Centro Nacional de Pesquisa em Energia e Materiais
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Laboratory and Data Analysis Procedures for Determination of Biomass Nanoscale Porosity Employing Calorimetric Thermoporometry

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Abstract

This Technical Memorandum describes in detail the laboratory and data-analysis procedures of calorimetric thermoporometry. The method has been employed in CTBE/CNPEM for routine characterization of biomass nanoscale porosity in aqueous environments.

Keywords: thermoporometry; cellulose; biomass; porosity; accessibility.

Resumo

Este Memorando Técnico descreve em detalhe os procedimentos laboratoriais e de análise de dados para realização de termoporometria calorimétrica. O método tem sido empregado no CTBE/CNPEM para caracterizações rotineiras da porosidade nanométrica de biomassa em ambientes aquosos.

Palavras-chave: termoporometria; celulose; biomassa; porosidade; acessibilidade.

Summary

1. Introduction

Lignocellulosic biomass is naturally nanostructured and nanoscale pores are an integral part of the biomass architecture. This is true for raw biomass as well as for wide range of processed biomass and derived cellulosic materials. In aqueous environments such pores are filled with water. In addition, water causes matrix swelling that enhances porosity. For this reason, biomass nanoscale porosity in aqueous environment is typically much greater and not comparable to the porosity of the same biomass in a dry state. Noteworthy, for most biological conditions and aqueous biomass processing, it is the wet nanoscale porosity that matters. Wet nanoscale porosity is critical, for instance, to infer enzyme accessibility, which requires biomass pores larger than the few nanometers size of the enzymes.

Calorimetric thermoporometry is an interesting technique to determine biomass nanoscale porosity in aqueous environments. Its workhorse instrument is a benchtop differential scanning calorimeter (DSC) coupled to a cooling module. Several research groups have been employing thermoporometry to probe nanoscale pores of cellulosic biomass (Maloney, T. C., Paulapuro, H., 1999; Luukkonen, P., Maloney, T., Rantanen, J., Paulapuro, H., Yliruusi, J., 2001; Fahlén, J., Salmén, L., 2005; Park, S., Venditti, R. A., Jameel, H., Pawlak, J.J., 2006; Pihlajaniemi, V., Sipponen, M. H., Liimatainen, H., Sirviö, J. A., Nyyssölä, A., Laakso, S., 2016). In CTBE/CNPEM we initially worked to improve the analytical method (Driemeier, C., Mendes, F. M., Oliveira, M. M., 2012) and to automate several laboratory and data analysis procedures. This allowed us to intensify the use of thermoporometry (Driemeier, C., Mendes, F. M., Oliveira, M. M., 2012; Driemeier, C., Oliveira, M. M., Curvelo, A. A., 2016) and to actually bring the technique to a new level.

Here, we describe in detail our laboratory and data analysis procedures of calorimetric thermoporometry. These procedures have been successfully employed in over a thousand samples during the five years in which thermoporometry has been used as routine analysis in CTBE/CNPEM. This Technical Memorandum complements the analytical developments previously published by our research group (Driemeier, C., Mendes, F. M., Oliveira, M. M.,

2012; Driemeier, C., Oliveira, M. M., Curvelo, A. A., 2016). With the detailed descriptions given here, we expect to promote further understanding, usage and adoption of calorimetric thermoporometry.

2. Laboratory procedures

2.1 Instrument

Thermoporometry has been performed using a DSC model Q200 from TA Instruments. The DSC is coupled to a RCS90 cooling unit and an autosampler (Figure 1). Although all the experience of CTBE/CNPEM has been based on this instrument, we expect similar instruments should perform comparably. Noteworthy, our experience has shown that aging of the instrument may be critical for its performance in thermoporometry.

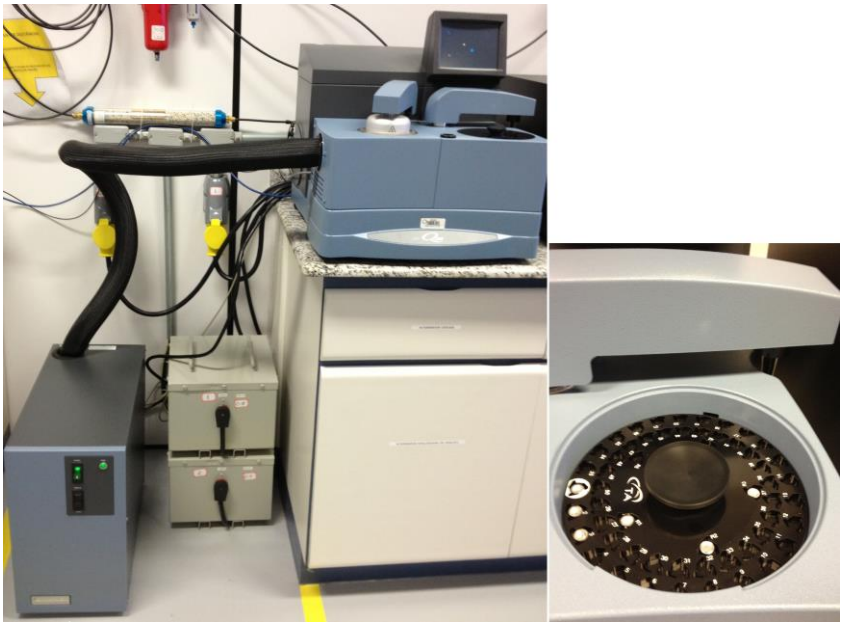
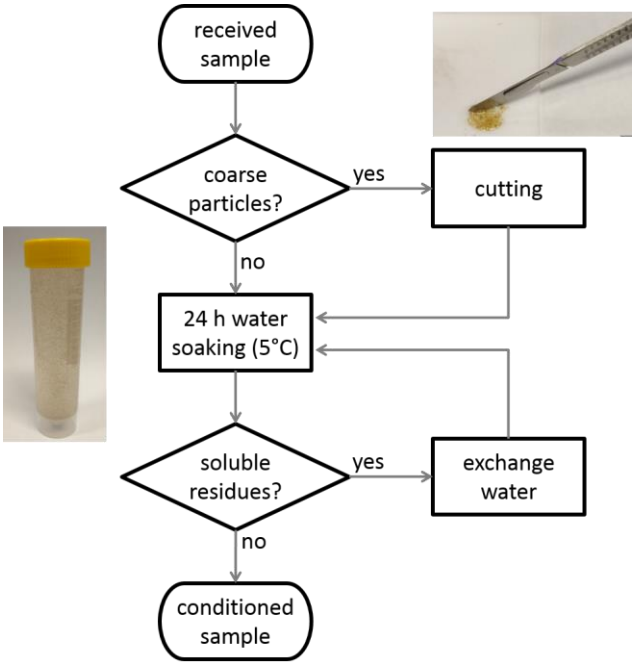


Figure 1: Differential Scanning Calorimeter Q200 with RCS90 cooling unit (left) and detail of the autosampler (right).

2.2 Sample conditioning

Samples are conditioned to satisfy three criteria: (i) a representative aliquot must fit into the DSC pan; (ii) the sample must be free of water-soluble compounds that may bias ice melting temperature; and (iii) the sample must be saturated with water.

To satisfy criterion (i), samples need to have particle sizes below ~1 mm. If samples are coarser, millimeter-sized representative particles are manually cut using a blade. Mechanical grinding and milling is usually not recommended because it may alter the porosity that will be measured. To satisfy criterion (ii), samples are soaked in a dilute aqueous suspension (~100-300 mg of solid in 50 mL of deionized water) during 24 h. This step aims at diluting any water-soluble compound present in the solid matrix. Water is exchanged and the soaking step is repeated if there is any evidence of residual water-soluble compounds. Samples are kept refrigerated (~5°C) during water soaking. After that, samples are saturated with water and therefore criterion (iii) is also satisfied. Flowchart 1 summarizes sample conditioning.



Flowchart 1: Sample conditioning.

2.3 Sample preparation in the DSC pans

Wet solid aliquots are collected from the refrigerated suspensions and left on a clean glass surface until they thermalize with the environment. Then, the aliquot is inserted into an aluminun Tzero™ pan (P/N: 901683.901) later covered with a Tzero™ hermetic lid (P/N: 901684.901) (Figure 2).

Figure 2: DSC pan (left) and hermetic lid (right).

The weights of the pan, lid, and sample are measured five times as part of the laboratory procedure, as will be detailed later. These measurements are performed in an analytical balance with readability of 0.01 mg. The mass of sample aliquot M_{spl} inserted into the pan shall weight preferentially 10-20 mg. M_{spl} is the sum of solid mass and water mass ($M_{spl} = M_{dry} + M_{water}$), with M_{dry}:M_{water} ratio preferentially between 1:3 and 1:5. The total weight is verified in the balance as the pan is prepared. The M_{dry}:M_{water} ratio is determined later in the analytical procedure, but with practice the analyst develops enough sensitivity to achieve the desired range. After inserting the aliquot into the pan, the pan is sealed with the hermetic lid, using the Tzero™ Sample Press Kit (P/N: 901600.901) with the accessory for hermetic lid (P/N: 901608.904) (Figure 3). Flowchart 2 summarizes the preparation of sample pan.

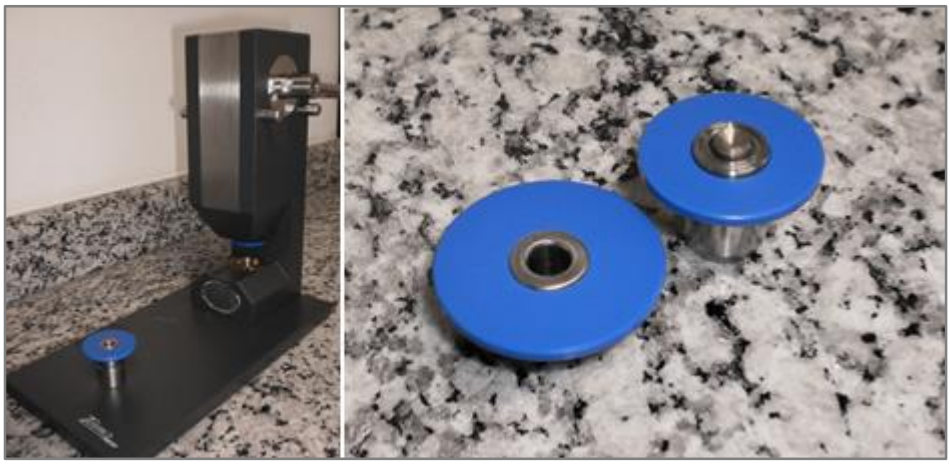
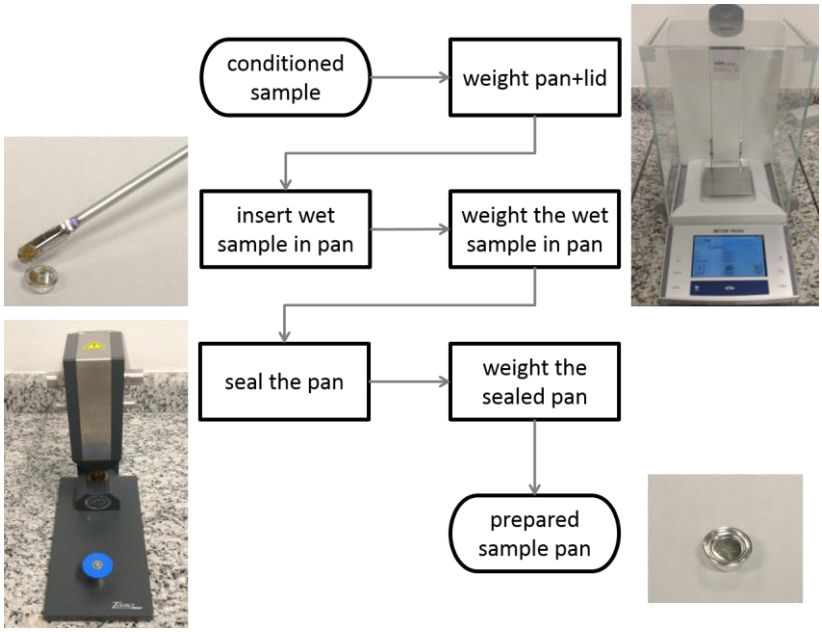


Figure 3: Press kit with accessory for hermetic lid.



Flowchart 2: Preparation of sample pan.

2.4 Procedures in the DSC instrument

The DSC instrument is calibrated following manufacturer recommendations. A sapphire standard (P/N: 970345.901) is used for baseline calibration. Indium metal is used for temperature and cell constant calibrations. The complete calibration is performed once a month or earlier if necessary. Indium melting is repeated in a daily basis to verify temperature calibration (tolerance ±0.05°C). The reference and sample pans are inserted in the DSC cell (Figure 4) using the robotized arm of the autosampler.

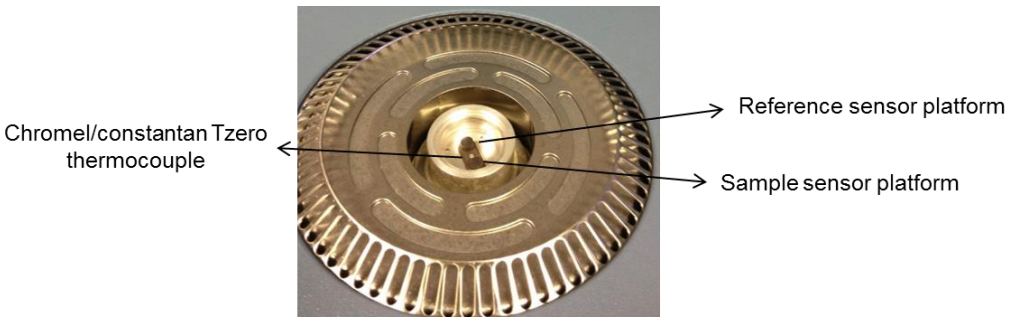


Figure 4: DSC cell.

The initial DSC instrument setting has the following values: set point temperature of 40.00°C, flange temperature between -90.00 and -80.00 °C, and sample purge flow of 50.00 mL/min. A thermoporometry run in the DSC uses a heating sequence from -70°C to 0.5 °C. The sequence comprises a set of temperature ramps, each ramp followed by an equilibration isotherm (see Appendix). The instrument program and an example of measured thermogram are shown in Figure 5.

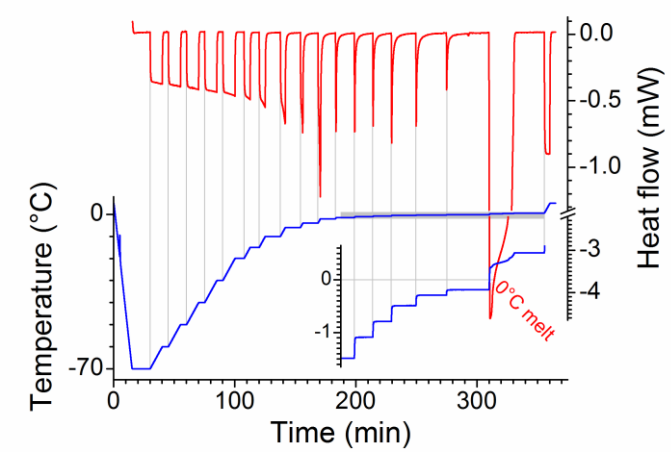
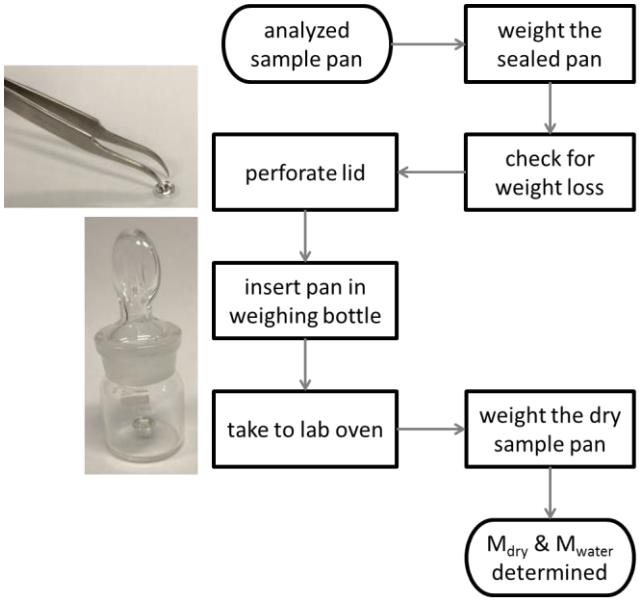


Figure 5: DSC temperature program (blue) and example of measured heat flow (red). Reproduced from Driemeier et al., 2016.

2.5 Determination of M_{dry} and M_{water}

After the DSC run, the sealed set (pan + lid + enclosed sample) is weighted again to verify possible mass loss during the DSC run (tolerance ±0.05 mg). The lid is then gently perforated with a tweezer and the set is inserted in a weighing bottle and transferred to a laboratory oven at 105°C for 90 minutes. The set is cooled down for 30 minutes in a desiccator and then weighted again for determination of the mass loss during drying, which corresponds to the water mass M_{water} present in the analyzed sample pan. Concomitantly, sample dry mass M_{dry} is determined by subtracting M_{water} from measured sample wet mass M_{spl} ($M_{dry} = M_{spl} - M_{water}$). Flowchart 3 summarizes the procedures for determination of M_{dry} and M_{water} in the DSC pan.



Flowchart 3: Determination of sample dry mass (M_{dry}) and water mass (M_{water}).

2.6 Complementary data and analyses schedule

Two additional parameters are required for data analysis. One is the temperature derivative of the specific heat capacity of the non-transforming sample component, dc_{nt}/dT (Driemeier, C., Mendes, F. M., Oliveira, M. M., 2012). The non-transforming sample component includes the solid and the non-freezing water. This parameter was measured in several samples of cellulosic biomass containing 10-20% water. The value $dc_{nt}/dT = 0.0065\ J\,g^{-1}\,K^{-2}$ is considered a good approximation for practical purposes and it is systematically used in our analysis.

The other parameter is the corrective factor κ (kappa) applied to measured heats (Driemeier, C., Mendes, F. M., Oliveira, M. M., 2012). In the typical schedule of thermoporometry analyses, the instrument is calibrated (see section 2.4) and a set of samples is analyzed within the one-month validity of the calibration. Samples of pure deionized water (~5 samples) are included in the sample set. κ is determined from these water samples by requiring that the specific enthalpy of ice melting reproduces reference value (334 J/g). Hence, κ is a correction to the instrument cell constant determined in the indium melting calibration described in section 2.4. This correction improves the method

accuracy because the reference to indium melting is replaced by an internal reference to ice melting.

2.7 Recorded analysis components

An overview of the acquired data is given in Table 1, including measured and calculated parameters as well as annotations. Analysts shall record these components to have good traceability of the thermoporometry results.

Table 1: Analysis components recorded by the analyst

Component	Format	Units	Origin	Comments
Preparation	text	-	annotation	description
# Pan+Lid	numeric	-	annotation	id of pan-lid set
Mass: Pan+Lid empty	numeric	mg	measured	see flowchart 2
Mass: Wet Sample (M_{spl})	numeric	mg	measured	see flowchart 2
Mass: Pan+Lid+Sample	numeric	mg	measured	see flowchart 2
# Pan+Lid(end)	numeric		annotation	id of weighing bottle
Mass: Pan+Lid+Sample(end)	numeric	mg	measured	see flowchart 3
$M_{difference}$	numeric	mg	calculated	check weight loss (see flowchart 3; tolerance ±0.05 mg)
Mass: Pan+Lid+DrySample	numeric	mg	measured	see flowchart 3
M_{dry}	numeric	mg	calculated	see flowchart 3
M_{water}	numeric	mg	calculated	see flowchart 3
# Calibration Water	numeric	-	annotation	id of water calibration set
kappa	numeric	-	measured	cell constant correction (from water calibration set)
dc_{nt}/dT	numeric	$Jg^{-1}K^{-2}$	measured	0.0065 as default
Result file	text	-	annotated	file name of raw thermogram and analyzed profile

3. Data analysis

3.1 Calculation and notation

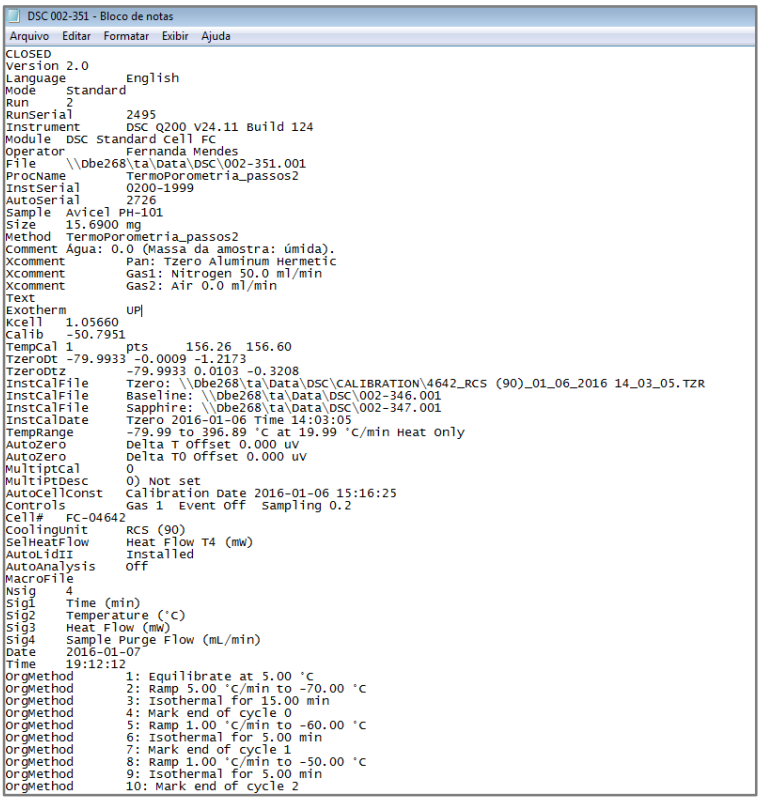
Data analysis is executed by a macro coded in Microsoft Excel. This macro file will be made available on request to the authors. The calculations run by the macro are detailed in Driemeier et al. (2012) and additional details can be learned by inspection of the macro file. Variables used in the calculations are summarized in Table 2. Notations used in Driemeier et al. (2012) and in the macro are indicated.

Table 2: Variables used in the thermoporometry data analysis

Variable description	Units	Notation	
		Driemeier et al. 2012	*MS Excel Macro*
Mean temperature	°C	$\overline{T}$	T_{mean}
Raw measured heat	mJ	Q'_i	Q_{raw}
Corrected measured heat: $Q_i = \kappa Q'_i$	mJ	Q_i	Q_{corr}
Specific heat capacity of ice	$J\,g^{-1}K^{-1}$	c_i^{ice}	c_{ice}
Specific enthalpy of ice melting	$J\,g^{-1}$	q_i	q_{ice}
Sample heat capacity	$J\,K^{-1}$	C_i	C_{spl}
Ice mass	mg	M_i	M_{ice}
Pore diameter	nm	x	Diameter
Freezing bound water	$g\,g^{-1}$	FBW	FBW
Pore area	$m^2\,g^{-1}$	A	Area
Non-freezing bound water	$g\,g^{-1}$	NFW	NFW
Corrective factor of cell constant		κ	kappa
Temperature derivative of the specific heat capacity of the sample non-transforming mass	$J\,g^{-1}\,K^{-2}$	dc^{nt}/dT	dc_{nt}/dT
Sample dry mass	mg	M^{dry}	M_{dry}
Sample water mass	mg	$M^{spl} - M^{dry}$	M_{water}
Non-transforming mass	mg	M^{nt}	M_{nt}

3.2 Macro inputs

The DSC instrument generates a .txt file with standardized format (Figure 6). This .txt file is an input of the macro (Figure 7). The macro also requires the input of M_{dry}, M_{water}, dc_{nt}/dT, and *kappa* (Figure 8).



DSC 002-351 - Bloco de notas

Arquivo Editar Formatar Exibir Ajuda

```
CLOSED
Version 2.0
Language        English
Mode    Standard
Run     2
RunSerial       2495
Instrument      DSC Q200 V24.11 Build 124
Module  DSC Standard Cell FC
Operator        Fernanda Mendes
File    \\Dbe268\ta\Data\DSC\002-351.001
ProcName        TermoPorometria_passos2
InstSerial      0200-1999
AutoSerial      2726
Sample  Avicel PH-101
Size    15.6900 mg
Method  TermoPorometria_passos2
Comment Água: 0.0 (Massa da amostra: úmida).
Xcomment        Pan: Tzero Aluminum Hermetic
Xcomment        Gas1: Nitrogen 50.0 ml/min
Xcomment        Gas2: Air 0.0 ml/min
Text
Exotherm        UP
Kcell   1.05660
Calib   -50.7951
TempCal 1       pts     156.26  156.60
TzeroDt -79.9933 -0.0009 -1.2173
TzeroDtz        -79.9933 0.0103 -0.3208
InstCalFile     Tzero: \\Dbe268\ta\Data\DSC\CALIBRATION\4642_RCS (90)_01_06_2016 14_03_05.TZR
InstCalFile     Baseline: \\Dbe268\ta\Data\DSC\002-346.001
InstCalFile     Sapphire: \\Dbe268\ta\Data\DSC\002-347.001
InstCalDate     Tzero 2016-01-06 Time 14:03:05
TempRange       -79.99 to 396.89 °C at 19.99 °C/min Heat Only
AutoZero        Delta T Offset 0.000 uV
AutoZero        Delta T0 Offset 0.000 uV
MultiptCal      0
MultiPtDesc     0) Not set
AutoCellConst   Calibration Date 2016-01-06 15:16:25
Controls        Gas 1  Event Off  Sampling 0.2
Cell#   FC-04642
CoolingUnit     RCS (90)
SelHeatFlow     Heat Flow T4 (mW)
AutoLidII       Installed
AutoAnalysis    Off
MacroFile
NSig    4
Sig1    Time (min)
Sig2    Temperature (°C)
Sig3    Heat Flow (mW)
Sig4    Sample Purge Flow (mL/min)
Date    2016-01-07
Time    19:12:12
OrgMethod       1: Equilibrate at 5.00 °C
OrgMethod       2: Ramp 5.00 °C/min to -70.00 °C
OrgMethod       3: Isothermal for 15.00 min
OrgMethod       4: Mark end of cycle 0
OrgMethod       5: Ramp 1.00 °C/min to -60.00 °C
OrgMethod       6: Isothermal for 5.00 min
OrgMethod       7: Mark end of cycle 1
OrgMethod       8: Ramp 1.00 °C/min to -50.00 °C
OrgMethod       9: Isothermal for 5.00 min
OrgMethod       10: Mark end of cycle 2
```

Figure 6: Example of DSC data in .txt file. Only part of the file is shown.

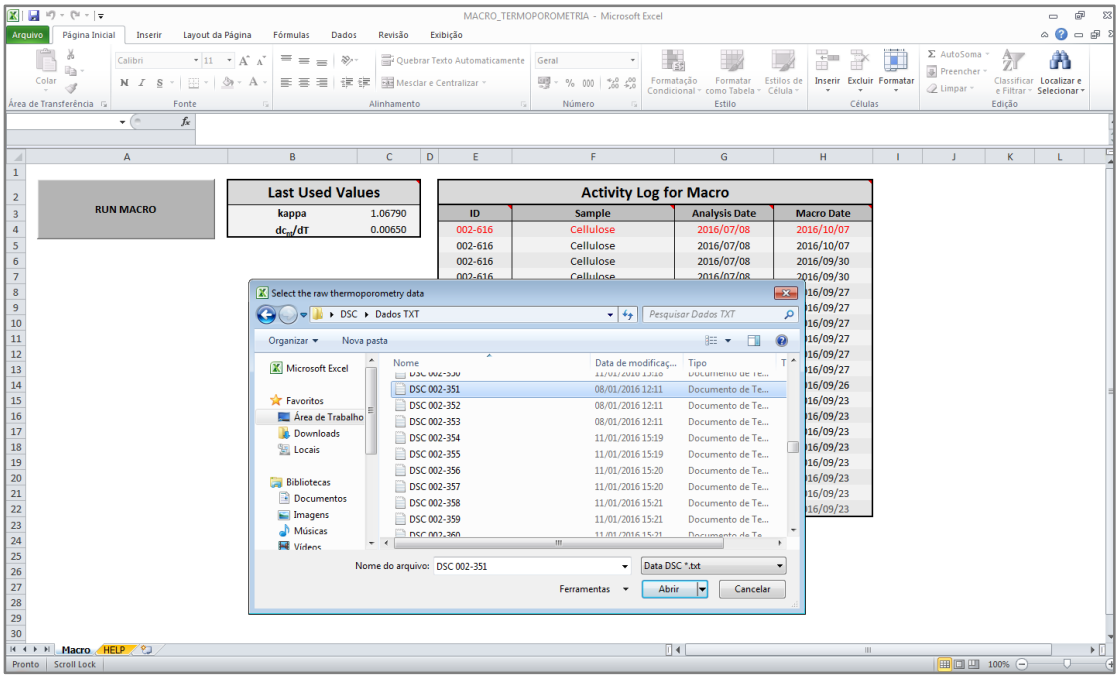


Figure 7: Macro screen for input of .txt file recorded by the DSC instrument.

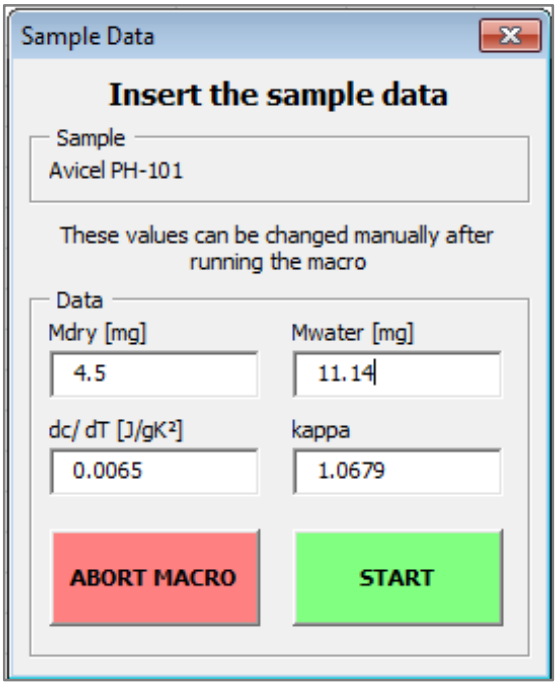


Figure 8: Macro screen for input of M_{dry}, M_{water}, dc_{nt}/dT, and kappa.

3.3 Heat flow baseline and determination of Q_{raw}

The first calculation of the macro is the determination of the heat flow baseline (Figure 9). Baseline is first determined automatically, taking the end of the equilibrating isotherms as reference points. After automatic baseline determination, the user has the option to manually edit the baseline (in column M: “Corr”). Time integration of the heat flow (referenced to baseline) yields the raw measured heat Q_{raw} (column N) for each of the programmed heating steps.

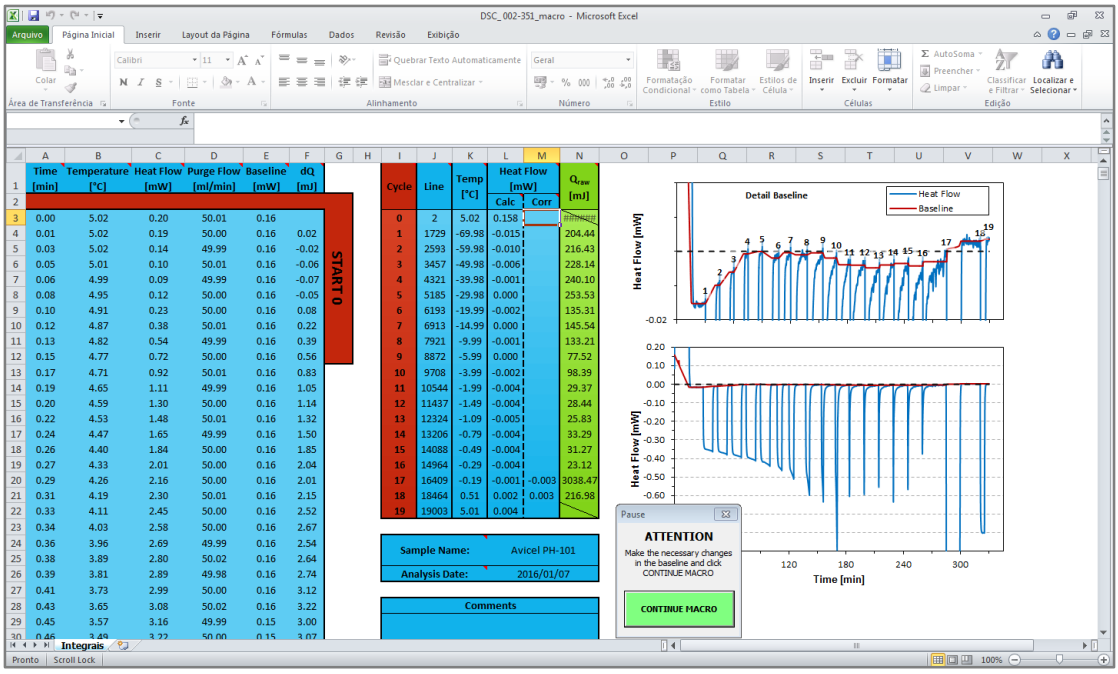
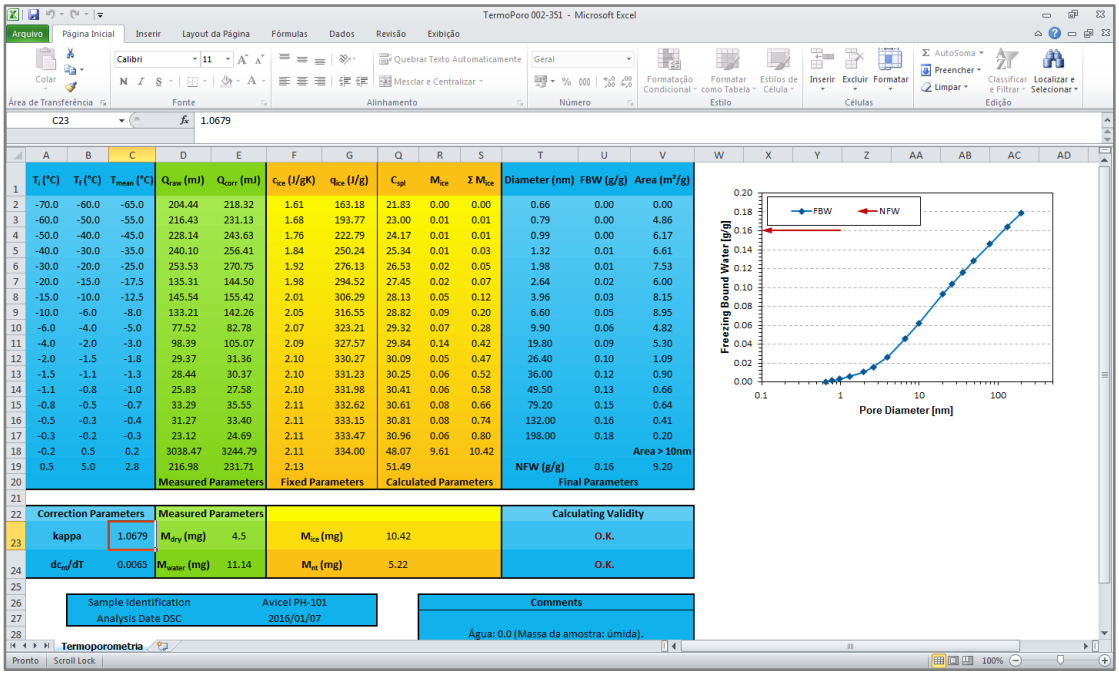


Figure 9: Macro screen showing determination of heat flow baseline (red line in graphs) and Q_{raw} (green column).

3.4 Determination of the FBW profile

The macro creates a new spreadsheet for the determination of the FBW profile (Figure 10). FBW is given in units of g water per g dry matter and is determined as function of pore diameter (1-200 nm). The spreadsheet receives Q_{raw} determined in the previous step, as well as the macro input parameters M_{dry}, M_{water}, dc_{nt}/dT, and *kappa*. In addition to the FBW profile, the spreadsheet calculates the specific area (in units of m^2/g) associated with the FBW profile. More specifically, the area of pores greater than 10 nm is calculated as an indicator of the surface area accessible to enzymes.



T_i (°C)	T_f (°C)	T_{mean} (°C)	Q_{raw} (mJ)	Q_{corr} (mJ)	c_{ice} (J/gK)	q_{ice} (J/g)	C_{spl}	M_{ice}	Σ M_{ice}	Diameter (nm)	FBW (g/g)	Area (m^2/g)
-70.0	-60.0	-65.0	204.44	218.32	1.61	163.18	21.83	0.00	0.00	0.66	0.00	0.00
-60.0	-50.0	-55.0	216.43	231.13	1.68	193.77	23.00	0.01	0.01	0.79	0.00	4.86
-50.0	-40.0	-45.0	228.14	243.63	1.76	222.79	24.17	0.01	0.01	0.99	0.00	6.17
-40.0	-30.0	-35.0	240.10	256.41	1.84	250.24	25.34	0.01	0.03	1.32	0.01	6.61
-30.0	-20.0	-25.0	253.53	270.75	1.92	276.13	26.53	0.02	0.05	1.98	0.01	7.53
-20.0	-15.0	-17.5	135.31	144.50	1.98	294.52	27.45	0.02	0.07	2.64	0.02	6.00
-15.0	-10.0	-12.5	145.54	155.42	2.01	306.29	28.13	0.05	0.12	3.96	0.03	8.15
-10.0	-6.0	-8.0	133.21	142.26	2.05	316.55	28.82	0.09	0.20	6.60	0.05	8.95
-6.0	-4.0	-5.0	77.52	82.78	2.07	323.21	29.32	0.07	0.28	9.90	0.06	4.82
-4.0	-2.0	-3.0	98.39	105.07	2.09	327.57	29.84	0.14	0.42	19.80	0.09	5.30
-2.0	-1.5	-1.8	29.37	31.36	2.10	330.27	30.09	0.05	0.47	26.40	0.10	1.09
-1.5	-1.1	-1.3	28.44	30.37	2.10	331.23	30.25	0.06	0.52	36.00	0.12	0.90
-1.1	-0.8	-1.0	25.83	27.58	2.10	331.98	30.41	0.06	0.58	49.50	0.13	0.66
-0.8	-0.5	-0.7	33.29	35.55	2.11	332.62	30.61	0.08	0.66	79.20	0.15	0.64
-0.5	-0.3	-0.4	31.27	33.40	2.11	333.15	30.81	0.08	0.74	132.00	0.16	0.41
-0.3	-0.2	-0.3	23.12	24.69	2.11	333.47	30.96	0.06	0.80	198.00	0.18	0.20
-0.2	0.5	0.2	3038.47	3244.79	2.11	334.00	48.07	9.61	10.42			Area > 10nm
0.5	5.0	2.8	216.98	231.71	2.13		51.49			NFW (g/g)	0.16	9.20
			Measured Parameters		Fixed Parameters		Calculated Parameters			Final Parameters		

Correction Parameters		Measured Parameters				Calculating Validity
kappa	1.0679	M_{dry} (mg)	4.5	M_{ice} (mg)	10.42	O.K.
dc_{ice}/dT	0.0065	M_{water} (mg)	11.14	M_{int} (mg)	5.22	O.K.

Sample Identification	Avicel PH-101
Analysis Date DSC	2016/01/07

Comments
Água: 0.0 (Massa da amostra: úmida).

Figure 10: Final spreadsheet with determination of the FBW profile (graph).

4. Conclusions

In CTBE/CNPEM we acquired significant experience in the characterization of biomass wet nanoscale porosity by calorimetric thermoporometry. Our laboratory and data analysis procedures have been successfully employed in over a thousand samples during more than five years. These procedures are described in detail in this Technical Memorandum.

5. References

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Appendix: DSC Instrument Program

OrgMethod	1: Equilibrate at 5.00 °C
OrgMethod	2: Ramp 5.00 °C/min to -70.00 °C
OrgMethod	3: Isothermal for 15.00 min
OrgMethod	4: Mark end of cycle 0
OrgMethod	5: Ramp 1.00 °C/min to -60.00 °C
OrgMethod	6: Isothermal for 5.00 min
OrgMethod	7: Mark end of cycle 1
OrgMethod	8: Ramp 1.00 °C/min to -50.00 °C
OrgMethod	9: Isothermal for 5.00 min
OrgMethod	10: Mark end of cycle 2
OrgMethod	11: Ramp 1.00 °C/min to -40.00 °C
OrgMethod	12: Isothermal for 5.00 min
OrgMethod	13: Mark end of cycle 3
OrgMethod	14: Ramp 1.00 °C/min to -30.00 °C
OrgMethod	15: Isothermal for 5.00 min
OrgMethod	16: Mark end of cycle 4
OrgMethod	17: Ramp 1.00 °C/min to -20.00 °C
OrgMethod	18: Isothermal for 7.50 min
OrgMethod	19: Mark end of cycle 5
OrgMethod	20: Ramp 1.00 °C/min to -15.00 °C
OrgMethod	21: Isothermal for 7.50 min
OrgMethod	22: Mark end of cycle 6
OrgMethod	23: Ramp 1.00 °C/min to -10.00 °C
OrgMethod	24: Isothermal for 12.50 min
OrgMethod	25: Mark end of cycle 7
OrgMethod	26: Ramp 1.00 °C/min to -6.00 °C
OrgMethod	27: Isothermal for 12.50 min
OrgMethod	28: Mark end of cycle 8
OrgMethod	29: Ramp 1.00 °C/min to -4.00 °C
OrgMethod	30: Isothermal for 12.50 min
OrgMethod	31: Mark end of cycle 9
OrgMethod	32: Ramp 1.00 °C/min to -2.00 °C
OrgMethod	33: Isothermal for 12.50 min
OrgMethod	34: Mark end of cycle 10
OrgMethod	35: Ramp 1.00 °C/min to -1.50 °C
OrgMethod	36: Isothermal for 15.00 min
OrgMethod	37: Mark end of cycle 11
OrgMethod	38: Ramp 1.00 °C/min to -1.10 °C
OrgMethod	39: Isothermal for 15.00 min

OrgMethod	**40: Mark end of cycle 12**
OrgMethod	**41: Ramp 1.00 °C/min to -0.80 °C**
OrgMethod	**42: Isothermal for 15.00 min**
OrgMethod	**43: Mark end of cycle 13**
OrgMethod	**44: Ramp 1.00 °C/min to -0.50 °C**
OrgMethod	**45: Isothermal for 20.00 min**
OrgMethod	**46: Mark end of cycle 14**
OrgMethod	**47: Ramp 1.00 °C/min to -0.30 °C**
OrgMethod	**48: Isothermal for 25.00 min**
OrgMethod	**49: Mark end of cycle 15**
OrgMethod	**50: Ramp 1.00 °C/min to -0.20 °C**
OrgMethod	**51: Isothermal for 35.00 min**
OrgMethod	**52: Mark end of cycle 16**
OrgMethod	**53: Ramp 1.00 °C/min to 0.50 °C**
OrgMethod	**54: Isothermal for 45.00 min**
OrgMethod	**55: Mark end of cycle 17**
OrgMethod	**56: Ramp 1.00 °C/min to 5.00 °C**
OrgMethod	**57: Isothermal for 5.00 min**
OrgMethod	**58: Mark end of cycle 18**