

## TESTING OF DRYING PROCESS MODEL FOR ACOUSTICALLY LEVITATED DISACCHARIDE AQUEOUS SOLUTIONS

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**ABSTRACT.** The paper reports experimental results collected for testing a drying process model for acoustically levitated droplets. More specifically, the so-called  $D^2$  versus time law is tested on droplets of homologous disaccharide aqueous solutions. For assessing the validity of the model, the determination of the droplet diameter is performed by a direct video camera monitoring and by an OH/CH area ratio analysis of InfraRed spectra collected as a function of time. For such a test, aqueous solutions of trehalose and sucrose, which show different surface tension values, are investigated. The experimental findings, obtained by the two independent employed approaches, reveal, for both the samples, a good agreement with the theoretic model previsions for the initial time trend. Furthermore, in agreement with literature data, they furnish a value of the surface tension, which is higher for trehalose in respect to sucrose.

### 1. Introduction

Nowadays, drying processes and evaporation kinetics of levitated droplets are considered complex mechanisms. Although many theoretical models on the drying behavior of suspended droplets have been formulated, only few experimental data are available. Previous investigations often provide only integral information while specific information about the individual physical processes taking place are missing. In general, levitation (Caccamo *et al.* 2017; Cannuli *et al.* 2018b,c,d; Caccamo and Cannuli 2019) is a contactless technique that permits to remove sample-holder interplays and decreases contamination in order to study a solution with a high control degree. Among these techniques, acoustic levitation has the advantage of not requiring specific properties of the sample and can treated very small object. It allows obtaining very high concentrations of mixtures starting from solutions, impossible to achieve without the use of levitation. In particular, the importance of acoustic levitation resides in the access to supercooled liquids regularly and supersaturated solutions under well-controlled conditions. Furthermore, the latest updates in acoustic levitation allow container-less studies on supercooled liquids and are fit for analysis of non-equilibrium processes. The present technology assent only to process small samples for very short periods and not long-lasting experiments with samples of a few hundred milligrams; with the acoustic levitation, it is possible thanks to the smallness of the positioning forces and

the elimination of the buoyancy-driven convection. In this context, in acoustic levitation it is fundamental to implement an intuitive model of drying processes occurring when a single droplet is suspended in the air; furthermore, the observation of a single droplet upon drying often enables to track the physical and chemical changes and to get information on the investigated systems. The acoustic levitator is an instrument suitable for the study of droplets kinetics. Also in biophysics field, aqueous solutions (Magazù *et al.* 1989; Jannelli *et al.* 1996; Magazù 1996; Lokotosh *et al.* 2000) disaccharides (Magazù *et al.* 1998a, 2007b; Minutoli *et al.* 2008; Pagnotta *et al.* 2008), proteins (Magazuù *et al.* 2010; Magazù *et al.* 2011b; Barreca *et al.* 2013; Fenimore *et al.* 2013), polymers (Gonzalez-Tello *et al.* 1994; Branca *et al.* 1998a; Faraone *et al.* 1999a,b; Chen *et al.* 2005) and polyols (Jonsson *et al.* 1998a,b; Li *et al.* 1998; Chelli *et al.* 1999; Grandori *et al.* 2001; Magazù *et al.* 2007a; Zondervan *et al.* 2007), can be analyzed by the acoustic levitation technique for the study of hydrogen-bonded phenomena. Experimental analyses have been carried out for such systems, starting from highly diluted to highly concentrated compounds. For this study, the analyzed samples are diluted aqueous solvents of two homologous disaccharides, more specifically, trehalose and sucrose solutions, in a concentration of 50% water and 50% pure sample. So far a significant experimental material has been accumulated on the properties of disaccharides aqueous solutions, obtained by inelastic light diffusion and incoherent neutron dispersion (Magazù 2000; Branca *et al.* 2003b; Magazù *et al.* 2008a,b, 2011a, 2013a). It is known that to understand such mechanisms determining the effectiveness of trehalose and sucrose, it is critical to characterize the structural and dynamical properties of such solutions (Branca *et al.* 1998b, 2002b, 2005). The combination of acoustic levitation and IR spectroscopy can present several advantages, it offers a method to check the dehydration process of the diluted trehalose and sucrose mixtures as a function of time and high concentration range. Furthermore, it furnishes a way for testing a drying process theoretical model. In this paper, the  $D^2$ -versus time law is tested on homologous disaccharide aqueous solutions of trehalose and sucrose, levitated by an acoustic levitator to validate a drying process theoretical model. In particular, droplet diameter measurements are performed by a direct monitoring through video camera and laser pointing and by an OH/CH area ratio analysis of InfraRed spectra collected as a function of time. The obtained experimental results reveal a good agreement with the theoretical model previsions for the initial time trend and in addition, they furnish a value of the surface tension which is higher for trehalose in respect to sucrose.

## 2. Drying process theoretical model

Mechanisms of droplet drying are enough complex, in particular they involve a period in which the drying process is constant and it follows the well-known  $D^2$ -Law, followed by a period where the rate of drying falls. In general, the evaporation rate of solutions is variable and it follows a non-linear dependence of the square diameter with time. In particular, on levitated droplets evaporation in levitators with strong acoustic fields it was shown that the acoustic streaming in the gas furnishes a convective mechanism bigger than the Stefan flow or natural convection. The acoustic streaming dominates the evaporation process in levitators and in the case of disaccharides aqueous droplets experimental data on  $D^2(t)$  were correlated by a linear function reminiscent of the  $D^2$ -Law (Figure 1).

The D<sup>2</sup>-Law predicts that the square of the droplet diameter decreases linearly with time. Few important assumptions must be considered for a droplet in suspension, such as:

- heat and mass transfer between liquid and gas phase are diffusion controlled;
- a spherical symmetry is considered;
- no radiation effects take place;
- constant and uniform temperature occurs.

Assuming that, the density of the liquid droplet is  $\rho_{droplet}$ , the density of the gas at the droplet surface is  $\rho_{gas}$ , the diffusion coefficient is  $D_g$  and the Spalding transfer number  $B_M$ . The mass flux of vapor leaving the droplet surface can be calculated as:

$$\dot{m}_v = \pi D \rho_{vapor} D_g Sh \left( \frac{Y_{vap,s} - Y_{vap,\infty}}{1 - Y_{vap,\infty}} \right) \tag{1}$$

where  $Y_{vap}$  is the mass fraction at the droplet surface which is supposed to be uniform. The outer boundary is noted with the subscript  $\infty$  and represents the condition far away from the droplet. The rate change of liquid droplet mass is expressed as follows:

$$\dot{m}_v = - \frac{dm_d}{dv} \tag{2}$$

$$\frac{dm_d}{dv} = -\rho_{droplet} \frac{dV}{dt} = -\rho_{droplet} \frac{\pi dD_s^3}{4 dt} \tag{3}$$

$D_s^3$  can be divided in a constant diameter, that is  $D$  and in a variable diameter as function of time,  $D_s^2$ :

$$D_s^3 = D_s^2 \cdot D \tag{4}$$

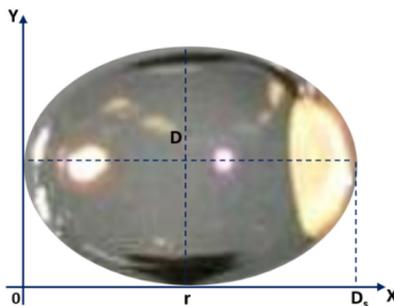


FIGURE 1. Droplet diameter, that can be divided in a constant diameter, that is  $D$  and in a variable diameter as function of time,  $D_s^2$ .

So, it is possible to write eqn.(3) as  $\frac{dm_d}{dv} = -\rho_{droplet} \frac{\pi D}{4} \frac{dD_S^2}{dt}$ , but  $\dot{m}_v = \dot{m}_d$ , so, comparing eqn. (1) with the eqn. (3) the equation for the diameter becomes:

$$\pi D \rho_{vapor} D_g Sh \left( \frac{Y_{vap,s} - Y_{vap,\infty}}{1 - Y_{vap,\infty}} \right) = -\rho_{droplet} \frac{\pi}{4} \frac{dD_S^2}{dt} \quad (5)$$

$$\frac{dD_S^2}{dt} = -4 \frac{\rho_{gas}}{\rho_{droplet}} D_g Sh \left( \frac{Y_{vap,s} - Y_{vap,\infty}}{1 - Y_{vap,\infty}} \right) = -4 \frac{\rho_{gas}}{\rho_{droplet}} D_g Sh \ln(1 + B_M) \quad (6)$$

For diffusion-controlled evaporation the film thickness goes into infinity, hence  $Sh_0 = 2$ . The modified Sherwood number  $Sh$  takes the value 2 and after substituting the value of  $Sh_0$  in eqn. (1), eqn. (4) becomes:

$$\frac{dD_S^2}{dt} = -8 \frac{\rho_{gas}}{\rho_{droplet}} D_g \ln(1 + B_M) \quad (7)$$

$$\int \frac{dD_S^2}{dt} dt = \int -8 \frac{\rho_{gas}}{\rho_{droplet}} D_g \ln(1 + B_M) dt \quad (8)$$

in which  $B_M$  is the Spalding mass transfer coefficient.

The integration of this equation for an initial condition  $D_0$ , i.e. at  $t = 0$ , gives the well-known  $D^2$ -Law that describes the temporal evolution of droplet surface of pure liquid:

$$D_S^2 = D_{0S}^2 - \beta t \quad (9)$$

where  $\beta$  is the evaporation rate coefficient:

$$\beta = 8 \frac{\rho_{gas}}{\rho_{droplet}} D_g \ln(1 + B_M) \quad (10)$$

In addition to the evaporation rate, another important parameter in droplet evaporation is the lifetime of the droplet, also called evaporation time  $\tau_{e}nd$ , which can be determined from eqn. (6) with  $D|_{t=\tau_{e}nd} = 0$ :

$$\tau_{e}nd = \frac{D_0^2}{\beta} \quad (11)$$

### 3. Investigated samples

The investigated samples are diluted aqueous solutions of trehalose (known as  $\alpha$ -D-glucopyranosyl- $\alpha$ -D glucopyranoside) and of sucrose (known as  $\alpha$ -D-glucopyranosyl- $\beta$ -D-fructofuranose) in a concentration range of 50% water and 50% pure sample, with a chemical structure shown in Figure 2. Trehalose is a not reducing disaccharide of glucose, due to the linkage that occur between the two-glucopyranose rings at the reducing end of the glycosylic residues, while sucrose is a disaccharide of  $D$ -fructose and  $D$ -glucose connected at their reducing groups (Branca *et al.* 1999a; Magazù *et al.* 1999b, 2007b; Minutoli *et al.* 2008; Pagnotta *et al.* 2008). By understanding the particular interplays that occur between sugars, water and biological systems, it is possible to explain the reason for which disaccharides are good protectants (Magazù *et al.* 1998b; Branca *et al.* 1999b; Magazù *et al.* 1999a; Ballone *et al.* 2000; Magazù *et al.* 2008c, 2016).

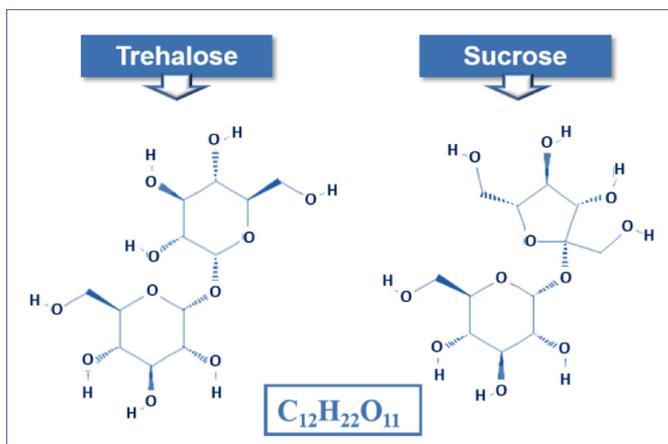


FIGURE 2. Molecular structure of trehalose, also known as  $\alpha$ -D-glucopyranosyl- $\alpha$ -D glucopyranoside and sucrose, known as  $\alpha$ -D-glucopyranosyl-  $\beta$ -D-fructofuranose. They have the same molecular formula  $C_{12}H_{22}O_{11}$  and same molecular weight of  $342.30g/mol$ .

Furthermore, from the study of the properties of different sugars, it is possible to know the reason for which some disaccharides are more valid than others. In this context, trehalose and sucrose are the most widely employed disaccharides to stabilize the biological materials, such as cells, proteins and membranes. They have the same chemical formula ( $C_{12}H_{22}O_{11}$ ) and molecular weight of  $342.30g/mol$ , but different structures. In fact, trehalose owns two symmetrical connected glucose rings, while sucrose only a rigid fructose ring. From a general point of view, sucrose is one the most studied sugars over the last hundred years, as evidenced by the famous work published in 1906 by Einstein about the Brownian motion. A large number of studies on the effects of sucrose have been carried out for over a hundred years and this number continues to grow each year. Several recent empirical studies have examined the thermo-physical properties and the structure of aqueous solutions of trehalose and sucrose, proving the benefits obtained by the addition of these two disaccharides to biological systems, besides the hydrogen-bonding capabilities (Branca *et al.* 2002a; Minutoli *et al.* 2007; Barreca *et al.* 2010; Magazù *et al.* 2010a,b). Comparisons of their relative efficacy have often found trehalose to be the more effective protectant. Notwithstanding the abundance of data and the extraordinary bioprotective and osmoregulative properties of trehalose are well empirically defined, the underlying molecular mechanisms remain cryptic and the understanding of this disaccharide remains inadequate (Varga *et al.* 2008; Magazù *et al.* 2013b; Migliardo *et al.* 2014). The combination of acoustic levitation and IR spectroscopy can offer a method to check the dehydration process of the diluted trehalose and sucrose mixtures as a function of time and high concentration range. Furthermore, it furnishes a way for testing the drying process theoretical model.

#### 4. Instrumental set-up and testing procedures

Acoustic levitation of droplets is a new tool for container-less material processing. It is employed in conjunction of IR spectroscopy in studies of drying processes and as theoretical model testing, in addition to the directly measure performed by means of a video camera. In particular, the levitation device, shown in Figure 3, is composed by a levitation apparatus, an ultrasonic power amplifier, an acoustic controller circuit and a video camera.

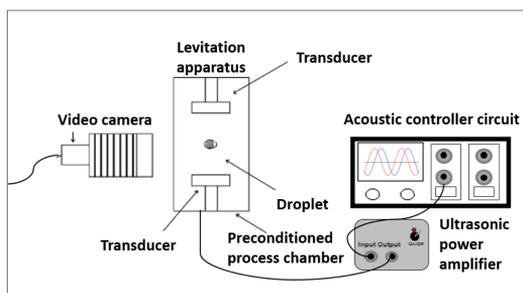


FIGURE 3. Levitation device scheme, constituted by a levitation apparatus, placed inside a preconditioned process chamber, an ultrasonic power amplifier, an acoustic controller circuit and a video camera.

The levitation apparatus, placed inside a preconditioned process chamber for the moisture and temperature control, is constituted by two transducers, mounted on a rigid vertical axis in opposite position, generating two acoustic standing waves of 22 KHz. They can levitate liquid droplets in each node formed by the conjunction of the two sinusoidal waves, thanks to a pressure gradients and interferences. The acoustic waves are generated by a piezoelectric crystal, which gives rise to a stationary acoustic radiation force, irradiated by the transducers, when the distance between them is an integral multiple of the half wavelength. Furthermore, two acoustic absorbing foam disks are glued onto the face of each transducer to reduce instabilities in the levitated sample. The droplet is introduced in the middle of the transducers using a micropipette injection. The ultrasonic power amplifier, consisting of a capacitive load for the two transducers, variable as function of frequency and it is powered by a power supply of  $\pm 40V$ . There is also an oscilloscope to monitor the amplitude of the waves and to measure the current to the transducers. The acoustic controller circuit, furnishes the drive signal to the ultrasonic power amplifier. It hooks the resonant frequency of the transducers with a phase lock loop and maintains near zero the phase angle between the drive current and the voltage. Finally, a video camera is employed for video recordings and images and for the sample positioning. It also allows the diameter determination by a direct monitoring. The second device of the instrumental set-up is an IR Spectrometer Vertex 70v, by Bruker Optics. It operates in conjunction with the levitator to collect absorption spectra in an off-line status. The spectra are constituted by 128 interferograms, ensuring a spectral resolution of  $4cm^{-1}$ . Furthermore, each spectrum is corrected for atmospheric water background, baseline and area normalization. A Toughbook PC is employed for recording all video, images and data and for measuring the diameter, throughout a specific software.

The measurement of the droplet diameter as a function of time, i.e. the drying process of a single levitated droplet within of an acoustic levitator, can be performed with three different procedures: a mathematical theoretical formulation, a direct and indirect measurement. More specifically, the theoretical formulation is the one already discussed in section 1. The direct measurement consists in a monitoring of the droplet in time with a video camera, connected to a PC that records and measures the droplet dimensions in real time. Finally, the indirect measurement consists on the joint use of acoustic levitation and IR spectroscopy techniques for studying aqueous solutions that lose water in time (Branca *et al.* 2000; Migliardo *et al.* 2013b; Caccamo and Magazù 2016, 2017a,b; Caccamo *et al.* 2018b). The latter is an alternative and innovative indirect measurement for radius evaluation through the analysis of the  $OH/CH$  area ratio of the solution IR spectra collected as a function of time. More specifically, at first, a mapping of the  $OH/CH$  area ratio as a function of different solution concentration values is carried out; then the  $OH/CH$  area ratio for the acoustically levitated droplets for different levitation time is calculated and finally by using density data it is possible to determine the diameter evolution as a function of time and hence to test the  $D_2$  versus time law of the drying process theoretical model. The direct and indirect measurement methods can be considered a double validation to the drying process model.

## 5. Experimental results for theoretical model testing

The drying process theoretical model is tested before by a direct measurement and then by the conjunction use of acoustic levitation and IR spectroscopic technique. The measurements were performed on disaccharide solutions that were analyzed as substances with high solubility in water in order to achieve a high solid content in the sample solutions. The solubility of the disaccharide in water is ca. 200g/100ml at 25°C. In this study, the investigated systems were trehalose and sucrose aqueous solutions, manufactured by Sigma-Aldrich, as a function of disaccharide weight fraction percentage. The solutions were prepared by mass, using an analytical balance with  $\pm 0.01\text{mg}$  accuracy. Levitated solutions were also analyzed in this study. Double-distilled water was used in making the solutions. Surface tension measurements were carried out using a standard thermostated stalagmometer, calibrated with distilled water ( $\sigma = 72.8\text{mN/m}$  at  $T = 20^\circ\text{C}$ ). A constant temperature bath was used to control the temperature of the solutions to an accuracy of  $\pm 0.1^\circ\text{C}$ . Density measurements were performed by standard pycnometer technique. Measurements for each solution were repeated four times. The surface tension of a liquid mixture is an important property, which plays an important role in affecting the mass and heat transfer at the interface. Droplets of 1.5mm of radius were inserted into the pressure node of the sound field using a micropipette injection. The Sound Pressure Level (SPL) was set up between 155 and 160 dB and the experiments were performed three times. The direct measurement was performed by means of a Hitachi KP-HD20A video camera, with a resolution of 1944 H x 1092 V (2.1 megapixels) and by laser pointing, studying the  $D^2$  vs  $t$  data of trehalose and sucrose aqueous solutions as a function of time, that follow a linear trend for 20 minutes, showing an ability to avoid total water evaporation since a percentage of water is strongly bonded with the disaccharide (Branca *et al.* 2003a; Migliardo *et al.* 2013a; Magazù *et al.* 2018; Cannuli *et al.* 2019). Such a property plays a key role in bio-protection. Another analysis that allows to extract information on the drying

process of aqueous solution of the disaccharide is to consider the intramolecular region of spectra. The mathematical procedure to evaluate concentration is a mapping of the *OH/CH* band area ratio as a function of different solution concentration values. In summary, an *OH/CH* band area ratio measure for the acoustically levitated droplets for different levitation time is performed and then, by using density data, it is possible to determine the diameter evolution as a function of time. In such a way, it is possible to test the  $D^2$  law of the drying process theoretical model. In this context, spectroscopy techniques, such as IR spectroscopy, in conjunction with acoustic levitation, permit to determine the *OH/CH* stretching area as function of time. In general, from the *OH/CH* area, the concentration values can be determined as follow:

$$\Phi(t) = \frac{n_{disaccharide}M_{disaccharide}}{n_{disaccharide}M_{disaccharide} + n_{water}M_{water}} \quad (12)$$

with  $\Phi(t)$ =weight concentration as function of time;  
 $M_{disaccharide}$ =molar mass of disaccharide= 343.2g/mol;  
 $n_{disaccharide}$ =number of moles of disaccharide= const;  
 $M_{water}$ =molar mass of water= 18g/mol;  
 $n_{water}$ =number of moles of water, variable with time.

Concentration values were obtained by comparing levitated spectra, calculated to the concentration values, as a function of time and the agreement was very positive. The hypothesis that was made at this point is that it is just water to evaporate and to contribute to the drying process of the droplet. The density measured is represented as a function of the concentration:

$$\rho = \rho(\Phi) \quad (13)$$

Figure 4 shows the density of trehalose (on the left) and of sucrose (on the right) aqueous solutions as a function of disaccharide weight percentage [%] at T=25°C. It increases as disaccharide content increases.

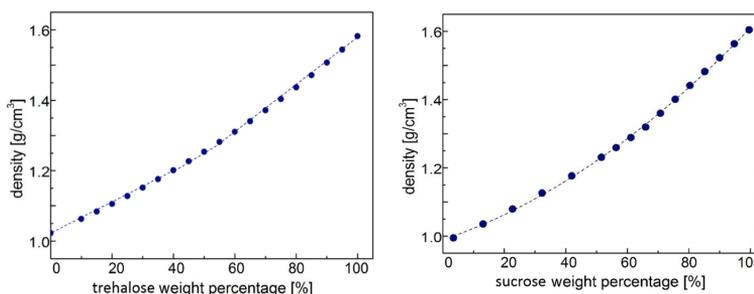


FIGURE 4. Trehalose (on the left) and sucrose (on the right) aqueous solutions density as function of disaccharide concentrations at T=25°C.

From the density formulation, represented by the ratio between the total mass and the volume, i.e.:

$$\rho(t) = \frac{M(t)}{V(t)} \quad (14)$$

it is possible to calculate the volume  $V(t)$ .

The total mass is equal to:

$$M(t) = n_{disaccharide}M_{disaccharide} + n_{water}M_{water} \quad (15)$$

where the hypothesis made is that only the number of the water moles change as a function of time. By using density data, it is possible to determine the diameter evolution as a function of time and hence to test the  $D^2$  versus time law of the drying process theoretical model in the following way:

$$V(t) = \frac{4}{3}\pi r^3 \quad (16)$$

$$r(t) = \sqrt[3]{\frac{3V(t)}{4\pi}} \Rightarrow D = 2r(t) \quad (17)$$

Determining the diameter of the droplet, it is possible to compare it with the  $D^2$ -law:

$$D^2 = D_0^2 - \beta t \quad (18)$$

The spectrum analysis allowed to understand how the sample loses the contribution of the water as a function of time, in the specific case, after 30 minutes the links between the disaccharide and  $H_2O$  become so strong that they result inseparable. The humidity of the room is maintained constant thanks to a dehumidifier. Wavelet analysis (Daubechies 1990; Li and Nozaki 1997; Grinsted *et al.* 2004; Chen *et al.* 2009; Magazù *et al.* 2012; Caccamo *et al.* 2016; Marchese *et al.* 2017; Caccamo *et al.* 2018a; Cannuli *et al.* 2018a), was employed for removing noise from the spectra. Figure 5 shows the area of the OH-stretching and CH-contribution ratio of levitated trehalose (on the left) and sucrose (on the right) aqueous solutions at  $T=25^\circ\text{C}$  as a function of time for an initial concentration value of 0.50. By comparing the spectra area ratio as a function of concentration and the spectra area ratio as a function of time it is possible to estimate that the mixture concentration values. In the insert, the area of the OH-stretching and CH-contribution as a function of disaccharide concentration is reported.

As it can be seen, in both disaccharides, the value of area ratio decreases with the time increasing. By comparing the spectra area ratio as a function of concentration and the spectra area ratio as a function of time it is possible to estimate the mixture concentration values. The agreement between the two data-set is satisfactory. Finally, by using density data it is possible to determine the diameter evolution as a function of time and hence to test the  $D^2$  versus time law of the drying process theoretical model. Figure 6 shows the  $D^2(t)$  curve of trehalose (on the left) and of sucrose (on the right) aqueous solutions. In particular, a linear decrease of the radius squared until the critical point appears where a crust form. The transition is not a sharp break, but changes over a long time form a bend in the curve.

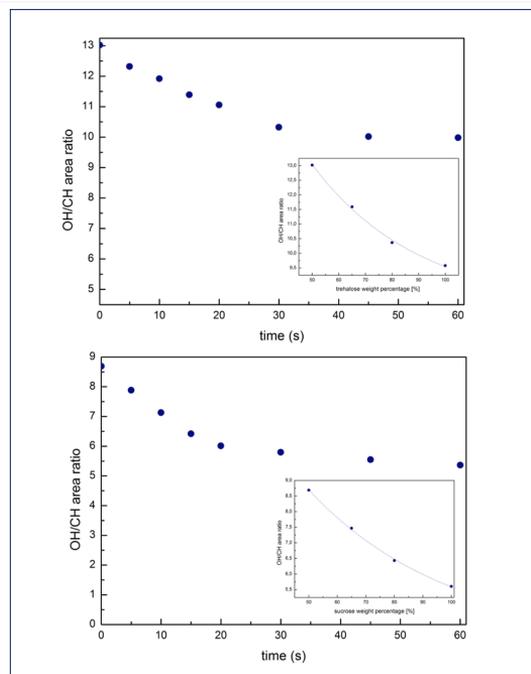


FIGURE 5. OH/CH area ratio for levitated trehalose (on the left) and sucrose (on the right) aqueous solutions at  $T=25^{\circ}\text{C}$  in a concentration range of 50% water and 50% pure sample. In the insert, the area of the OH-stretching to the CH-contribution ratio as a function of disaccharide concentration at a temperature value of  $T=25^{\circ}\text{C}$ , is reported.

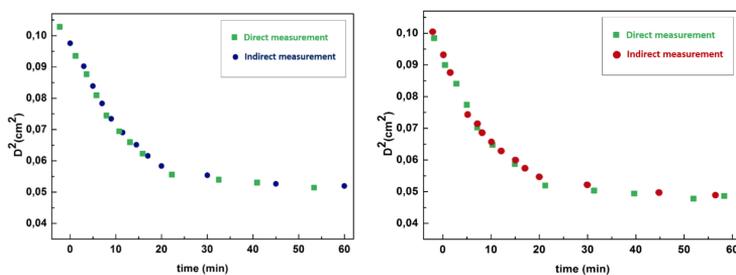


FIGURE 6. Drying behaviour of trehalose (on the left) and of sucrose (on the right) solutions droplets at  $25^{\circ}\text{C}$  dependent on the disaccharide content ( $D^2(t)$ ). The blue points represent the trend of indirect measurement, while the green points, the trend of the direct measurement, that are in accord.

The experimental results show as, both in the case of trehalose and sucrose aqueous solutions, the drying model prediction is in accord in the first trend. The variation of the droplet diameter, as a function of time, is equal both in the theoretical model, that in direct and undirect measurement. The experimental findings, obtained by the two independent employed approaches, reveal, for both the samples, a good agreement with the theoretical model previsions for the initial time trend. Furthermore, they furnish a value of the surface tension which is higher for trehalose in respect to sucrose.

## 6. Conclusions

In this paper, the experimental results for testing a theoretical model describing the drying process of an acoustically levitated droplet of a solution following two different approaches were reported. In particular, the so-called  $D^2$  versus time law was tested on trehalose and sucrose aqueous solutions. The theoretical model showed experimental evidence both for the diameter determination, performed by the direct monitoring with the video camera, and by evaluating the diameter from the analysis of the OH/CH area ratio of the solution InfraRed spectra collected as a function of time. It was shown how acoustic levitation, combined with spectroscopic techniques, allowed to explore a wide disaccharide concentration range and to test the dependence of the diameter law as a function of lag time, i.e.  $D^2$  versus time. By these analyses, it emerged that the behaviour of  $D^2$  vs t follows a linear trend for about 20 minutes, reaching then a plateau at longer time. The result was confirmed by the analysis of the intramolecular region, showing that trehalose is more able to avoid total water evaporation than sucrose, this result showed that trehalose owns more protectant properties. Furthermore, they furnished a value of the surface tension which is higher for trehalose in respect to sucrose.

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