (min)	Temp of DMSO solution
0	27.5
1	31.8
2	50.2
3	68
4	79.6
5	86.7
6	91
7	93.7
8	95.3
9	96.2
10	96.8
16	97.5

Figure 2. Time Versus Temperature Profile for 5-ml Vial Concentrator



The results of this experiment indicate that ~4.5 minutes are required for the DMSO solution to reach 85 °C.

A time delay of 4 minutes should therefore be incorporated into the drying macro to allow the vial to reach a temperature above the azeotrope temperature of the acetonitrile–water solution prior to initiating elution of the fluoride.

C. Fluoride drying simulations to verify the benefit of incorporating a time delay and adding a vacuum system.

The fluoride drying and precursor addition steps used for the vial-based production of FLT were simulated as follows: 1 mL of DI water replaced the [F-18]fluoride solution. The kryptofix/potassium carbonate solution was replaced with 10% water in acetonitrile (0.45 ml) and the precursor in acetonitrile was replaced with just acetonitrile (0.5 ml).

The results for the determination of residual water in the final precursor solution using the standard drying sequence with adjusted time delay but without Vacuum are given in Table 3 below. The results of the mock drying experiments indicated that the water content while low is quite variable.

Table 3. Mock FLT Fluorinations using Standard Drying macro on 5 ml Vial with Silicon Cap

Run	ppm Water
1	304.57
2	217.81
3	223.68
Avg± %Std	248.69 ±39.6%

In addition to improving the run-to-run reproducibility of tracer production, reducing the water content will also result in less precursor being sacrificed to water in the fluoride solution. Addition of vacuum drying was then evaluated using the same simulations as in the previous experiment but with the addition of the vacuum drying macros. The results for the determination of the water in the final precursor solution using the standard drying sequence are given in Table 4 below.

Table 4 Mock FLT Fluorinations Using Vacuum Drying System on 5-ml Vial With Silicon Cap

Run	ppm Water
1	138.09
2	129.81
3	168.31
Avg± %Std	145.40 ±16.5%

The results of the addition of the vacuum drying to the system not only reduced the water content in the final precursor solution by almost half but also significantly decreased water content variability.

D. Test of the incorporation of fluoride into the FLT precursor with bacuum drying with silicon cap.

Using the addition of the vacuum drying to the 5-ml vial system, two test reactions were made using 20 mg of precursor/reaction. The % incorporation of fluoride was determined using radioTLC on silica plates with 90% acetonitrile/water as the mobile phase. The results of the testing are given in Table 5. The previous studies of fluoride incorporation into the FLT precursor using the 3-ml vial are included for reference.

Table 5. % Incorporation of Fluoride Into FLT Precursor with Silcone Cap

Reaction Number	%RCY by TLC	Vial Size
1	43.71	3
2	52.21	3
4	40.34	3
5	53.62	3
6	44.79	3
7	**58.33**	**5**
8	**70.61**	**5**
Average	51.94	
Std. Dev	9.60	

5-ml vial, PEEK cap, and Concentrator 2

E. Fluoride drying simulations using fluoride drying with added time delay and PEEK Cap on 5-ml vial

The fluoride drying and precursor additions were simulated using the same conditions as outlined for the silicone cap. The results for the determination of the water in the final precursor solution using the standard drying sequence are given in Table 6 below.

Table 6. Mock FLT Fluorinations Using Standard Drying Macro on 5-ml Vial With PEEK Cap

Run	ppm Water
1	678.73
2	836.01
3	392.74
Avg ± Std	635.83 ± 183.5

The results of the mock drying experiments indicated that the water content while low is quite variable. The addition of the vacuum drying system was then tested using the same simulations, with the addition of the vacuum drying macros. The results for the determination of the water in the final precursor solution using the standard drying sequence are given in Table 7 below.

Table 7. Mock FLT Fluorinations Using Vacuum Drying System on 5-ml Vial With PEEK Cap

Run	ppm Water
1	240.96
2	200.66
3	279.5
Avg± %Std	240.37 ± 13.4

The results of the addition of the vacuum drying to the system gave less than half the water content in the final precursor solution and a significant decrease in the variability of the water content.

F. Test of the incorporation of fluoride into the FLT varying precursor concentration.

Three test reactions were made using 20 mg of precursor/reaction and two reactions were performed using 10 mg/reaction and the % incorporation was determined using radioTLC in silica plates with 90% acetonitrile/water as the mobile phase. The results of the testing are given in Table 8.

Table 8. % Incorporation of Fluoride Into FLT Precursor Using 5-ml Vial with PEEK Cap

Reaction Number	%RCY by TLC	Precursor mg/ run
1	31.19	20
2	40.29	20
3	31.99	20
4	35.8	10
5	48.01	10
Average	37.46	
Std. Dev	6.2	

G. Summary of results for the 5-ml vial with the silicone and PEEK Caps

a) The 5-ml vial does not seem to have adversely impacted radiosynthesis of the model radiotracer FLT.
b) There is the need to add an additional time delay to allow the 5-ml vial to reach a temperature to ensure efficient removal of water. This same program can be used with both the 3- and 5-ml vials.
c) The use of nitrogen gas alone can reduce the water content of the fluoride to levels which can be used for radiosynthesis. However, the use of nitrogen gas alone leads to high variability in the final water content.
d) The use of vacuum further reduces both the water content and the variability of residual water in the final fluoride solution.

Macros Used in these Tests:

For the 3-ml vial

3-ml vial program
Fast_drying_c
Vac_Pump_Test
Vac_Pump2
Precursor_Add FLT

For the 5-ml vial

5-ml vial program
FLT_Fluoride_Drying
Vac_Pump_Test
Vac_Pump2
Precursor_Add FLT