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NIR spectroscopy coupled with multivariate data analysis in the prediction of the characteristics of mannitol lyophilized cakes

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Abstract

Mannitol is used in freeze-dried products as a bulking agent, consistency enhancer, or stabilizer, having the lowest hygroscopicity among the excipients commonly used as consistency agents, therefore it can be used in the formulation of unstable preparations. Lyophilization is a complex method of drying a solution at low temperature and low-pressure that addresses especially thermolabile substances, such as proteins. The end-product is a lyophilized powder, which can be administered parenterally after reconstitution with suitable solvents.

The objective of this study was to evaluate freeze-dried products obtained from mannitol solutions of different concentrations. Mannitol solutions of increasing concentrations of 2.5%, 5%, 7.5%, and 10% were prepared by dissolving mannitol in distilled water. The prepared solutions were freeze-dried after a preliminary DSC analysis, in which the thermal phenomena that occurred during lyophilization were identified. Freeze-dried preparations were analyzed by different methods: macroscopic analysis by visual assessment and comparison with data from the literature, texture analysis by which several properties of these preparations were studied (hardness, deformation, mechanical work, adhesive strength, fracture resistance, and the number of fractures), evaluation of reconstitution time and porosity. The mannitol used in the lyophilization process, being partially amorphous, required differential calorimetric analysis to establish its glass transition and to avoid the collapse of the preparations during lyophilization. Finally, NIR spectroscopy was used to predict the characteristics of the freeze-dried powders in a non-invasive manner, without prior sample preparation.

Keywords: lyophilization, mannitol, NIR, MVDA

INTRODUCTION

Lyophilized products are the dosage forms of choice when the preparation of stable parenteral products is desired. Freeze-drying increases product stability, being one of the most used drying techniques (1,2). It consists of three consecutive stages: freezing, primary,

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and secondary drying (3). Parenteral dosage forms regulations for excipients are strict, also the number of suitable excipients is limited (4). The freeze-dried products for parenteral administration meet the pharmacopeial requirements for the uniformity of dosage units, uniformity of content and uniformity of mass (5).

Bulking agents in lyophilized formulations are used for improving solubility and stability and promote controlled or prolonged drug delivery.

Mannitol represents one of the most frequently used bulking agents in lyophilization alongside glycine, lactose, and sucrose (2). It can exist in five different physical forms, out of which two of them (the amorphous mannitol and mannitol hemihydrate) are metastable and tend to transform into anhydrous crystalline forms during lyophilization or product shelf life (6). Mannitol needs to be in an amorphous form to have a stabilization effect as the long-term stability of the product can be affected by the incomplete crystallization during the freezing step (2).

Differential scanning calorimetry (DSC) is one of the most widely used analytical techniques applied to determine qualitative and quantitative information on the thermal properties of solid materials such as the melting and degradation temperatures, glass transition temperature, melt and crystallization enthalpy, specific and latent heats, and polymorphism (7,8). The selection of sample mass, sample heating, and cooling rate control is critical for DSC test result accuracy (8). Another application of DSC is the evaluation of physicochemical interactions between the active pharmaceutical ingredient (API) and excipients to identify the most compatible combinations (9).

Near infrared (NIR) spectroscopy offers the chemical information from the spectral features and allows to inspect vials variability (1). The advantages offered by NIR spectroscopy are the possibility of continuously monitoring the chemical and physical characteristics during freeze-drying, in a non-invasive and non-destructive manner (10).

This study was performed following a request from the local pharmaceutical industry to formulate a freeze-dried generic product for parenteral delivery containing as active pharmaceutical ingredient a non-steroidal anti-inflammatory (NSAID). The original product contained mannitol as a filler, so this manuscript presents a preliminary study aiming to test the behaviour of freeze-dried mannitol solutions of different concentrations regarding their reconstitution capacity, mechanical properties, and porosity. Further, a NIR-chemometric method was developed to predict some of the physical characteristics using a non-invasive method, NIR spectroscopy coupled with multivariate data analysis (MVDA).

MATERIALS AND METHODS

Materials

Mannitol (Pearlitol® 200M) was purchased from Merck & Co (Darmstadt, Germany).

Methods

Mannitol solution preparation

50 ml of four mannitol solutions were prepared, of 2.5%, 5%, 7.5% and 10% (w/V). They were divided into 25 vials of 2 ml each.

Differential scanning calorimetry (DSC)

The DSC measurements were performed by a Mettler-Toledo (Mettler-Toledo GmbH, Greifensee, Switzerland) to determine the glass transition temperatures (Tg') and the crystallization events of the prepared solutions in their frozen states (before freeze-drying). From each of the liquid formulations, 15-25 mg were loaded into aluminum pans with pierced lids and cooled from 25 to -55°C at a rate of 10°C/min, then reheated to 25°C at a rate of 20°C/min.

Freeze-drying

For each mannitol solution, 15 samples of 2 ml were taken using an automatic pipette and placed in lyophilized vials. Mannitol solutions were lyophilized using VirTis Advantage Plus (SP Scientific, Gardiner, NY, USA). A fast-freezing regime was applied to -48°C at a speed of 1°C/min (Figure 1). For the complete solidification of the product, this temperature of -48°C was kept constant for 6 hours. The primary drying was performed at a temperature of -25°C, the pressure of 150 mTorr, for 40 hours. Secondary drying took place at a temperature of 20°C, pressure of 350 mTorr, for 2.5 hours.

Reconstitution time

The reconstitution time was measured twice, first right after freeze-drying and second after one year of

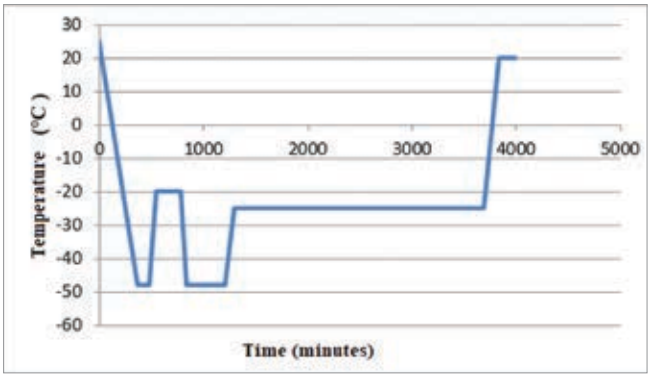

FIGURE 1. Freeze-drying process parameters

keeping in the desiccator at a temperature of 20 ± 1°C. The reconstitution time was determined manually by injecting the solvent into the lyophilized preparation vial. 2 ml of distilled water was used for the reconstitution and the time necessary for the complete mannitol dissolution was recorded. A mean of five measurements and standard deviation (SD) were calculated.

Porosity

To calculate the porosity of the preparations, the diameter and height of the samples were measured. Using the average of the 2 measurements the sample volume was calculated with the following formula: $V=\pi(d/2)^2xh$), where V-volume, d-diameter, h-height. The weight of the freeze-dried product was calculated by the difference of weight for each sample vial and the empty vials.

The density of the preparation was calculated with the formula: $\rho = m / V$, where ρ - the density, m - the weight of the freeze-dried product.
The porosity was determined from the equation $\varepsilon=(1-\rho)/\rho a$, where ε – porosity and $\rho a=1.514$ g/cm^3 – the true density of mannitol.

Texture analysis

Texture analysis was performed using Brookfield TexturePro CT V1.5 (Brookfield Engineering, SUA) equipped with an acrylic probe (TA10), directly into the freeze-drying vials. Constant pressure was applied at a load of 1 g and a speed of 0.2 mm/s, until a target distance of 7 mm. Load vs. distance profiles were recorded for 5 samples of each concentration and several parameters were calculated: hardness, deformation, hardness work, adhesive strength, brittleness, and the number of fractures, as means of five measurements and standard deviations.

NIR-spectroscopy

A portable MicroNIR PAT-U (Viavi Solutions, California, SUA) spectrometer was used. The apparatus was equipped with Linear Variable Filter technology, which allows the reduction of the device's size and the direct measurement through the base of the vial. Spectra were recorded in reflectance mode, over the full range of the spectrometer, 950 to 1650 nm, with a resolution of 6 nm. Each spectrum represented the average of 200 scans, recorded with an integration time of 7 ms/scan. All parameters have been set using the device's own software, JDSU (California, USA). Five samples (vials) were evaluated from each concentration, five spectra being recorded directly through each lyophilized vial, resulting in a total of 100 spectra.

MVDA

The data was imported and analyzed using the SIMCA 14.0 software (Sartorius Stedim, Sweden). Principal Component Analysis (PCA) was performed to identify which spectral domain is specific for changes in the physical characteristics of the cake. The multivariate prediction model was developed using the Orthogonal Partial Least Squares (OPLS) method, which had the purpose to separate the X – specific spectral systematic variation into predictive and orthogonal (uncorrelated) fractions (11).

RESULTS AND DISCUSSION

Differential scanning calorimetry (DSC)

Thermal events for 2.5% and 10% mannitol solutions were analysed by DCS. The thermogram of the 2.5% mannitol solution is presented in Figure 2 with an average Tg' at -29.36° C, and the onset of Tg' at -30.96° C. In Figure 3 the DSC thermogram of the 10% mannitol solution with an average Tg' at -29.36° C is presented, and the onset of Tg' at -30.55° C.

Following the DSC analysis and the presented thermograms, it can be observed that at -10° Cthe crystallization of mannitol solutions, which is an exothermic process, took place. After crystallization, the temperature drops to -55° Cwhere the preparation is completely frozen. Then the heating process has begun and at -30° C the amorphous mannitol becomes glassy, a state in which the product had a lower viscosity. During the primary drying of lyophilization at this temperature the structure can collapse, due to the lack of rigidity. The primary drying should take

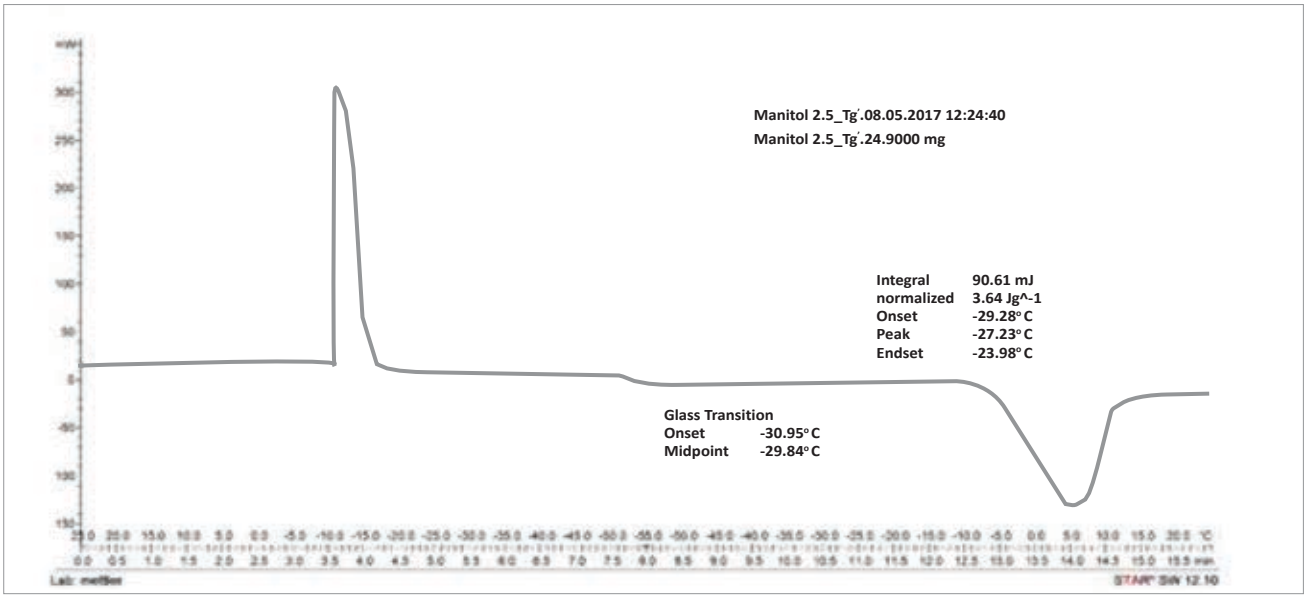

FIGURE 2. Thermogram of 2.5% mannitol solution

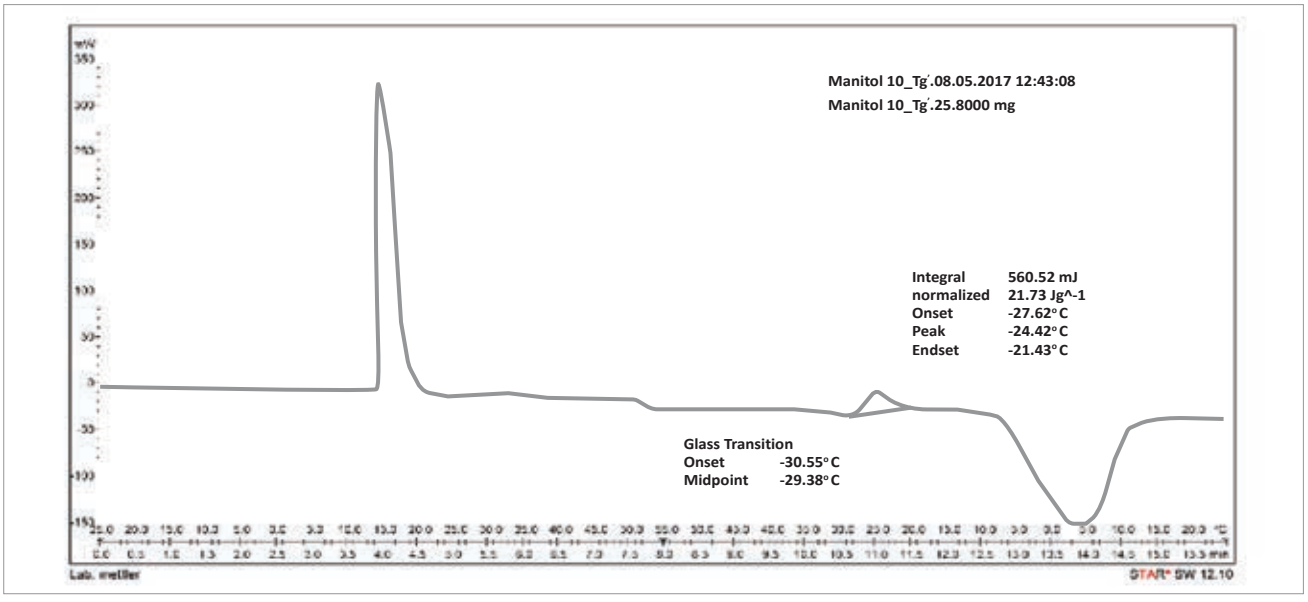

FIGURE 3. Thermogram of 10% mannitol solution

place at a lower temperature compared to the glass transition, when the structure is rigid, for example below -32°C. At such a low temperature, the primary drying proceeds much more slowly. As such, an annealing step was applied at -20°C during the freezing stage, which would produce complete crystallization of mannitol and eliminate the glass transition (Figure 1). Therefore, a higher temperature, -25°C, could be chosen for the primary drying. Beyond the temperature of -25°C the endothermic melting of the preparation begins.

Reconstitution time

Table 1 shows the reconstitution times obtained for each of the lyophilized products. The evaluation of the reconstitution time was made immediately after preparation and after keeping the samples in the desiccator for 1 year. The results showed that increasing mannitol concentration, the time required for dissolution increases. In addition, significant

TABLE 1. Reconstitution time of the freeze-dried products

Mannitol concentration (%)	Reconstitution time 0 (s)	Reconstitution time 1 year (s)
2.5%	16.574 ± 1.063	13.6 ± 1.396
5%	20.408 ± 1.663	17.236 ± 1.642
7.5%	33.89 ± 3.993	16.012 ± 2.416
10%	28.862 ± 4.651	21.78 ± 2.264

TABLE 2. Porosity of the freeze-dried products

Mannitol concentration (%)	Porosity (g/cm³)
2.5%	0.6442 ± 0.0013
5%	0.6296 ± 0.0011
7.5%	0.6132 ± 0.00048
10%	0.5971 ± 0.0016

differences ($p < 0.05$) are observed between time 0 and time 1. Reconstitution is faster after holding in the desiccator, due to the elimination of the residual moisture.

Porosity

Table 2 shows the results of the porosity of the four freeze-dried products with different mannitol contents. The results showed that the 2.5% mannitol cake presented a more porous structure, whereas the 10% mannitol product showed the lowest porosity. As expected, increasing the solid content resulted in a porosity decrease. As high porosity or high surface area allows fast water immersion in the freeze-dried cake, it should have an impact on reconstitution behaviour. When comparing the two sets of results, the one for porosity with the reconstitution time, the correlation between the two parameters is revealed: high porosity facilitated reconstitution.

Texture analysis

The freeze-drying technique proved to be a major step in the development of stable parenteral products since the residual moisture is reduced, the stability of the formulation increases, and the reconstitution time is shortened due to the porous structure of the cake. At the same time, the brittle structure may be subjected to cracking and powdering during handling and transportation (12).

The application of the texture analysis technique to study the compressive mechanical properties of the lyophilized products directly in glass vials has been previously described as a sensitive and reliable method (13). Thus, the analysis of the intact cake may provide useful information about the mechanical strengths and texture properties such as tendency to fractures and elasticity. The analysis of the freeze-dried products directly in lyophilization vial avoids the distortion of the samples and water absorption during manipulation (13).

The load vs. time profiles of the four mannitol samples are presented in Figure 4. An increase of the load can be observed with the increase of the mannitol content in the lyophilized products.

The results showed an important influence of the mannitol content on the mechanical properties of the dried cakes. As shown in Table 3, the hardness increased while the mannitol concentration increased. There was an increase in the mechanical work required to apply the force as the mannitol concentration increased. The highest value of adhesiveness was obtained for the sample with 10% mannitol and the lowest for the one with 5%. The variation of the adhesiveness did not correlate with that of the mannitol content. The brittleness varied

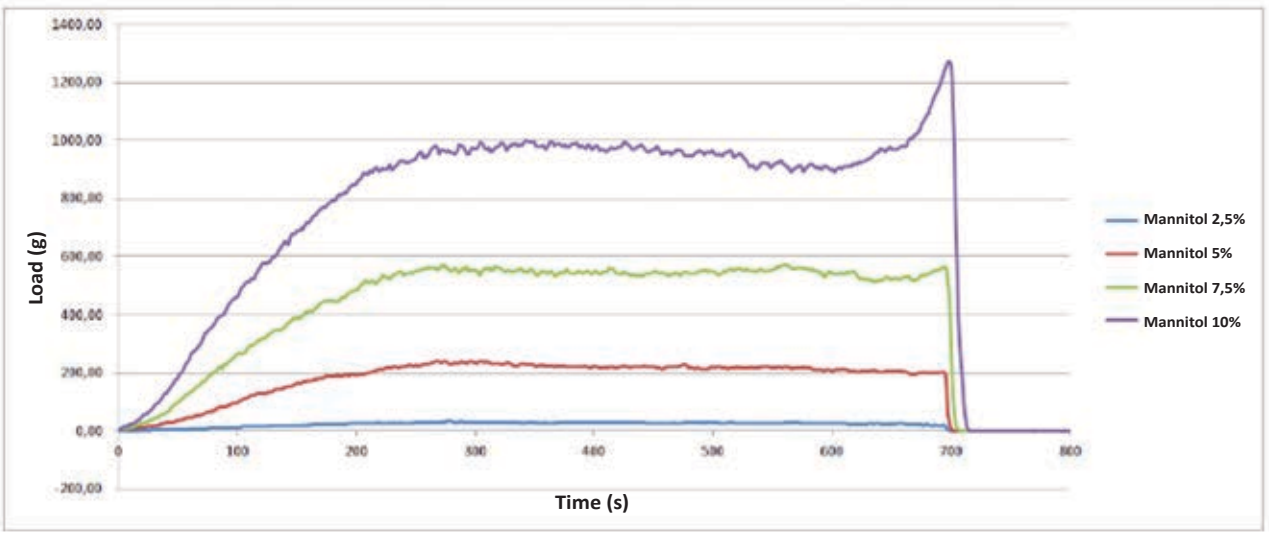

FIGURE 4. Texture profiles of lyophilized mannitol products

TABLE 3. Parameters calculated from the texture analysis of lyophilized preparations

Sample	Hardness (g)	Deformation (mm)	Hardness work (mJ)	Adhesive force (g)	Brittleness (g)	Number of fractures
Mannitol 2.5%	37.5 ± 5.5	2.8 ± 0.24	1.7 ± 0.24	2.9 ± 1.1	1.4 ± 1.0	149 ± 7
Mannitol 5%	253.7 ± 58.2	3.6 ± 1.28	12.5 ± 3.08	1.9 ± 0.7	40.6 ± 40.1	80 ± 9
Mannitol 7.5%	625.3 ± 147.9	4.9 ± 2.20	30.6 ± 6.85	2.6 ± 0.8	241.4 ± 94.9	66 ± 7
Mannitol 10%	1330.8 ± 278.6	6.7 ± 0.65	56.3 ± 8.05	4.0 ± 0.8	479.2 ± 145.2	47 ± 17

proportionally to the hardness for samples with low concentrations of mannitol, high values of the number of fractures were obtained.

Mannitol showed its advantage to reinforce the structures of the lyophilized matrix, consistent with the results of other reports investigating the mechanical strength and the tendency to fractures of freeze-dried products (14,15). Additionally, mannitol has been reported to improve the appearance of the cake by forming intact and porous structures, with superior appearance properties when compared to glucose, lactose, dextran 20, or sucrose (14).

NIR-spectroscopy

The spectral evaluation began with PCA, pursuing two objectives, to establish the most suitable spectral preprocessing method and to identify the spectral areas sensitive to changes in properties of interest i.e., mannitol concentration and cake porosity.

Several spectral preprocessing methods have been tested, over the whole registered spectral range as well as over the ranges representing the most spectral variation. By applying the 1st order Savitzky-Golay derivative baseline effects and linear trends were eliminated and the resolution of the spectra was improved. The model has been reduced to the spectral range representing the most significant variation, 1100 to 1300 nm (Figure 5). One more spectral domain, between 1450 and 1600 nm, registered high variations in intensity. However, this domain did not reflect the properties of interest and did not improve the predictive performance, therefore it has not been included into the final model.

The statistical values reflecting the performance of the PCA model developed by considering the spectral range from 1100 to 1300 nm are presented in table 4. The calculated cumulative R2X representing the data correlation was 0.969, for a model with two main components explaining 50.5% and 46.4% of the spectral intensity variation.

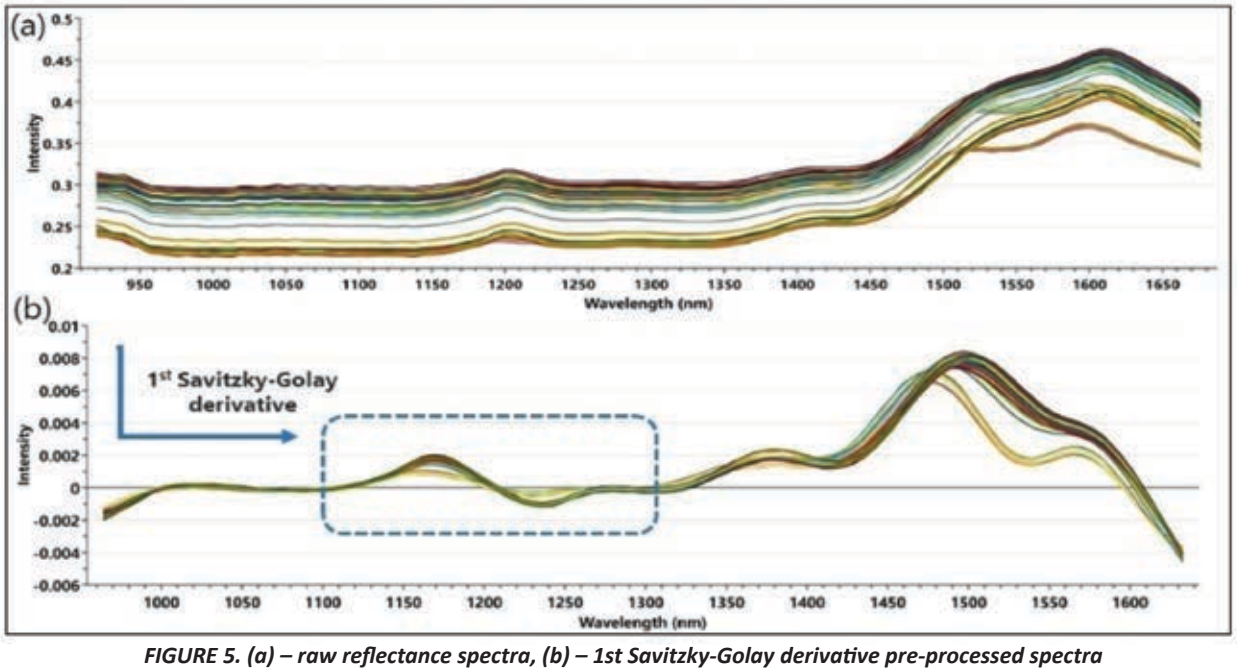

FIGURE 5. (a) – raw reflectance spectra, (b) – 1st Savitzky-Golay derivative pre-processed spectra

TABLE 4. PCA model parameters

Principal components	R^2X	R^2X cumulative	Value	Q^2	Q^2 cumulative
1	0.505	0.505	17.2	0.463	0.463
2	0.464	0.969	15.8	0.936	0.966

TABLE 5. Observed vs predicted mannitol concentration and porosity

Concentration (%)			Porosity (g/cm3)		
Observed	Predicted	Recovery (%)	Observed	Predicted	Recovery (%)
2.5	2.562	102.5	0.644	0.644	100.0
5.0	5.873	117.4	0.629	0.622	98.9
7.5	7.567	100.9	0.613	0.612	99.8
10.0	8.696	87.0	0.597	0.606	101.5

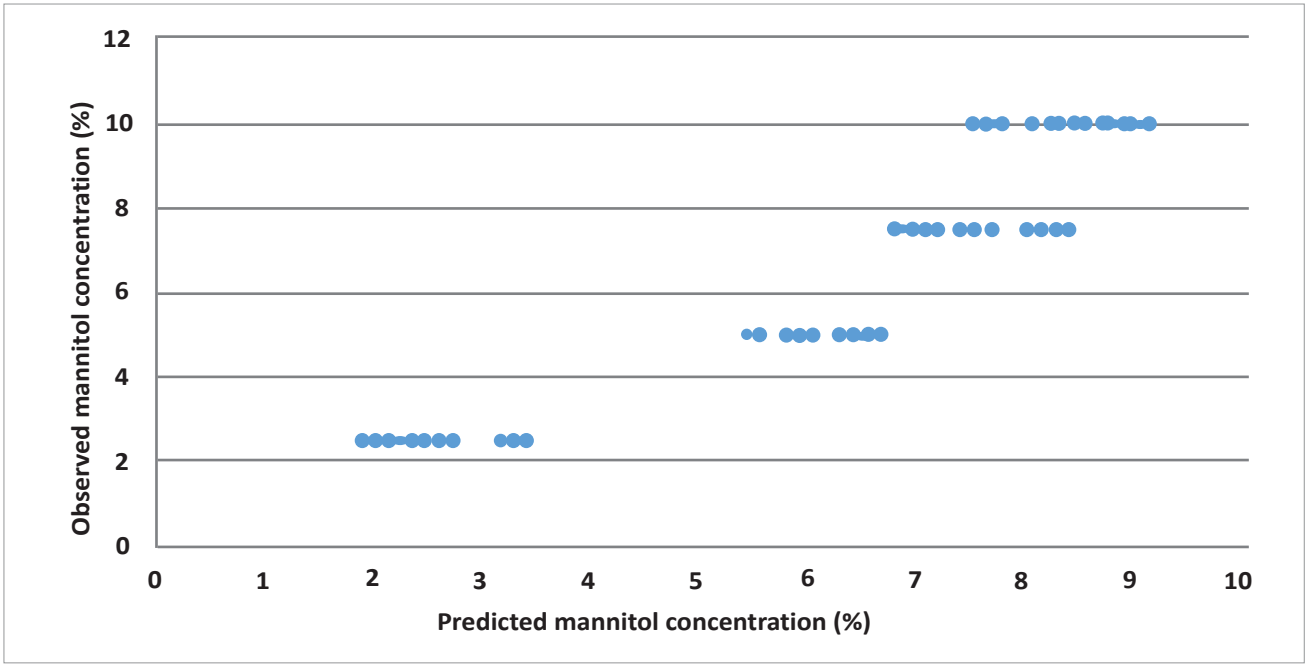

FIGURE 6. Observed vs. predicted – mannitol concentration

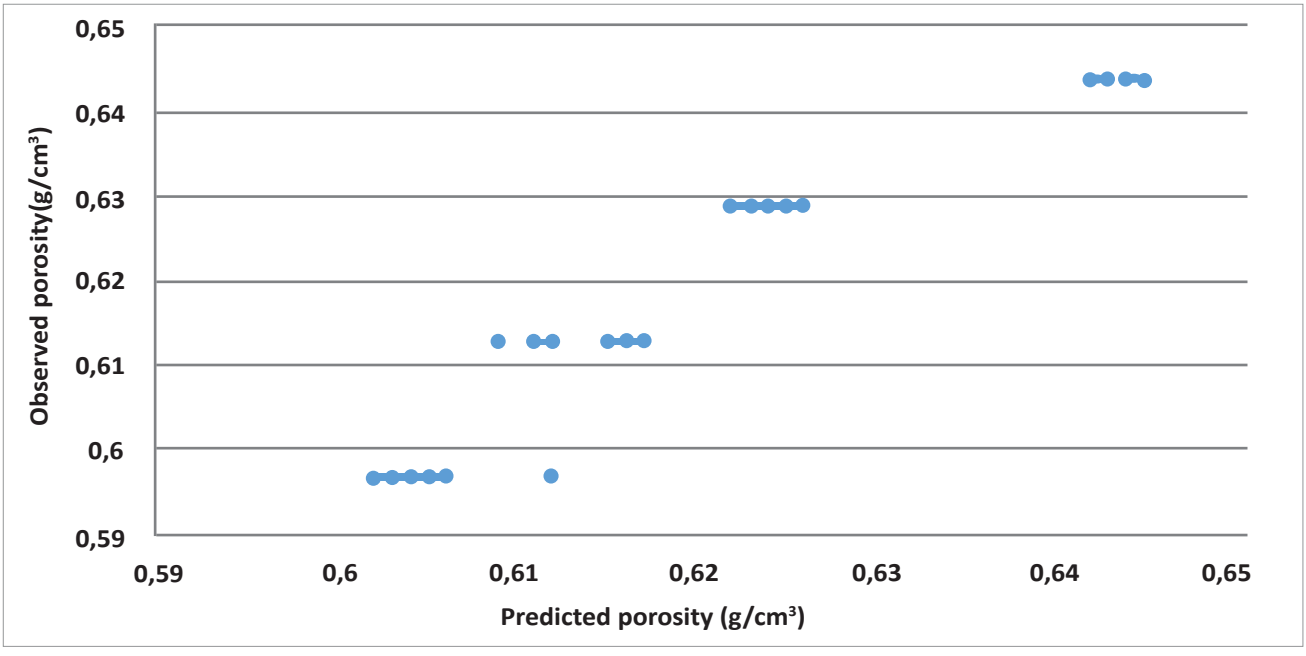

FIGURE 7. Observed vs. predicted – porosity

The OPLS prediction model was developed with the purpose to estimate mannitol concentration and cake porosity, its defining parameters being presented in the Table 5. The predictive fraction of the model explaining 78% of the spectral variation, while the orthogonal (uncorrelated) fraction just 21.3%, thus resulting a cumulative R2X of 0.993.

The measured and NIR – predicted values of the two properties of interest are illustrated in the two following figures (Figure 6, Figure 7) and numerically presented in Table 6.

The cake's porosity prediction model delivered accurate results, the estimated values differing by a maximum of 1.5 % units from the measured ones, for all sample concentrations.

Regarding the mannitol concentration prediction capacities of the NIR method, an overprediction can be observed for the 5 % samples where the predicted values lay at an average of 5.8 %. Another model limitation can be observed for the most concentrated samples. The 10 % mannitol solution is underpredicted at only 8.7 %. However, the prediction sets for all concentration were relatively precise, with relatively low coefficients of variation, of maximum 6.37 %, and no outliers.

The limitation of the technique's sensitivity can be explained by the fact that generally, the NIR absorption bands are wide, register weak intensity and overlap each other, also the spectra include physical and chemical information about all sample components. For this reason, multivariate data analysis and chemometric processing are almost always required to correlate the spectral domain with the samples' properties of interest. Even so, the data analysis does not always have the capacity to lead to the development of very sensitive NIR methods (16,17).

One other fact inducing the methods limitations could be the spectral acquisition in reflectance mode. This measurement mode is typically applied for solid samples, as scattering and absorbance of the particles on the surface of the sample cause changes of the signal intensity. Besides the diffuse reflectance recording mode, as the low absorption coefficient of the NIR spectroscopy allows increased penetration of the sample, the direct analysis in transmittance would also be possible. This particular technique, performed by using other types of NIR spectrometers, allows the radiation beam to pass through the lyophilizate cake and could provide spectra containing more representative information (18). Therefore, a parallel reflectance and transmittance spectral analysis and prediction methods development could potentially make the subject of further comparative studies.

CONCLUSIONS

In this paper, the mechanical and physical properties of the lyophilized mannitol preparations were analyzed. Mannitol is the most used excipient in the preparation of lyophilized products as a diluent or stabilizer and was used as a model substance, ensuring an easy and quick restructuring of the finished product. The important influence of the mannitol concentration on the mechanical properties, the reconstitution time and the porosity were demonstrated following the performed analysis. The increase in mannitol concentration led to harder structures, with high fracture resistance, prolonged reconstitution time and low porosity. Finally, the NIR spectroscopy evaluation of lyophilized samples led to the development of a valid predictive model for estimating the concentration and porosity of lyophilized preparations.

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