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DETERMINATION OF HEAT TRANSFER COEFFICIENTS OF BIOLOGICAL SYSTEMS DURING COOLING IN LIQUID NITROGEN UNDER FILM AND NUCLEATE POOL BOILING REGIMES

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Abstract. The cryopreservation process consists of reducing the temperature of the sample to a point where biological stability is achieved. In particular the measurement of the temperature change of the sample is important to calculate cooling rates and to determine if a sample is vitrified or undergoes phase change transition. As soon an object is plunged into liquid nitrogen it enters into a film boiling regime due to the large temperature difference between the object and the liquid nitrogen (LN2). This determines a heat flux from the object to LN2 causing the latter to boil in the immediate vicinity of the object and creating a pocket of nitrogen vapor around the object which acts as an "insulator" and retards further heat transfer. Film boiling is also referred to as the "Leidenfrost effect". Boiling curves for a specific cryobiological system are scarcely found in the literature due to the small dimensions of the devices used in the process and the experimental limitations. The experimental information such as the time-temperature curve allows the prediction of the surface heat transfer coefficients that govern the cooling process: film, transition and nucleate boiling. In order to predict the surface heat transfer coefficient for each boiling regime the mathematical modeling of the partial differential equations that represent the energy transfer must be implemented, applying convective boundary conditions. In this work the different heat transfer coefficients and the boiling curve of straws filled with ice (at an initial temperature between -2ºC to -9ºC) were experimentally measured when they were immersed in liquid nitrogen; this allowed to determine the existence of different boiling regimes. The application of a numerical finite element program using the software COMSOL was used to predict time-temperature curves and to obtain the surface heat transfer coefficients that control each boiling regime. Independent experiments were carried out using straws that contained a biological fluid (semen+extender), which were initially at room temperature, to further validate the different surface heat transfer coefficients for film and nucleate pool boiling. The program takes into account the variable thermo-physical properties of the biological sample. This constitutes a highly non-linear mathematical problem, as the freezing process evolves with a variable surface heat transfer coefficients as the different boiling regimes occur. The program was experimentally validated contrasting experimental temperatures vs. time with numerical predictions. The numerical program is an important tool in order to correctly assess the heat transfer process and optimize the cryopreservation of straws filled with biological fluids.

1 INTRODUCTION

The knowledge of the thermodynamic process that a biological sample experiences during the cryopreservation procedures is of major importance. Specifically, the measurement of temperature changes in the sample can be used to calculate cooling rates and to determine whether a sample is vitrified or undergoes phase transition. When dealing with liquid nitrogen (LN2), having a saturation temperature at atmospheric pressure, T_{sat} =-196 ºC, the temperature difference between the fluid and the sample (which can be at room temperature), is large enough to cause boiling of the liquid entering into the film boiling regime (Bui and Dhir, 1985; Suryanarayana and Merte, 1972). This determines a heat flux (q) from the object to LN2 creating a pocket of nitrogen vapor around the solid which acts as an "insulator" retarding further heat transfer. Film boiling is also referred to as the "Leidenfrost effect". The object will cool down, rather slowly due to the low heat transfer rates and the "minimum heat flux" point will be reached at the Leidenfrost temperature (T_L). Vapor film will then break off while the heat flux progressively increases as transition to the nucleate boiling regime is established. This event is characterized by a steep increase of the heat flux up to a point called the "maximum heat flux" (Bui and Dhir, 1985; Nishio and Ozu, 1988).

The boiling curve which corresponds to variation of the heat flux (q) as a function of the excess wall temperature ($\Delta T=T_{wall}$ - T_{sat}) depends on several relevant factors such as: i) the type of cryogenic fluid used and its thermal properties, ii) the material in contact with the fluid and its roughness which affects the nucleation sites and the T_L, iii) the relative position of the solid in the cryogenic fluid (vertical, horizontal, or in angle), and iv) the geometry of the solid (plates, sphere, cylinders, or irregular shapes).

There is a lack of experimental information concerning boiling curves in devices such as plastic straws containing biological samples. Plastic French straws are widely used in cryobiology and immersion in liquid nitrogen (LN2) is a common procedure in cryopreservation of biological samples. Specifically, there exists no certain information whether the cooling process is governed entirely by a film type or by a nucleate boiling regime, or a combination of both processes. Additionally it is important to assess the range of ΔT where each regime develops.

The objectives of the present study were: i) to experimentally determine the time-temperature curve of an ice-filled French straw when immersed in LN2 and to define the boiling regimes that govern the process by applying a numerical finite element program that calculates the variable surface heat transfer coefficients; ii) to validate the h values using a French straw filled with a model biological fluid system (bovine semen-extender) which undergoes freezing upon cooling with thermo-physical properties that are temperature dependent.

2 MATERIALS AND METHODS

2.1 Experimental Measurements

Cylindrical plastic straws used for bovine semen cryopreservation were obtained from AB Technology, Inc., Pullman, Washington, USA). The average external diameters, length and thickness were D=2.805mm, L=124mm, e=0.21mm, respectively.

In order to determine the surface heat transfer coefficients at the straw-LN2 interface, the straw was filled with ultra-pure, reverse-osmosis filtered water (Milli-Q, Milipore

Corporation, MA, USA) that freezes at 0 ±0.3ºC. The time-temperature curve was recorded using a thermocouple type T (Copper-Constantan) inserted in the central axis of the straw, containing initially pure water. The straw containing pure water was slowly swirled in Nitrogen vapor over liquid nitrogen in a Dewar tank to generate the ice crystals. In contrast with the experiments done in the past for wires or tubes (Nukiyama, 1934, Drew and Mueller, 1937), in the case of cryopreservation devices such as French straws nor the temperature or heat flux can be controlled during the experiment. The ice has well-known thermophysical properties in the cooling range of -2 to -194ºC (Choi and Okos, 1986; Choi and Bishof, 2010). Once the freezing plateau region was completed the straw was maintained in a thermostatic bath using a solution of ethylene glycol-water (30%v/v) at a temperature below -2ºC and above -9 ºC. After equilibration the straw was rapidly plunged in liquid nitrogen using the Dewar canisters. The advantage of using ice in the straw is to avoid phase changes and in this way the ice sample contained in the straw experienced only cooling during the temperature range of -3 ºC to -194ºC. The thermocouple was connected to an acquisition device (TESTO, Germany). Figure 1 shows the Dewar container with the Testo used in the experiments and how the thermocouple was coupled and mounted to the straw.

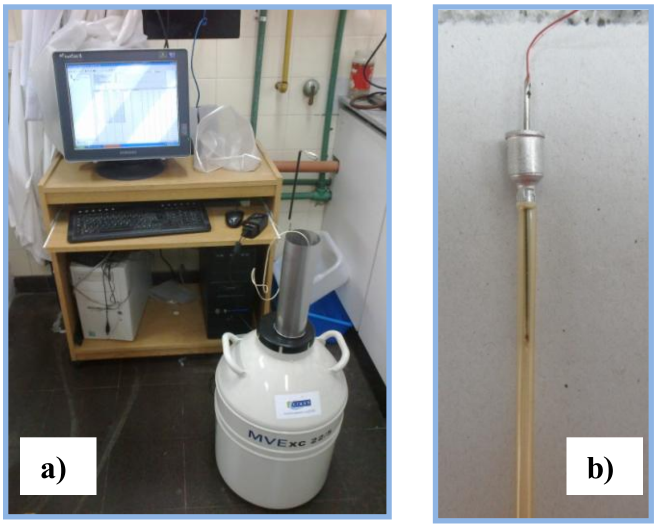

Figure 1: a) Dewar container filled with LN2 and Testo Acquisition Device connected to a PC b) Straw initially filled with pure water with the thermocouple threaded into the needle and mounted into the straw to avoid radial movement during the experiment.

2.2 Model system

The system (plastic straw and internal material) can be described as two concentric finite cylinders of different substances: the inner material being either ice or biological cells (semen + extender) and the plastic straw. The partial differential equations that represent the heat transfer in the fluid that is submitted to the freezing process (Eq. 1) and the plastic support (Eq. 2) considering radial and axial coordinates have been thoroughly described in Santos et al., 2013 a, b and are as follows:

$$\rho(T)Cp(T)\frac{\partial T}{\partial t}r = \frac{\partial}{\partial r}\left(k(T)r\frac{\partial T}{\partial r}\right) + \frac{\partial}{\partial z}\left(k(T)r\frac{\partial T}{\partial z}\right) \qquad (1)$$

$$\rho_p \, Cp_p \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r}\left(k_p \, r \frac{\partial T}{\partial r}\right) + \frac{\partial}{\partial z}\left(k_p \, r \frac{\partial T}{\partial z}\right) \quad (2)$$

where: T is temperature, ρ is the density, Cp specific heat, k thermal conductivity.

The subscripts s corresponds to the ice or biological fluid and p to the plastic material. It can be noticed that the ice and biological suspension have thermal properties that are temperature dependent due to the presence of the phase change transition. In the case of the plastic support the thermo-physical properties (k_p, ρ_p ,Cp_p) are considered constant. The modeling of the biological fluid constitutes a highly non-linear mathematical problem due to the abrupt change of the thermal properties with temperature during phase change transition (Santos et al., 2013a). The initial temperature condition was considered uniform in both material domains.

The convective boundary condition at the interface plastic support-LN2 is:

$$-k_p \nabla T.n = h(T_{wall} - T_{ext}) \quad (3)$$

where h is the surface heat transfer coefficient, k_p is the plastic thermal conductivity, T_{wall} is the variable surface wall temperature at the interface plastic support-liquid nitrogen, T_{ext} is the external temperature (in this case the saturation temperature of LN2 at atmospheric pressure), n is the normal outward vector, and ∇T is the temperature gradient evaluated at the surface.

A triangular mesh using Lagrange elements of order 2 was applied to discretize the domains. The number of elements that constituted the mesh for the biological fluid and plastic support domains were 30656 and 23808, respectively (Fig. 2). All the numerical runs were tested for their computational speed; the maximum CPU time was less than 10 min using a PC Intel(R) Core(TM) i3 with a processor speed of 2.93 GHz and a RAM of 4GB.

The time discretization scheme used was a Backward Euler Differentiation (minimum order 1 and maximum order 5) with a tuning step having a maximum of 1 s and a minimum initial starting value of 0.0001s.

The numerical program calculates de temperature profile as a function of time, in the straw and the fluid during the freezing process, especially the temperature at the wall which is used in the prediction of the heat flux by applying the convective boundary condition.

Figure 3 shows the temperature at an axial position $z=65x10^{-3}$ m (half height of the plastic straw) for different radial points and experimental times.

A subroutine that enables the introduction of a variable coefficient h with time was coded in Matlab language in the main program which was originally generated in the commercial software COMSOL AB. This subroutine allows the prediction of an h value for each boiling regime. Different heat transfer coefficients were introduced to simulate the temperature-time curve for the straw; then, experimental and predicted temperatures for each proposed h were compared. The heat transfer coefficient that minimized the Residual Sum of Squares given by Eq. (4) was selected.

$$RSS = \sum \left(T_{exp} - T_{pred}\right)^2 \quad (4)$$

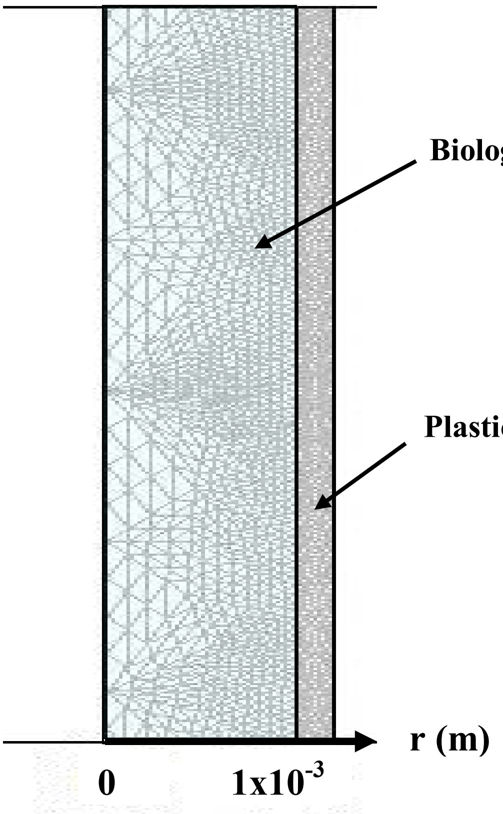

Figure: 2 Spatial discretization of the biological fluid and plastic support domains using triangular elements. Revolution body with a revolution axis at r=0 m.

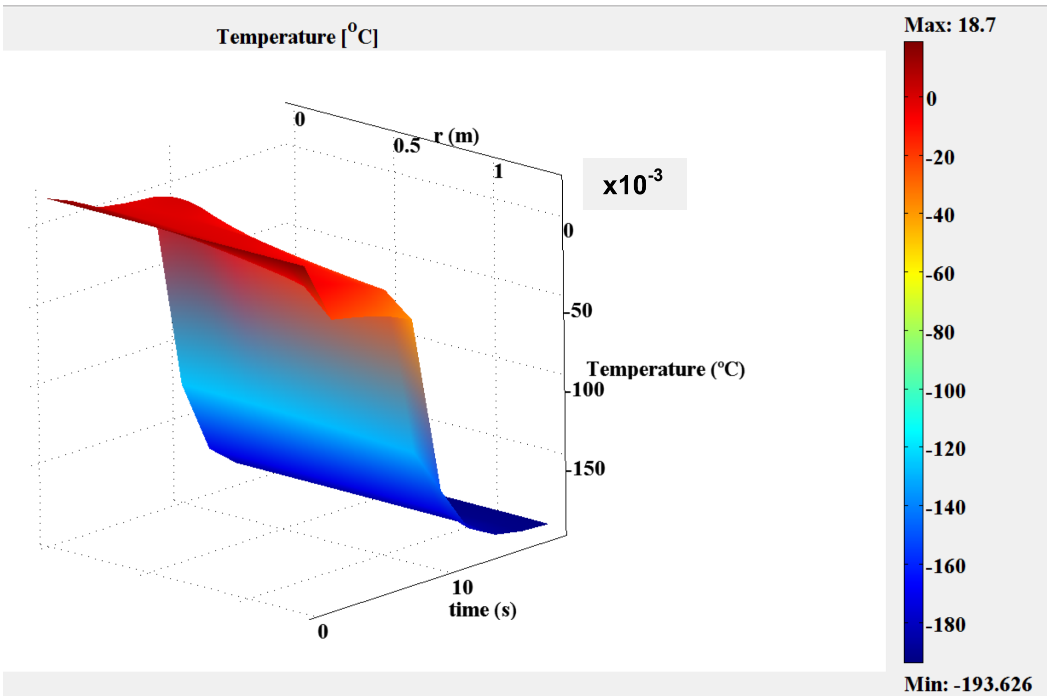

Figure 3: Temperature predictions in the biological fluid and plastic support domains at an axial position $z=65x10^{-3}$ m as a function of radial position (r) and time (t)

2.3 Thermo-physical Properties used in the numerical program

In the case of ice-filled straws the thermo-physical properties used as input in the numerical program were as follows: the average specific heat was considered 1461.7 J/kgºC for temperatures below 0ºC. The thermal conductivity and density of ice are shown in Table 1 (Choi and Okos, 1983; Choi and Bishof, 2010).

T (ºC)	k (W/m ºC)	ρ (kg/m^3)
0	2.22	916.2
-5	2.25	917.5
-10	2.30	918.9
-15	2.34	919.4
-20	2.39	919.4
-25	2.45	919.6
-30	2.50	920.0
-35	2.57	920.4
-40	2.63	920.8
-50	2.76	921.6
-60	2.90	922.4
-70	3.05	923.3
-80	3.19	924.1
-90	3.34	924.9
-100	3.70	925.7
-110	4.10	----
-120	4.30	----
-130	4.70	----
-140	5.20	----
-150	5.60	----
-180	----	934.0

Table 1: Thermal conductivity and density of ice at different temperatures.

The thermo-physical properties of the semen+extender were calculated based on the composition of the biological fluid expressed in wet basis mass fraction: carbohydrates = 0.098, fat = 0.031, and protein = 0.027. The moisture content of the semen+extender was experimentally measured and found to be 84.4 %. Choi and Okos (1986) equations were applied to estimate the thermal conductivity and density (Fig. 4a and 4b).

Specific heat and latent heat of ice melting of the semen + extender mixture were measured by using a Differential Scanning Calorimeter (DSC) (TA Instruments, New Castle, Delaware, USA) model Q100 controlled by a TA 5000 module with a quench cooling system under a nitrogen atmosphere at 20 mL/min.

Samples of semen+extender suspension were enclosed in sealed aluminum pans. An empty pan was used as a reference sample. Pans were heated at 2 ºC/min from -150 to 20 ºC, with isothermal periods at the initial and final temperatures. In order to measure the specific heat, three scans were carried out: one for the sample, one for a standard (sapphire), and one for the empty sample pan. In these scans the reference holder contained an empty pan. Distilled water was also scanned using the same program to verify equipment calibration. The specific heat was calculated following the ASTM E1269 procedure and McNaughton and Mortimer (1975) recommendations. The latent heat of melting (ΔH_m) was determined as indicated by Roos (1986). The ΔH_m was calculated by integrating the peak of the melting curve; this value was used to estimate the unfrozen water fraction in the material. The temperature integration limits of the peak were chosen when a clear separation between curve and base line was detected

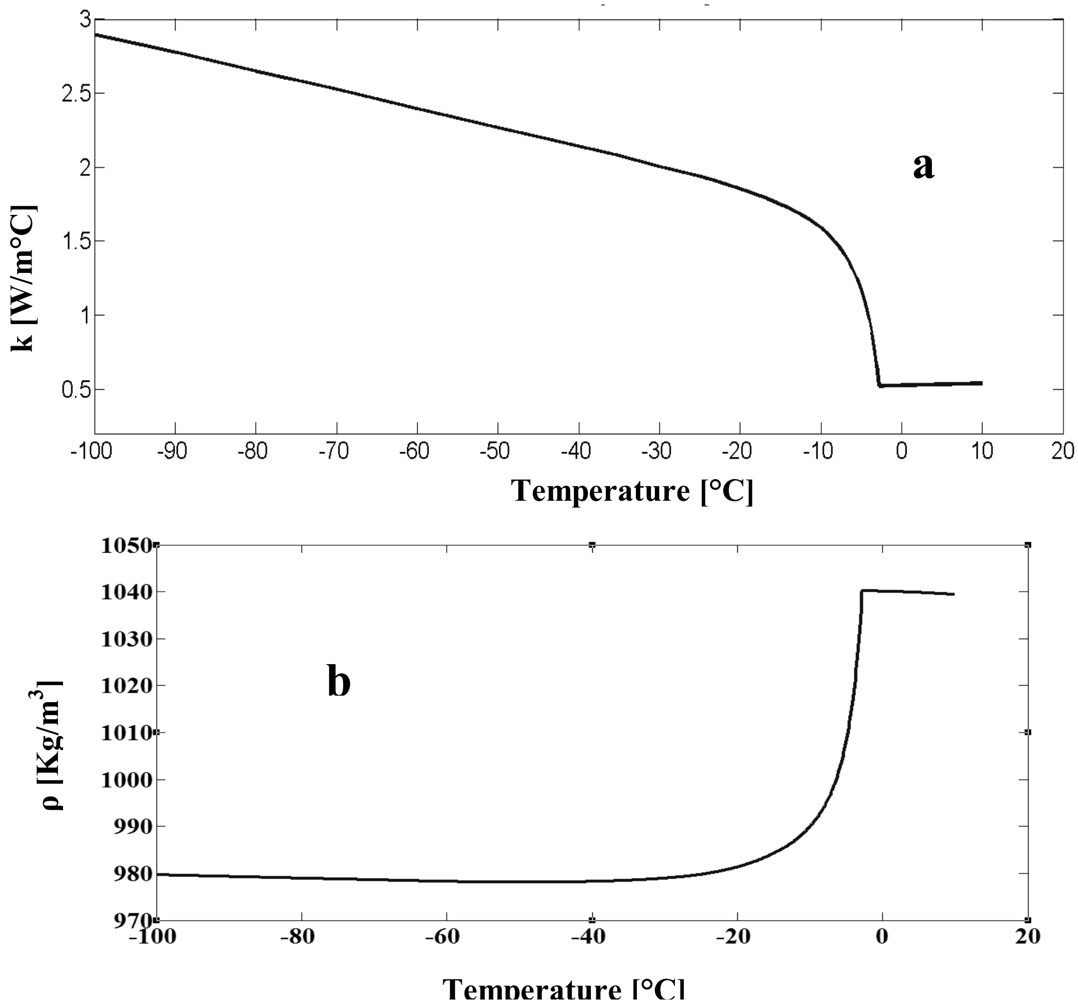

Figure 4: Estimated thermal conductivity (a) and density (b) of semen+extender sample as a function of temperature

Experimental data of the apparent specific heat, where the sensible heat is merged with the latent heat, produced a curve with a large peak around the freezing point. However for mathematical modeling purposes the specific heat capacity function has to rise smoothly to a peak over a finite range of temperature (Neeper, 2000). Therefore based on experimental data a Cp vs. Temperature curve was constructed using a Gaussian and Heaviside function (Comsol AB Multiphysics, 2005; Neeper, 2000) maintaining the experimental values of the initial freezing point, the range of temperature change, the latent heat of melting. The equation used in the numerical program to represent the specific heat as a function of temperature is as follows:

$$Cp(T) = Cp_{ff} + \frac{\Delta H_m}{Ts} \quad f(H_{ea}) + D\,\Delta H_m \tag{5}$$

Where Cp_{ff} is the specific heat of the fully frozen state, Ts is the peak temperature point (equivalent to the μ media in the gaussian curve) and D is a gaussian curve defined as

$$D(T) = \frac{e^{\left(\frac{(T-T_m)^2}{dT^2}\right)}}{\sqrt{\pi dT^2}} \tag{6}$$

where dT is the half width of transition, that is the temperature difference from melt within which 84% of the latent heat occurs (Neeper, 2000); $f(H_{ea})$ is the Heaviside function which is a built in function in COMSOL Matlab environment that has continuous second order derivatives. This function enables the numerical finite element software (Comsol AB Multiphysics, 2005) to successfully deal with the abrupt change in the apparent specific heat of the sample with temperature. The specific heat of the biological fluid obtained by DSC and using Eq. 5-6 can be observed in Fig. 5.

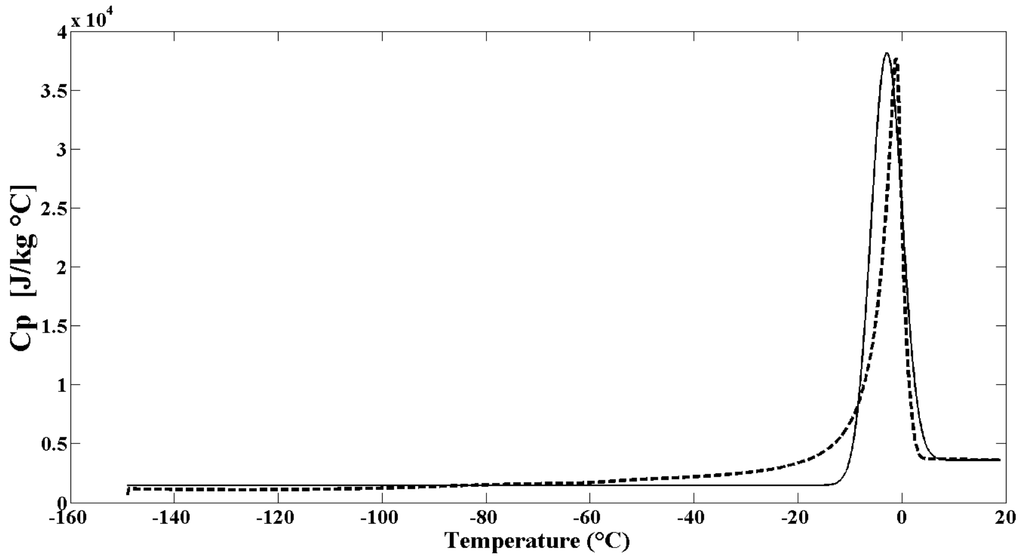

Figure 5: Apparent specific heat of semen+extender sample experimentally obtained by DSC (- -) and using the Heaviside and Gaussian functions (▬) .

3 RESULTS AND DISCUSSION

3.1 Determination of the surface heat transfer coefficients using ice-filled French straw

The experiments with straws containing ice, that were plunged in liquid nitrogen, allowed the rigorous determination of the surface heat transfer coefficients avoiding any phase change in the cooling process (temperatures of ice ranging between -2 and -196ºC). Fig. 6a shows the experimental temperatures versus the predicted values assuming two different hypothesis: i) considering film boiling regime during the entire cooling process (using a single and constant h value); ii) assuming film boiling for the first stage followed by nucleate boiling regime (higher h value); Fig. 6b shows a replicate run. All temperature measurements using thermocouples have an experimental absolute error of 0.5ºC.

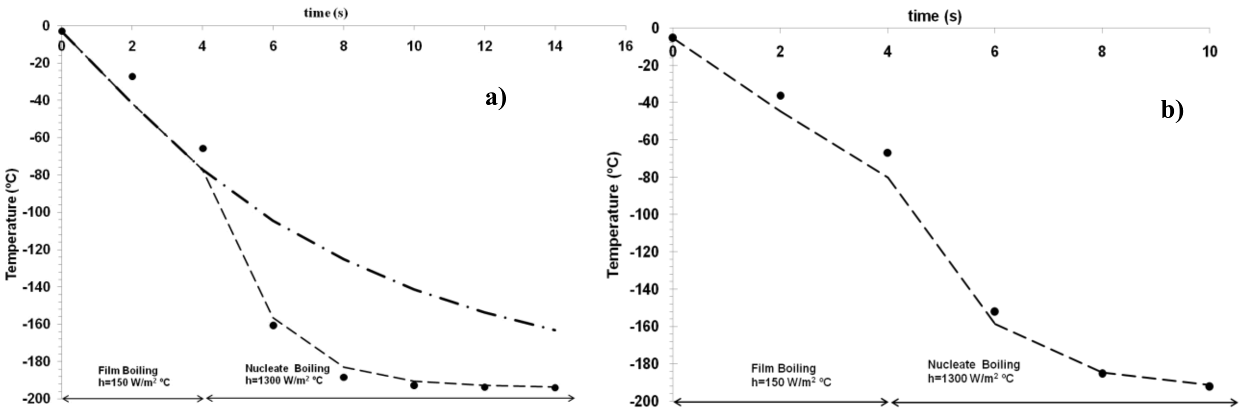

Figure 6: a) Experimental time-temperature curves in the straw containing ice (●) at r= 0.0008 m, L =0.060 m. Predicted temperatures obtained from the computational simulation with variable h values (- -); b) results of a duplicate run. The curve (▬ ● ▬) shown in a) was obtained considering a single value of h=150 W/m^2 ºC (film boiling regime) over the entire experiment.

As can be observed there is a lack of agreement between the experimental and predicted temperatures when a single constant value of h was used over the entire cooling curve (Fig. 6a). However good agreement was achieved considering two regime stages during the cooling of straws. Note that since ice was used from the beginning, no plateau region corresponding to the latent heat of phase change is observed. The cooling curve showed an abrupt change in the slope which is attributed to the transition of film into nucleate boiling regime. This change in the cooling slope was also reported in other experiments with stainless steel blocks immersed in liquid Nitrogen (Jin et al., 2009). The change in the rate of cooling can be attributed to the presence of two different regimes, first film and then nucleate boiling. During pool film boiling the excess wall temperature (ΔT) is at its maximum and the h that best fitted experimental results was h=150 W/m^2 ºC. During nucleate boiling a rapid drop of the temperature was observed and the h value of 1300W/m^2 ºC gave the best fit to the experimental temperatures.

3.2 Validation of the previously obtained surface heat transfer coefficients using French straws filled with semen+extender.

Fig.7a shows experimental time-temperature values and the finite element numerical predicted curves for French straws filled with semen+extender assuming the same hypothesis as in Section 3.1: i) considering film boiling regime during the entire cooling process (using a single constant h value); ii) assuming film boiling for the first stage (h =150 W/m^2 ºC) followed by nucleate boiling regime (h = 1300W/m^{2}ºC).

As it can be noted, good agreement between experimental and predicted values was observed when assuming film boiling for first stage (h=150 W/m^{2}ºC) and nucleate boiling regime (h=1300 W/m^{2}ºC) for the second stage. This observation reinforces the existence of two stages of boiling regime phenomenon. The curve shows a mild plateau region which can be attributed to the phase change transition of water into ice in the semen sample. Afterwards, an abrupt slope change in the cooling curve develops and a rapid drop in the temperature is observed which corresponds to the transition of film to nucleate pool boiling similarly as it was explained for the ice system.

Fig. 7b shows another replicate obtained with French straws containing semen+extender fluid and the numerical predictions using the h values previously determined.

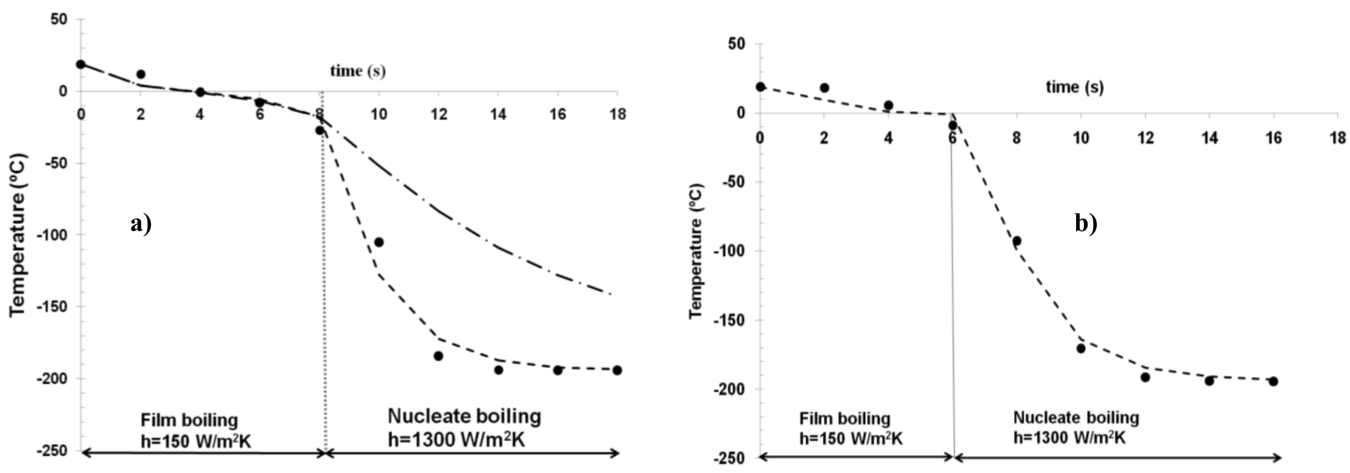

Figure 7: a) Experimental time-temperature curves (●) in the straw containing the biological fluid that undergoes freezing at r= 0.0008 m; L =0.060 m. Predicted temperatures using the program with variable h values (- -); b) Results from a similar experiment at r=0.0004m L=0.0604m. The curve (▬ ● ▬) shown in a) was obtained considering a single value of h=150 W/m2 ºC (film boiling regime) over the entire experiment

3.3 Heat flux as a function of excess wall temperature-Boiling Curve

The heat flux (q=h ΔT) as a function of the excess wall temperature ($\Delta T=T_{wall}-T_{sat}$) was determined using the predicted wall temperature of the straw (T_{wall}) and the h values (Fig. 6).

The heat flux for straws filled with ice (Fig. 8a) have a q_{min} value of 19555 W/m^2 which corresponds to an excess wall temperature value of ΔT=130.4ºC, being the wall temperature T_{wall} = -65.7ºC. The stable film boiling regime extends up to the point where the heat flux is at its minimum (q_{min}) corresponding to $(\Delta T)_{qmin}=T_{wall}-T_{sat}$. This $(\Delta T)_{qmin}$ for the tested straw-liquid nitrogen system was in the range of 130-140ºC, that corresponds to a critical wall temperature range (Leidenfrost temperature T_L) of -66 to -55ºC.

In the case of the straw filled with the biological fluid (Fig. 8b) the minimum flux, q_{min}= 21072 W/m^{2}was obtained at an excess wall temperature ΔT= 140.5ºC which corresponds to a wall temperature of -55.52 ºC. When the straw surface temperature becomes lower than this value there is a transition from film boiling to nucleate boiling and the heat flux increases up to a maximum value, afterwards it decreases and ΔT diminishes.

This $(\Delta T)_{qmin}$ depends on several important factors such as the type of cryogenic fluid used and its thermal properties, the material in contact with the fluid and its roughness which affect the nucleation sites and the transition or Leidenfrost temperature.

Additionally other factors are, the relative position of the immersed body in the cryogenic fluid (vertical, horizontal, or angle of surfaces in contact with the fluid), and the geometry of the solid (plates, sphere, cylinders, or irregular shapes). There are many reports in literature

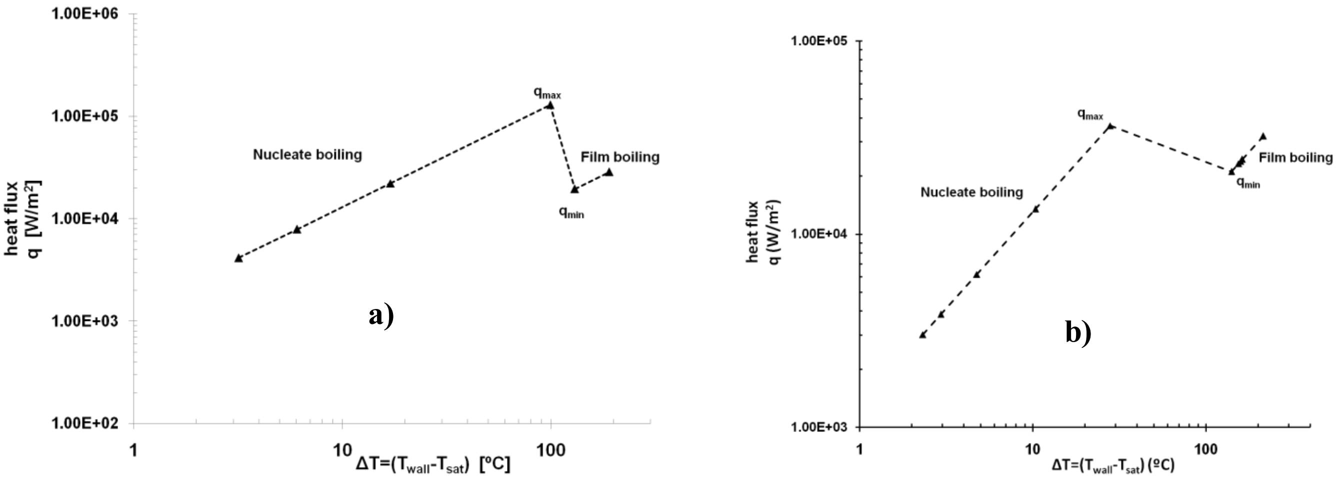

Figure 8: Boiling curves for French straws filled with a) ice b) biological fluid (semen+extender) when immersed in LN2.

concerning the $(\Delta T)_{qmin}$ for several systems and configurations. Table 2 shows the reported values of $(\Delta T)_{qmin}$ for several metallic objects when immersed in liquid nitrogen.

It can be remarked that in polished metals the $(\Delta T)_{qmin}$ values are lower than those for plastic straw since there are less active sites where bubble formation can take place. Roughness also triggers the nucleation of bubbles and thus tends to increase the temperature T_L. Another important issue to be considered is when using straws there is no control over the heat flux or temperature of the sample as the cooling process evolves, in contrast with the experiments with metal tubes or wires where the temperature and heat flux was the control variable, respectively.

System	Dimensions	$(\Delta T)_{qmin}$	Reference
Copper sphere	Diameter 2.89 cm	48	Chase et al. (2012)
Stainless steel block	6.0 x 6.0 x 1.75 cm	53	Jin et al. (2009)
Copper cylinder*	Diameter 5.08 cm Length 7.25 cm	25	Listerman et al. (1986)
Stainless steel cylinder*	Diameter 4.13 cm	76	Chao et al. (2003)
Stainless steel cylinder*	Not reported	80	Hui et al. (2006)
Copper spheres	Diameter2.54 to 0.635 cm	45	Lewis et al. (1965)
Platinum cylinder*	Diameter 0.60 cm	40	Sakurai et al. (1990)
Platinum wires	Diameter 0.1 to 1.0 mm	100	Kida et al. (1981)

* In this case, spatial position for cylinders (vertical/horizontal) was not reported.

Table 2:- Literature values of (delta T)$_{qmin}$ for several metallic objects immersed in liquid nitrogen.

4. CONCLUSIONS

Measurement of experimental time-temperature curves of plastic French straw filled with ice and immersed in LN2 was carried out enabling the estimation of the surface heat transfer coefficients by applying a numerical finite element program. A specific subroutine was coded in the program in order to calculate a variable h with time since two boiling regime, film followed by nucleate pool boiling, were detected to occur during the cooling process.

The existence of film and nucleate pool boiling regimes was validated by additional experiments using French straws filled with bovine semen+extender fluid, plunged in liquid nitrogen and comparing the time-temperature curves with the numerical predictions. Since this system freezes upon cooling, their temperature dependent thermophysical properties were taken into account in the computer code, as well as the variable surface heat transfer coefficients during the different boiling regimes. A good agreement was obtained between the experimental temperature profiles and numerical predictions during cooling of straws containing bovine semen + extender, confirming the reliability of the previously determined heat transfer coefficients (150 W/m^2K for film boiling and 1300W/m^2K for nucleate boiling).

Calculated values of heat flux (q) vs. ΔT, of straws placed vertically in liquid nitrogen were determined; this allowed the prediction of the Leidenfrost temperature, q_{min}, q_{max} and minimum ΔT necessary to sustain a film type behavior.

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