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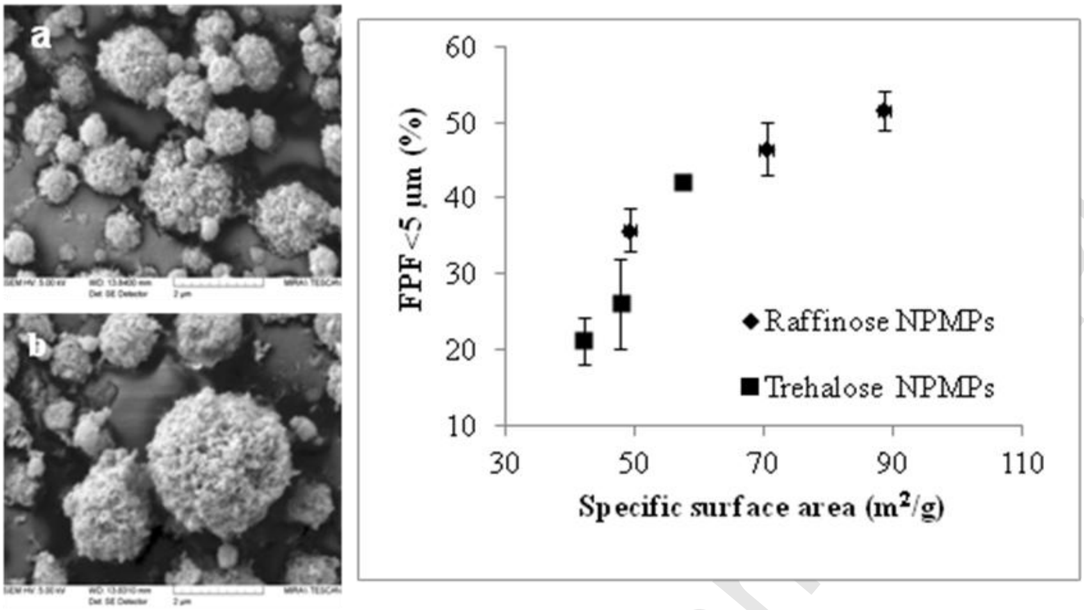
a
b
FPF <5 µm (%)
60
50
40
30
20
10
30
50
70
90
110
Specific surface area (m²/g)
◆ Raffinose NPMPs
■ Trehalose NPMPs

# Optimisation of spray drying process conditions for sugar nanoporous microparticles (NPMPs) intended for inhalation

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## ABSTRACT

The present study investigated the effect of operating parameters of a laboratory spray dryer on powder characteristics, in order to optimise the production of trehalose and raffinose powders, intended to be used as carriers of biomolecules for inhalation.

The sugars were spray dried from 80:20 methanol:n-butyl acetate (v/v) solutions using a Büchi Mini Spray dryer B-290. A $2^4$ factorial design of experiment (DOE) was undertaken. Process parameters studied were inlet temperature, gas flow rate, feed solution flow rate (pump setting) and feed concentration. Resulting powders where characterised in terms of yield, particle size (PS), residual solvent content (RSC) and outlet temperature. An additional outcome evaluated was the specific surface area (SSA) (by BET gas adsorption), and a relation between SSA and the *in vitro* deposition of the sugar NPMPs powders was also investigated.

The DOE resulted in well fitted models. The most significant factors affecting the characteristics of the NPMPs prepared, at a 95% confidence interval, were gas flow: yield, PS and SSA; pump setting: yield; inlet temperature: RSC.

Raffinose NPMPs presented better characteristics than trehalose NPMPs in terms of their use for inhalation, since particles with larger surface area resulting in higher fine particle fraction can be produced.

**Keywords:** Factorial design, Raffinose, Trehalose, Spray drying, specific surface area, Inhalation

## 1. INTRODUCTION

Pulmonary drug delivery using dry powder inhalers (DPI) is an important research area, impacting on the treatment of respiratory diseases such as asthma and, in recent years, as an alternative for systemic delivery of peptides and proteins, improving their bioavailability and effectiveness (Patton, 1996; Patton et al., 2004; Daniher et al., 2008). Where the protein/peptide to be used for pulmonary delivery is of low dose and high potency, it may be desirable to formulate it with a carrier material (inert excipient) to increase the volume of powder loaded and delivered from the DPI device. Protection of protein structure is critical in both processing and storage of the final formulation (Ní Ógáin et al., 2011).

Non-reducing sugars, such as raffinose and trehalose, possess properties which make them promising excipients for protection of biomolecules. They appear to be effective stabilizers of proteins in the amorphous state (Colaco et al., 1992; Johnson, 1997; Maury et al., 2005). Studies by Lopéz-Diéz et al. (2004), Maury et al. (2005), Yoshii et al. (2008) and Ní Ógáin et al. (2011) have established the protective action of trehalose and raffinose on protein integrity, avoiding loss of bioactivity. Carpenter and Crowe (1988, 1989) suggested the water replacement theory to explain the protective action of the sugar compounds, where the formation of H-bonding between the excipient and the biomolecule occurs when water is removed, maintaining the structural integrity of the peptide/protein. A second theory of protein protection through the use of such sugars was proposed by Franks et al. (1991) based on the formation of an amorphous glass during drying, which provides a rigid matrix around the protein molecules to restrict and stabilize their motion.

The DPI aerosol cloud should be constituted by particles with aerodynamic diameters between 1 and 3 μm with good dispersibility (good flow) to efficiently deliver the drug into the lower (alveolar) regions of the lungs (Johnson, 1997; Koushik and Kompella, 2004; Chow et al, 2007). Ní Ógáin et al. (2011) studied the *in vitro* deposition of spray dried trehalose and raffinose NPMPs powders using the Andersen Cascade Impactor (ACI). In this study it was determined that these powders had a mass median aerodynamic diameter of approximately 3 µm with a high fine particle fraction < 5 µm, making them suitable for pulmonary delivery.

Spray drying is a commonly used technique which may be employed to produce powders of fine particle size (Masters, 1991). The process consists of the atomization of a feed solution into droplets that dry rapidly because of their high surface area and intimate contact with the drying gas. Dried powder is protected from overheating by rapid removal from the drying zone. The final product can be removed from the air stream by cyclones and/or filters (Masters, 1991).

Micromeritic properties of the spray dried material, such as particle size, shape and density, can be controlled by altering spray drying parameters such as the inlet air temperature, liquid feed flow rate, the atomizer, or the viscosity, surface tension and feed solution concentration (Healy et al., 2008). When developing a new dry powder inhaler it is crucial to have a well characterised spray drying method and optimisation of the process involves an evaluation of the spray drying parameters, the liquid feed and product characteristics.

Billon et al. (2000), Stähl et al. (2002), Al-Asheh et al. (2003) and Tajber et al. (2009) have used factorial design studies to optimise spray drying processes, proving the usefulness of such statistical tools in understanding the process, saving time and reducing material costs.

Maltesen et al. (2008) carried out a design of experiment on the spray drying of insulin intended for inhalation on a Büchi Mini Spray dryer B-290 to understand the effects of process and formulation on powder/particle characteristics, such as yield, particle size, density, morphology and moisture content. Five variables were investigated: feed solution concentration, drying gas and feed flow rate, inlet air temperature and aspirator capacity. The variables with main effects on powder/particles characteristics were found to be feed concentration, inlet temperature and gas flow rate.

A limited number of studies have investigated the effects of spray drying parameters on the production of trehalose and raffinose powders for inhalation. Maury et al. (2005) reported that the most important spray drying parameter to improve trehalose powder yield, for non-porous particles spray dried from aqueous solutions, was the inlet temperature, with the nozzle (a two-fluid nozzle with cap-orifice diameter of 0.7 mm) and aspirator setting have little influence. Ní Ógáin et al. (2011) studied the production of trehalose and raffinose nanoporous microparticles (NPMPs) for inhalation, evaluating different ratios of methanol:n-butyl acetate (MeOH:BA) solvent system, and concluded that NPMPs spray dried from 80:20 (v/v) MeOH:BA displayed favourable micromeritic characteristics suggesting potential suitability for pulmonary delivery. The porous morphology of the particles was found to improve the aerosolisation properties compared to equivalent non-porous spray dried particles. Ní Ógáin et al. (2011) also demonstrated that a model protein could be incorporated into the carrier particles at a ratio of 1:4 (w/w) protein:carrier, while still retaining the characteristic porous morphology. However, the spray drying process developed to produce NPMPs of the two sugars was not optimised in terms of product characteristics or yield. Following on from the work of Ní Ógáin et al. (2011) our study investigates the effect of operating parameters of a Büchi B-290 laboratory spray dryer and feed solution concentration, in order to optimise the production of trehalose and raffinose NPMP powders, intended to be used as carriers of biomolecules for inhalation. A $2^4$ factorial design was undertaken with resulting powders characterised in terms of yield, particle size, thermogravimetric analysis and outlet temperature, as in a previous study in our laboratories (Tajber et al, 2009). An additional outcome evaluated was the specific surface area, as it is a reflection of porosity associated with porous particles such as the NPMPs (Healy et al., 2008).

Bosquillon et al. (2001) studied the effect of formulation excipients and physical characteristics of inhalation powders on their *in vitro* aerosolisation performance showing that fine particle fraction was affected by the excipient proportions and by the powder’s tapped density. Ní Ógáin et al. (2011) showed that raffinose and trehalose NPMPs had higher fine particle fractions (FPF) and higher specific surface areas (SSA) than the equivalent non-porous particle powders. Hence, a relation between SSA and the *in vitro* deposition of the sugar NPMPs powders was also investigated.

## 2. MATERIALS AND METHODS

### 2.1. Materials

d-Raffinose pentahydrate, d-(+)-trehalose dehydrate, phenol and concentrated sulphuric acid were purchased from Sigma, (Ireland). Solvents used were: n-Butyl acetate purchased from Merck (UK), methanol obtained from Lab Scan Analytical Sciences (Ireland) and, deionised water was obtained from Purite Prestige Analyst HP (Purite Limited, UK) water purification system.

### 2.2. Preparation of sugar NPMPs

Raffinose and trehalose were spray dried as solutions from 80% methanol/20% n-butyl acetate (v/v) using a Büchi Mini Spray dryer B-290 operating in the closed mode with an inert loop B295 accessory (Büchi, Switzerland). A 0.7 mm nozzle tip and a 1.5 mm diameter nozzle screw cap were used. Nitrogen (at 6 bar) was used as the drying gas in a co-current mode with aspirator capacity set to maximum (100%) as was the case in the previous studies conducted by Ní Ógáin et al. (2011). The remaining operating parameters were set according to the design of experiment. Spray dried particles were separated from the drying gas by a high-performance cyclone (Büchi, Switzerland), since previous studies have indicated an improved efficiency of this cyclone compared to a regular cyclone in collecting particles less than 2 µm in diameter (Brandenberger, 2002).

### 2.3. Experimental design

A randomised $2^4$ full factorial design with two replicates was devised to assess the effect of spray drying process parameters on powder production yield, particle size, specific surface area, residual solvent content and process outlet temperature. The parameters chosen to be studied were: (A) inlet temperature, (B) spray dryer airflow rate, (C) pump setting (feed solution flow rate), and (D) feed concentration. Each factor was studied at two levels: low and high (Table 1).

The setting for inlet temperature, pump setting and gas flow were based on preliminary one-factor-at-a-time studies where inlet temperature was varied between 90 and 140 ˚C and pump setting from 20 to 35%; practical work specifications were taken into account such as there being no condensation of the solution on the drying chamber during the process. The upper limit for feed concentration corresponds to the visually assessed limit of raffinose and trehalose solubility in the solvent mix used at room temperature.

The chosen factorial model was represented by:

$$Yi = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_{12} AB + \beta_{13} AC + \ldots \quad \text{(Eq. 1)}$$

where $\beta_n$ is the coefficient associated with factor, $n$, and the letters, $A$, $B$, $C$, etc., represent the factors (the spray drying parameters) in the model. Combinations of factors (such as *AB*) represent an interaction between the individual factors in that term. The equation coefficients were calculated using coded values, for purposes of direct comparison regardless of the factor magnitude. A low response is coded as -1 and a high response as +1 (Montgomery, 1997; Tajber et al., 2009).

Statistical analysis of variance, ANOVA, was performed to determine the significance (p-value) and impact (F-value) of each main factor as well as their interactions, using Minitab$^{TM}$ software (version 13.32). Parameters found to be significant at at least the 95% confidence level were included in the final prediction models.

## 2.4. Production yield

Yields were calculated by dividing the powder quantity collected by the quantity introduced into the feed solution, giving the yield per cent by weight (%).

## 2.5. Thermal Analysis

Thermogravimetric analysis (TGA) was used to determine the amount of residual solvent content (RSC) contained in samples after spray drying, as previously described (Ní Ógáin et al., 2011). All measurements were performed with a Mettler TG 50 (Switzerland) module linked to a Mettler MT5 balance Sample sizes of ~4 mg were used. To avoid interference from moisture in the air, the sample chamber was purged with dry nitrogen at a flow rate of 25 ml/min. A temperature range of 25 - 200°C for raffinose and 25-300°C for trehalose was employed with a heating rate of 10 °C/min. The TGA system was controlled by Mettler Toledo STARe software (version 6.10) and the RSC was defined as the weight loss in TGA between 25 and 130 °C.

## 2.6. Scanning Electron Microscopy (SEM)

SEM micrographs of spray dried materials were made by means of a MiraTescan XMU (Czech Republic). The samples were fixed on aluminum stubs using double-sided adhesive tape and sputter-coated with gold. Visualisation was performed at 5 kV and photo micrographs were taken at different magnifications in more than one region of the sample.

### 2.7. Particle size

The particle size (PS) distributions of the spray-dried powders were determined using a Mastersizer 2000 laser diffraction instrument (Malvern Instruments, UK) with a dry powder sample dispersion accessory (Scirocco 2000). Pressure was set at 2 bar and a vibration feed rate of 50% was used in order to achieve an obscuration between 0.5% and 6% (Ní Ógáin et al., 2011).  Samples were run at least in duplicate. Mastersizer 2000 software was used for data evaluation. The $d_{50}$ reported is the geometric median particle size of the volume distribution. The span of the volume distribution, a measure of the width of the distribution relative to the median diameter, was calculated according to Eq.2 (Maa et al., 1997):

$$\frac{\left[d_{(0.9)} - d_{(0.1)}\right]}{d_{(0.5)}} \qquad \text{(Eq. 2)}$$

### 2.8. Specific surface area

Specific surface area (SSA) of spray dried powders was measured by gas adsorption using a Micromeritics Gemini VI surface area and pore size analyzer (Micromeritics, U.K.). Adsorption measurements were performed with nitrogen gas as the analytical (adsorptive) gas and helium as the reference gas for free space measurements. Prior to analysis the samples were degassed under nitrogen gas, using a Micromeritics

SmartPrep degasser for 24h at 25°C to remove residual solvent content (Ní Ógáin et al., 2011). The evacuation conditions used in the analysis were as follows: rate of 500 mmHg/min, time 1 min. Equilibration time for adsorption was 10 seconds. The amounts of nitrogen gas adsorbed at a range of relative pressures, $0.05 < P/P_0 < 0.35$, were determined in order to calculate SSA by the Brunauer, Emmett and Teller (BET) method. Analysis was performed in triplicate for each sample.

## 2.9. *In vitro* aerosol deposition studies using the Next generation impactor (NGI)

The *in vitro* deposition of the dry powders was evaluated using a Next Generation Impactor (NGI, Copley Scientific Limited, Nottingham, UK) operated under pharmacopoeial conditions (Ph. Eur., 2011). As previously described by Tewes et al. (2010), the flow rate was adjusted to achieve a pressure drop of 4 kPa in the powder inhaler (Handihaler®, Boehringher Ingelheim, Ingelheim, Germany) and the time of aspiration was adjusted to obtain 4 l. The dry powder inhaler was loaded with a no. 3 hard gelatin capsule loaded with 20 ± 2 mg of powder for each test. After dissolution in an appropriate volume of water, particle deposition in the device, the throat and the stages and the filter was determined by the phenol-sulfuric acid colorimetric method in microplate format as previously described by Masuko et al. (2005). The sulfuric acid promotes the conversion of all non-reducing sugars to reducing sugars; these form a complex with the phenol turning the solution into a yellow-orange solution the absorbance of which is measured by UV spectroscopy at 490 nm (Fournier, 2001). Each test was repeated three times. The total amount of particles with aerodynamic diameters smaller than 5.0 µm was calculated by interpolation from the inverse of the standard normal cumulative mass distribution less than stated size cut-off against the natural logarithm of the cut-off diameter of the respective stages. This amount was considered as the fine particle fraction (FPF) (or respirable fraction) and expressed as a percentage of the emitted recovered dose (ED). The mass median aerodynamic diameter (MMAD) of the particles was determined from the same plot as the particle size corresponding to the 50% point of the cumulative distribution, and the geometric standard deviation (GSD) as $GSD = \sqrt{\frac{sizeX}{sizeY}}$, where $X$ is the particle size corresponding to the 84% point and size Y is the particle size corresponding 16% point of the cumulative distribution (Bosquillon et al., 2001).

## 3. RESULTS AND DISCUSSION

The coefficients of the final prediction models linking the spray drying and formulation parameters (in terms of coded factors) with responses are presented in Tables 2 and 3. To indicate the goodness of fit of these models the $R^2$ is also given in the latter tables. (The design matrix and the data collected from the particle characteristics evaluated for each sugar are provided in the supplementary material associated with this article as Tables 1S and 2S).

To establish whether the model equations can estimate the response values well, the observed versus predicted values for each outcome evaluated were plotted. Figure 1a and 1b show a good linear relationship between observed and predicted particle size for raffinose ($R^2$=0.95) and trehalose ($R^2$=0.94) spray dried powders, where experimental points obtained by navigating the design space, are close to the regression line. A similar relationship was observed for the remaining outcomes (data not shown).

In contrast to our results, studies by Baldinger et al. (2011) on the application of a two level full factorial design to the spray drying process of aqueous solutions with total solid concentration of 10% (w/v) containing a mixture of mannitol and trehalose in a mass ratio of 90:10 to produce inhalable dry powders, showed a poor linear relationship between the observed and predicted values obtained for the evaluated responses, using multivariate analysis as a statistical tool.

The latter study focused only on three parameters of the spray drying process: inlet temperature, atomization air flow rate and feed solution flow rate. The full factorial design presented in the current study, also evaluates the effect of the drying gas flow rate and concentration of the feed solution on the physicochemical characteristics of the sugar nanoporous microparticles. The difference in parameters investigated and in the feed solution formulation may be the reason for the difference between the two studies.

### 3.1. Production Yield

Spray dried raffinose yields varied between 30.2 and 71.6%, and trehalose yields between 28.8 and 64.9 %. In both studies the yield increased with a decrease in gas flow rate (*F*-value 39.61 ($p<0.0001$) for raffinose and, *F*-value 104.24 ($p<0.0001$) for trehalose), with this variable having the greatest effect. Lower gas flow reduces atomization energy producing larger particles, which are easier to capture in the cyclone (Technical data Büchi B-290, 2009; Stähl et al., 2002). Figure 2 supports this hypothesis showing a correlation between yield and particle size, where an increase in particle size results in an increase in yield.

Statistical analysis of the product yield values for raffinose powders demonstrated that among the main effects two have positive coefficients (an increase in the variable results in an increased response): pump setting and feed concentration, and one has a negative coefficient (an increase on the variable results in a decreased response): gas flow (Table 2). The inlet temperature was determined to be not significant ($p>0.05$). Stähl et al. (2002) and Tajber et al. (2009) also observed gas flow rate to have the largest effect on product yield, whereas Prinn et al. (2002) and Maltesen et al. (2008)

demonstrated that feed solution concentration had the highest impact on yield, and that gas flow was a less impacting variable.

Studies in technical data Büchi B-290 (2009) on the characterisation of the effect of variable parameters on spray dried powders demonstrated that gas flow and feed solution concentration have a large influence on the resulting particle size.

Considering the existence of a correlation between yield and particle size (Figure 2), it is expected that particle size would have an influence on powder yield.

An interaction between gas flow and feed concentration (F-value 9.50) was found to be significant ($p=0.007$) in the present study for raffinose and positively correlated, reinforcing the importance of these parameters.

Statistical analysis of trehalose powder yield values revealed two positive main effects: inlet temperature and pump setting, and two negative main effects: gas flow and feed concentration (Table 3). The inlet temperature, as well as the gas flow, had a strong impact on production yields (F-value 74.82, $p<0.0001$), consistent with results reported by Maury et al. (2005) for trehalose spray dried from aqueous solution, where high $T_{inlet}$ resulted in higher $T_{outlet}$ leading to drier and less sticky powders, increasing the production yield. Several interactions among operating variables were detected for trehalose productions yields: two factors, three factors and four factors (involving all main effects); showing how complex the spray drying process is and how one parameter will affect the response from the remaining parameters (Table 3 and Figure 3). The strongest interaction was between pump setting and feed concentration (CD) (F-value 27.24, $p<0.0001$) with a positive coefficient.

## 3.2. Particle size

Spray dried raffinose and trehalose powders consisted of small spherical and porous particles as shown by SEM (Figure 4). Particle size volume distributions in all cases were narrow and monomodal with low span values (between 1.2-2.3 for raffinose and 1.1-2.2 for trehalose). The median particle size ($d_{50}$) for raffinose particles was in the range of 1.4-4.6 µm and for trehalose particles was in the range 1.3-4.6 µm.

In the case of raffinose powders, ANOVA indicated that only the main effects were significant at the 95% confidence level and can be regarded as impacting on the size of particles. The process parameter with the greatest influence was the gas flow (F-value 267.39, $p < 0.0001$) followed by the feed concentration (F-Value 38.07, $p < 0.0001$). There are a number of reports in the literature which indicate that the size of particles in spray drying is controlled by feed concentration, where larger particles are obtained from more concentrated solutions and lower atomization levels (Elversson and Millqvist-Fureby, 2005; Technical data Büchi B-290, 2009). Lower gas flow reduces atomization energy producing larger particles, which are easier to capture in the cyclone (Stähl et al., 2002; Technical data Büchi B-290, 2009). From the model equation, a negative coefficient for gas flow and positive coefficient for feed concentration was found, which means, as gas flow decreases and feed concentration increases larger particles are produced, which is consistent with previous reports (Stähl et al., 2002; Al-Asheh et al., 2003; Tajber et al., 2009).

Statistical analysis of trehalose powders showed only three main effects: gas flow, pump setting and feed concentration were statistically significant with regard to particle size. F-values for these parameters are 229.24 ($p<0.0001$), 28.87 ($p<0.0001$) and 4.38 ($p=0.048$), respectively. Maury et al. (2005) reported that an increase in pump setting at constant atomizing air flow rate resulted in larger spray droplets, hence larger particles. The coefficients of the model equation are negative for gas flow and positive for pump setting and feed concentration, i.e., as gas flow decreases and pump setting and feed concentration increases, particle size increases which is consistent with previous studies (Stähl et al., 2002; Al-Asheh et al., 2003; Maury et al, 2005; Tajber et al., 2009). ANOVA also revealed a significant (at the 95% confidence level) interaction between two main effects: gas flow and pump setting (BC) (F-value 21.39, p=0.0001) (Table 3). In contrast to studies by Stähl et al. (2002), Al-Asheh et al. (2003) and Tajber et al. (2009), the inlet temperature had no significant effect on the particle size of raffinose or trehalose spray dried powders. The latter studies showed an increase in particle size with increasing inlet temperatures, due to agglomeration of particles at higher temperatures and to hardening of the droplet (Stähl et al., 2002; Al-Asheh et al., 2003).

### 3.3. Residual solvent content

Residual solvent content ranged from 1.6 to 4.6 % for raffinose powders and from 1.3 to 5.3 % for trehalose powders.

ANOVA showed that the statistical model developed for the residual solvent content values of raffinose powders was significant (F-value 5.17, p=0.0011). The inlet temperature was the only statistically significant main effect impacting on the residual solvent content (F-value 6.66, p=0.0201) and it had a negative coefficient. With increasing inlet temperature, more energy is supplied to the drying chamber leading to more efficient solvent removal from the droplets. This results in powders with lower residual solvent content (Maury et al., 2005; Technical data Büchi B-290, 2009). Also, several interactions were found below the established confidence level of 95% (Table 2 and Figure 6) and therefore deemed significant: a two-factor interaction between inlet temperature and pump setting (AC) (F-value 6.26, p=0.0201) and pump setting and feed concentration (CD) (F-value 16.32, p=0.0009), a three-factor interaction between gas flow, pump setting and feed concentration (BCD) (F-value 6.74, p=0.0195), and a four factor interaction between inlet temperature, gas flow, pump setting and feed concentration (ABCD) (F-value 25.35, p=0.0001).

The quantity of solvent to be evaporated is dependent on the inlet temperature and gas flow that affect the heat supply for droplet drying and on the pump setting and feed concentration that control the size and the solid and solvent content in the droplet. Raffinose NPMPs residual solvent content is the result of the effect of spray drying parameters on their own and of interactions between them.

ANOVA showed that the model established fitted the data obtained for trehalose powders well and was significant (F-value 10.86, p<0.0001). The main effects with major impacts are inlet air temperature (F-value 31.19, p<0.0001) and pump setting (F-value 46.10, p<0.0001). The impact of gas flow and feed concentration were not significant at the 95% confidence level. An increase in inlet air temperature will lead to a decrease in residual solvent content (negative coefficient, Table 3), in other words, an increased supply of heat energy will result in a more efficient drying. Pump setting is positively correlated with residual solvent content. When the pump speed is higher, more liquid is supplied to the drying chamber and more solvent vapour is generated, decreasing the exhaust temperature leading to a less efficient drying, hence higher residual solvent content (Maury et al., 2005; Technical data Büchi B-290, 2009). A lower pump setting should result in a higher outlet temperature and therefore more efficient drying, resulting in lower residual solvent contents (Figure 5).

Statistical analysis also revealed significant two-factor interactions between the following parameters: inlet temperature and gas flow (AB) (F-value 6.71, p=0.0197), inlet temperature and feed concentration (AD) (F-value 13.33, p=0.0022), gas flow and pump setting (BC) (F-value 6.70, p=0.0198) and pump setting and feed concentration

(CD) (F-value 29.77, p<0.0001) and a three-factor interaction was determined between inlet temperature, pump setting and feed concentration (ACD) (F-value 18.02, p=0.0006) (Figure 7).

### 3.4. Outlet temperature

The outlet temperature varied between 48 and 101 °C for raffinose spray drying, and between 49 and 103 °C for trehalose spray drying. This response was mainly affected by the inlet air temperature and pump setting, shown to be significant at the 95% confidence level, for both sugars (Table 2 and 3).

Increased inlet temperature leads to an increased supply of heat energy, leading to higher outlet temperatures (Tajber et al., 2009). Referring to the model equations for both sugars (Table 2 and 3), a positive correlation was found, i.e. higher setting of the inlet temperature resulted in higher outlet temperatures.

At a higher pump setting, more liquid is supplied to the drying chamber and more solvent vapour is generated, therefore decreasing the exhaust temperature (Maury et al., 2005; Büchi, 2009). The model equations, for raffinose and trehalose spray drying, showed negative coefficients for pump setting, i.e. as pump setting decreases, outlet temperature increases. Stähl et al. (2002) and Maltesen et al. (2008) reported the same effect for spray drying of insulin particles.

For the purpose of this study a high outlet temperature is desirable, leading to drier powders and higher yields. This can be seen on figure 5 which displays the relationship between the residual solvent content and the outlet temperature, where higher temperatures are observed to result in lower solvent residue content. A similar study by Billon et al. (2000) demonstrated the same relationship.

### 3.5. Specific surface area

Porous microparticles have potential advantages over non-porous materials as they have reduced interparticulate attractive forces and improved flow characteristics, low bulk densities and exhibit smaller aerodynamic diameters than their geometric diameters, facilitating greater deposition in the lower pulmonary region, with potential for improved efficiency of administration to the lungs in the dry form (Healy et al., 2008). The presence of porosity leads to high specific surface area (SSA) values (Papelis et al., 2003; Healy et al., 2008).

SSA values ranged from 25.53 to 80.34 $m^2/g$ for raffinose particles and from 17.52 to 59.02 $m^2/g$ for trehalose particles.

Evaluating results from raffinose powders, ANOVA calculations determined that the main effect with an impact on SSA was the gas flow (F-value 79.79, $p< 0.0001$). The other main effects (inlet temperature, pump setting and feed concentration) influenced the surface area at a lower level (12 to 17 fold less) with $p<0.05$. No interactions between factors were significant at $p<0.05$. The coefficients of the model equation are given in Table 2. Three of the main effects have a negative coefficient i.e. inlet temperature (A), pump (C) and feed concentration (D), indicating that when any of these factors decrease, particles with higher surface area are produced. One main effect has a positive coefficient i.e. gas flow (B), giving particles with higher surface area when at higher levels.

ANOVA evaluation of trehalose values showed that the main effects with a significant impact on SSA were inlet temperature (F-value 27.43, $p<0.0001$) and gas flow (F-value 47.35, $p<0.0001$). The strongest interaction was between inlet temperature and pump setting, with F-value of 44.15 ($p< 0.0001$).

According to Gregg and Sing (1982), when considering a non-porous particle, the surface area is inversely proportional to particle size; figure 8a and 8b show this relationship for surface area estimated from particle size data, assuming non-porous spherical particles of raffinose and trehalose. Taking into account the porosity of our particles, a correlation between actual measured surface area and particle size was investigated (Figure 7c and 7e). The correlation was found to be strong for raffinose particles ($R^2$=0.74, p<0.001) and weak for trehalose particles ($R^2$=0.28, p=0.0421), where, as particle size increases surface area decreases.

In this study we have observed that the particle size decreases with increasing gas flow, decreasing feed concentration and reduced pump setting. The existing relation between particle size and specific surface area would lead us to think that the same variables would have an impact on SSA. This hypothesis is valid for the studies on raffinose powders (Table 2), since the process parameters gas flow, pump setting and feed concentration impacted on SSA in the same way as on PS.

### 3.5.1. Effect of particle specific surface area on aerosolisation properties

The effect of the specific surface area (SSA) of particles on the fine particle fraction below 5 µm (FPF) of powders was studied for powders produced with the same particle size ($d_{50}$) and different SSA. Preliminary aerodynamic studies on the NPMPs established that particles with ~1.7 µm median particle size had an aerodynamic diameter of ~2 µm. NPMPs of raffinose and trehalose were therefore spray dried using the process parameters determined by applying the model equations to yield powders with a $d_{50}$ value of 1.7 µm but with SSA of minimum, average and maximum value in order to have three representative points within the design space.

For raffinose and trehalose NPMP powders a trend was observed where, as the SSA of porous particles increased a higher FPF could be achieved (Figure 9), reaching a FPF of more than 50% at the highest SSA values. Table 4 shows the obtained mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) for raffinose and trehalose powders.

Ní Ógáin et al. (2011) showed that raffinose and trehalose NPMP powders had higher FPF than the non-porous particles powders as well as higher SSA, by ~40 fold.

The main difficulty associated with inhalation of fine particle powders and their efficient delivery is the strong interparticle forces (mainly van der Waals forces) which make the cohesive bulk powder agglomerate (Daniher et al. 2008). The cohesion between non-porous particles by contact surfaces is proportional to the specific surface area (Chew et al., 2000).

Studies by Tabor (1977) demonstrated that surface roughness can greatly reduce the adhesion between solids, due to the high surface asperities, which can prize the surfaces apart and break the adhesions occurring at the lower asperities.

Our results lead us to believe that the high surface area of NPMPs, due to high porosity, resulted in particles presenting fewer areas of contact, leading to lower cohesion and easier dispersion, resulting in high FPFs.

## 4. Prediction of optimal process conditions

A theoretical optimisation can be performed using the statistical models obtained to find the optimal settings of the spray drying parameters to achieve a product with desired properties. Raffinose and trehalose NPMP production was optimised in order to obtain powders with minimum residual solvent content (RSC), particle size (PS) < 3 µm, high specific surface area (SSA) and yield ≥ 50%. A higher importance was ascribed to minimising residual solvent content, followed by maximising SSA The European Pharmacopeia classifies methanol as class 2 and butyl acetate as class 3 residual solvents (organic volatile chemicals that are used in the preparation of medicinal products and that have no therapeutic benefit (Ph. Eur., 2009). Hence it is important to minimise their levels in the sugar NPMPs powders in order for these to be suitable for human use. Also, we have shown that a higher value of SSA will lead to higher fine particle fraction and should optimise pulmonary delivery.

Predicted optimal settings for raffinose NPMPs were as follows: $T_{inlet}$ 150 °C, gas flow rate 50 mm (1052 l/h), pump rate 30% (8.5 ml/min) and 2.9% total solid concentration in the feed solution. For trehalose NPMPs the process conditions should be: $T_{inlet}$ 150 °C, gas flow rate 50 mm (988 l/h), pump rate 40% (11.4 ml/min) and 1% total solid concentration in the feed solution. Using these suggested process variables the resulting outlet temperature was predicted to be 87°C for raffinose and 86°C for trehalose. The powders produced were envisaged to present the following characteristics: yield 50%, PS ($d_{(50)}$) 1.8 μm, SSA 49.97 $m^2/g$ and RSC 2.2% for raffinose and yield 49%, PS ($d_{(50)}$) 1.7 μm, SSA 45.53 $m^2/g$ and RSC 2.3% for trehalose.

When tested, characteristics of the batches were, for raffinose NPMPs: yield 57.7±1.6 %, PS ($d_{(50)}$) 1.8±0.02 μm, SSA 58.16±0.51 $m^2/g$, RSC 2.6±0.34% and process $T_{outlet}$ was 85±1.4 °C; and for trehalose NPMPs: yield 57.1±2.4 %, PS ($d_{(50)}$) 1.6±0.04 μm, SSA 51.44±0.49 $m^2/g$, RSC 2.5±0.49% and process $T_{outlet}$ 86±0 °C. The results for outlet temperature, residual solvent and particle size were similar to the predicted values and within the standard deviation of the experimentally determined results. Yield and surface area values were higher than predicted. Thus, the outcome in terms of these latter parameters was even better than predicted by the model.

## 5. CONCLUSION

The design of experiment study undertaken resulted in well fitted models which highlighted the process variables impacting on the sugar NPMPs characteristics. Yield was affected by the gas flow and pump setting; particle size and specific surface area by the gas flow; residual solvent content and outlet temperature by the inlet temperature. Interaction between the process parameters were also found, demonstrating the complexity of the spray drying process.

The factorial models constructed could be used to optimise the spray drying process for the production of powders with suitable characteristics for pulmonary delivery, i.e. high yield, small particle size and low residual solvent.

Previous studies on porous microparticles did not examine the effect of a change in surface area on the *in vitro* deposition for dry powders intended for pulmonary delivery. In this study we compared powders with similar particle sizes but differing SSA and demonstrated a trend of increasing FPF with increasing SSA, attributable to the porosity of the particles.

Raffinose NPMPs had better characteristics than trehalose NPMPs in terms of dry powder inhalation, since particles with larger surface area, resulting in higher FPF, were produced.

## ACKNOWLEDGMENTS

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Table 1 Process variables evaluated in the factorial study.

| Parameters | | Low (-) | High (+) | Units |
|---|---|---|---|---|
| A - Inlet temperature | | 90 | 150 | °C |
| B – Gas flow | | 30 | 50 | mm |
| C – Pump setting | | 15 | 40 | % |
| D - Feed concentration | Trehalose | 0.5 | 1.5 | % |
| | Raffinose | 1 | 3.5 | |

Table 2 Raffinose: Coefficients of the model equations linking the significant (at least $p < 0.05$) spray drying parameters (in terms of coded factors) with responses. I - intercept ($\beta_0$); A - inlet temperature; B - gas flow, C – pump setting; D – feed solution concentration; SSA – specific surface area; RSC - residual solvent content.

| Term | Yield (%) | F-value | Term | PS $d_{(50)}$ (µm) | F-value | Term | SSA ($m^2/g$) | F-value | Term | RSC (%) | F-value | Term | Outlet temp. (°C) | F-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 52.22 | | I | 2.50 | | I | 46.80 | | I | 2.72 | | I | 74.28 | |
| B | -6.52 | 39.61 | B | -0.72 | 267.39 | A | -3.33 | 4.66 | A | -0.19 | 6.66 | A | 17.28 | 190.30 |
| C | 3.87 | 13.95 | C | 0.20 | 20.58 | B | 13.77 | 79.79 | AC | -0.18 | 6.26 | B | -3.09 | 6.10 |
| D | 3.42 | 10.92 | D | 0.27 | 38.07 | C | -3.36 | 4.74 | CD | 0.30 | 16.32 | C | -3.22 | 6.60 |
| BD | 3.19 | 9.50 | | | | D | -4.03 | 6.85 | BCD | -0.19 | 6.74 | | | |
| | | | | | | | | | ABCD | 0.37 | 25.35 | | | |
| $R^2$ | 0.84 | | $R^2$ | 0.96 | | $R^2$ | 0.87 | | $R^2$ | 0.83 | | $R^2$ | 0.93 | |

Table 3 Trehalose: Coefficients of the model equations linking the significant (at least $p < 0.05$) spray drying parameters (in terms of coded factors) with responses. I - intercept ($\beta_0$); A - inlet temperature; B - gas flow, C – pump setting; D – feed solution concentration; SSA – specific surface area; RSC - residual solvent content.

| **Term** | **yield (%)** | **F-value** | **Term** | **PS $d_{(50)}$ (µm)** | **F-value** | **Term** | **SSA ($m^2$/g)** | **F-value** | **Term** | **RSC (%)** | **F-value** | **Term** | **Outlet Temp. (°C)** | **F-value** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 47.98 | | I | 2.35 | | I | 43.21 | | I | 2.85 | | I | 73.13 | |
| A | 5.65 | 74.82 | B | -0.59 | 229.24 | A | -3.53 | 27.43 | A | -0.45 | 31.19 | A | 18.13 | 1649.02 |
| B | -6.67 | 104.24 | C | 0.21 | 28.87 | B | 4.64 | 47.35 | C | 0.54 | 46.10 | C | -5.00 | 125.49 |
| C | 2.49 | 14.51 | D | 0.082 | 4.38 | AC | 4.34 | 44.15 | AB | -0.21 | 6.71 | AC | -1.25 | 7.84 |
| D | -2.57 | 15.50 | BC | -0.18 | 21.39 | BC | 1.73 | 6.59 | AD | -0.14 | 13.33 | AD | -1.00 | 5.02 |
| BC | -2.47 | 14.32 | | | | BD | 2.34 | 44.15 | BC | -0.21 | 6.70 | CD | 1.13 | 6.35 |
| CD | 3.41 | 27.24 | | | | ABD | 1.87 | 6.59 | CD | 0.44 | 29.77 | | | |
| ABC | 1.48 | 5.10 | | | | | | | ACD | -0.34 | 18.02 | | | |
| ACD | 1.76 | 7.25 | | | | | | | | | | | | |
| ABCD | 1.46 | 4.98 | | | | | | | | | | | | |
| $R^2$ | 0.94 | | $R^2$ | 0.93 | | $R^2$ | 0.91 | | $R^2$ | 0.91 | | $R^2$ | 0.98 | |

Table 4 Mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD) and geometric median particle size ($d_{50}$) for raffinose and trehalose powders with increasing specific surface area (SSA).

| | **SSA ($m^2$/g)** | **MMAD (µm)** | **GSD** | **PS $d_{50}$ (µm)** |
|---|---|---|---|---|
| **Raffinose** | 49.26±0.42 | 3.3±0.14 | 2.1±0.09 | 1.7±0.02 |
| | 70.39±0.68 | 3.4±0.33 | 2.4±0.14 | 1.6±0.01 |
| | 88.66±0.79 | 3.6±0.51 | 2.6±0.09 | 1.8±0.01 |
| **Trehalose** | 42.28±0.30 | 8.4±1.37 | 3.4±0.53 | 1.7±0.07 |
| | 47.89±0.13 | 7.4±0.13 | 3.4±0.77 | 1.7±0.04 |
| | 57.41±0.73 | 4.3±0.35 | 2.8±0.09 | 1.7±0.02 |

Figure 1. Correlation between actual versus predicted particle size (a) for raffinose NPMPs and (b) for trehalose NPMPs.

Figure 2. Influence of particle size on yield of (a) raffinose NPMPs (b) trehalose NPMPs.

Figure 3. Impact of interactions between process variables on trehalose NPMPs yields: plot of interaction between (a) gas flow and pump setting and (b) feed concentration and pump setting; cube graph of interaction between (c) inlet temperature, gas flow and pump setting and (d) inlet temperature, pump setting and feed concentration.

Figure 4. SEM micrographs for raffinose NPMPs (x 30k) (left) and trehalose NPMPs (x 30k) (right).

Figure 5. Influence of outlet temperature on residual solvent content for (a) raffinose NPMPs and (b) trehalose NPMPs.

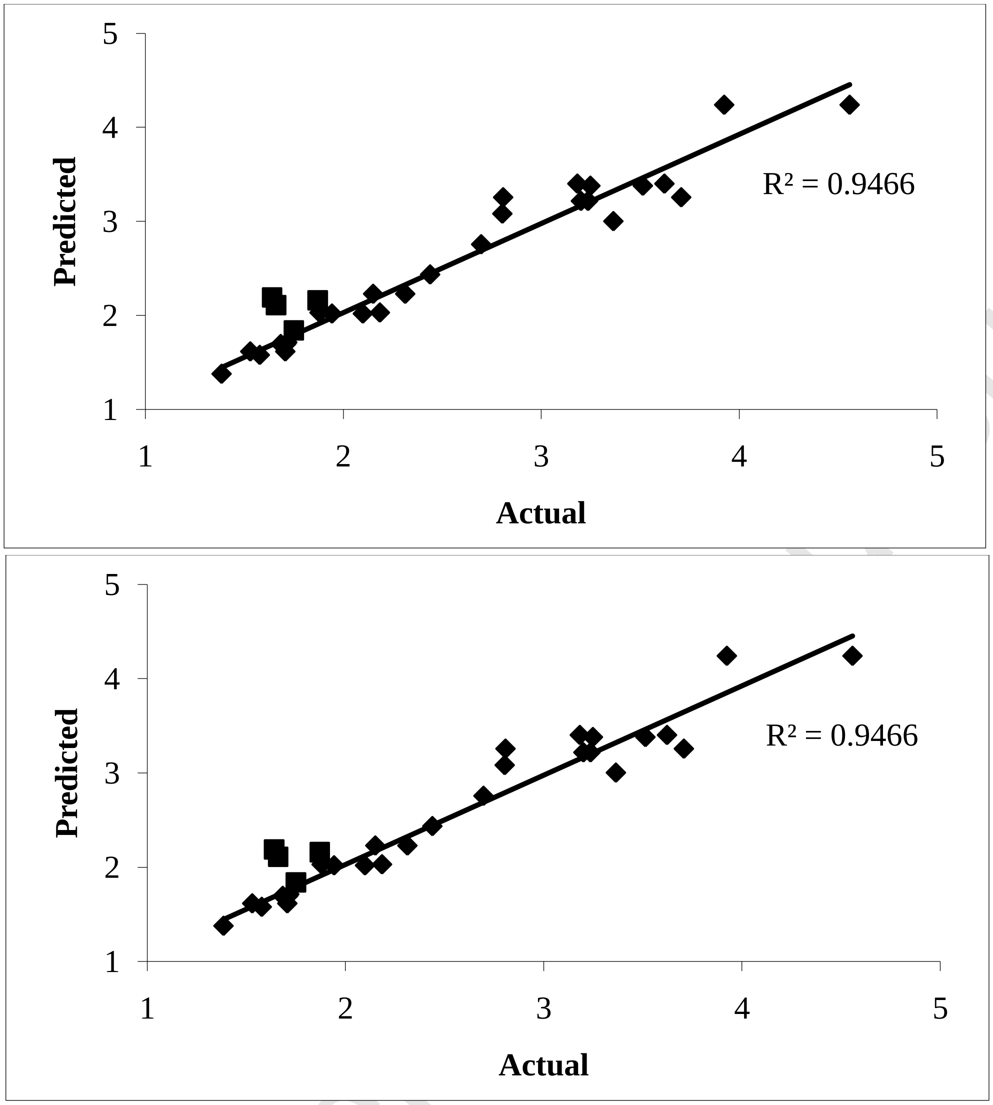
Figure 6. Impact of interactions between process variables on raffinose powder residual solvent content: plot of interaction between (a) inlet temperature and pump setting, (b) pump setting and feed concentration: (c) cube graph of interaction between pump setting, gas flow and feed concentration.

Figure 7. Impact of interactions between process variables on trehalose powder residual solvent content: plot of interaction between (a) inlet temperature and gas flow, (b) inlet temperature and

feed concentration (c) gas flow and pump setting and (d) pump setting and feed concentration; (e) inlet temperature, pump setting and feed concentration.

Figure 8. Relation between particle size and specific surface area for (a) raffinose particles where particles are assumed non-porous and the SSA is derived from particle size data, (b) trehalose particles where particle are assumed non-porous and the SSA is derived from particle size data (c) raffinose NPMPs, taking the actual measured SSA and (d) trehalose NPMPs, taking the actual measured SSA.

Figure 9. Effect of specific surface area on the fine particle fraction of spray dried raffinose NPMPs ($R^2$=0.973, p=0.0004) and trehalose NPMPs ($R^2$=0.978, p=0.0007).


5
4
3
2
1
Predicted
1
2
3
4
5
Actual
R² = 0.9466
5
4
3
2
1
Predicted
1
2
3
4
5
Actual
R² = 0.9466

a)

b)

Figure 1.

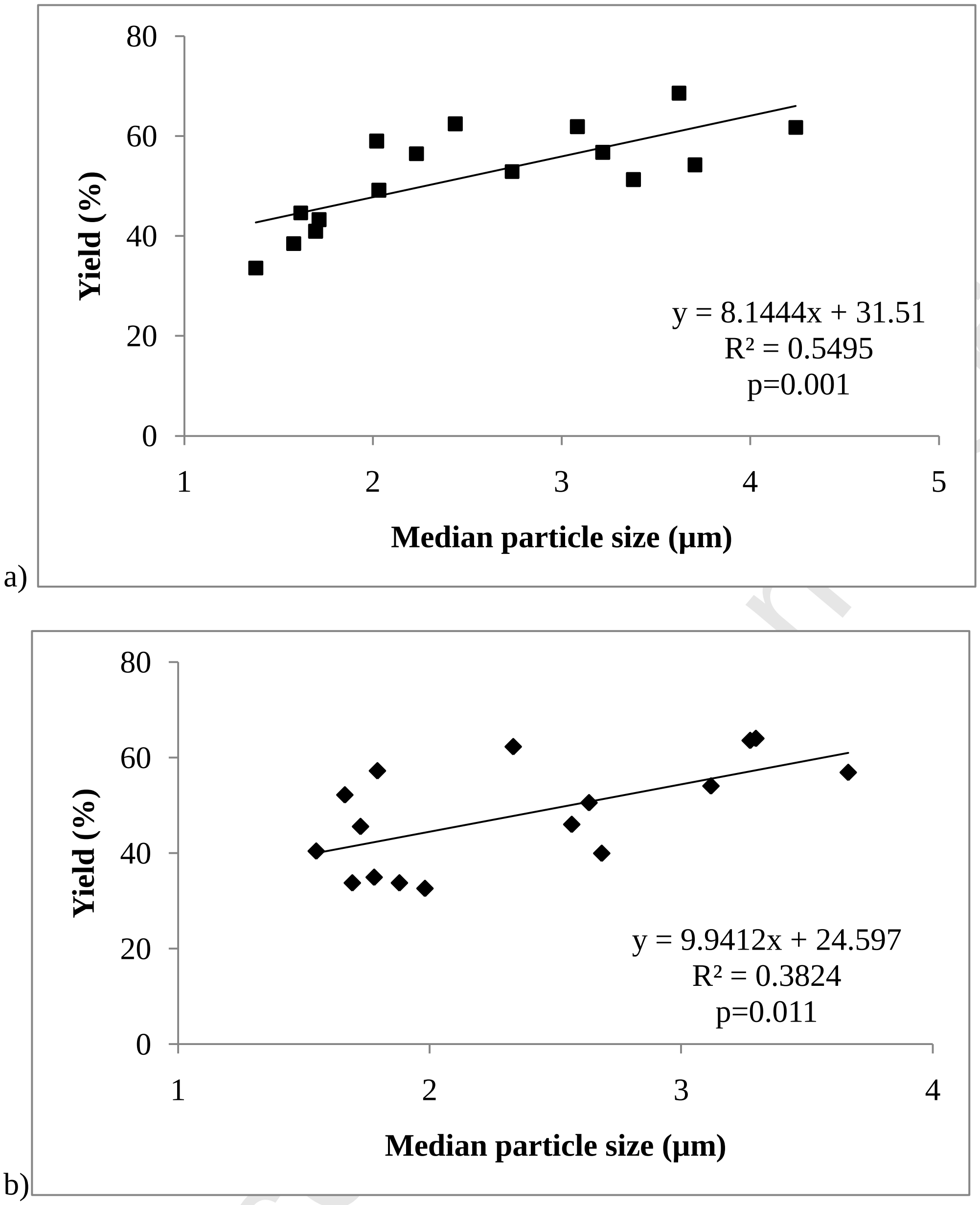

80
60
40
20
0
Yield (%)
1
2
3
4
5
Median particle size (µm)
y = 8.1444x + 31.51
R² = 0.5495
p=0.001
a)
80
60
40
20
0
Yield (%)
1
2
3
4
Median particle size (µm)
y = 9.9412x + 24.597
R² = 0.3824
p=0.011
b)

Figure 2.

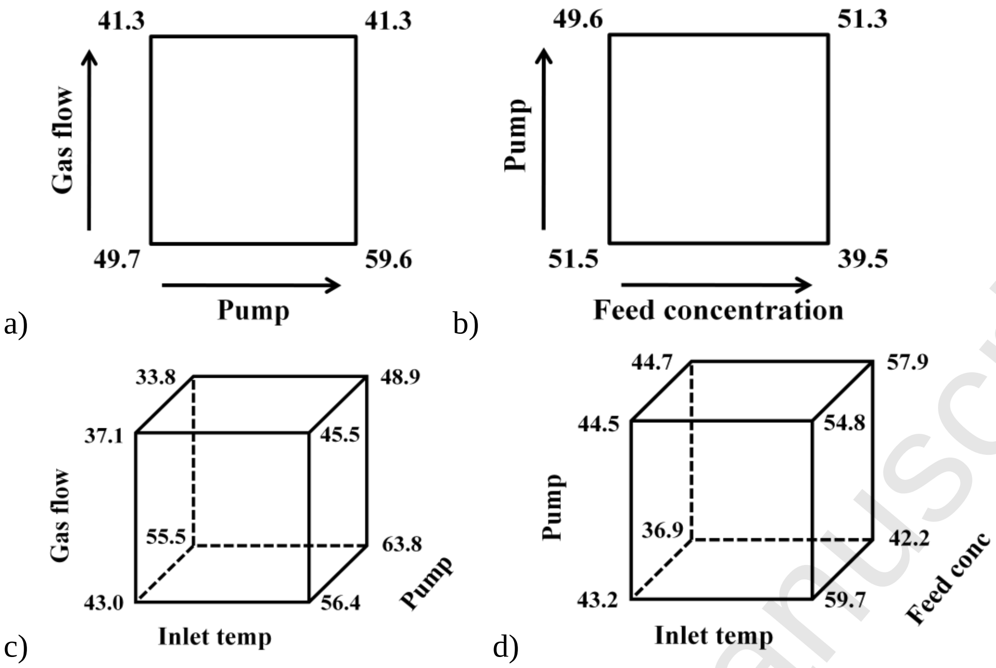


Figure 3.

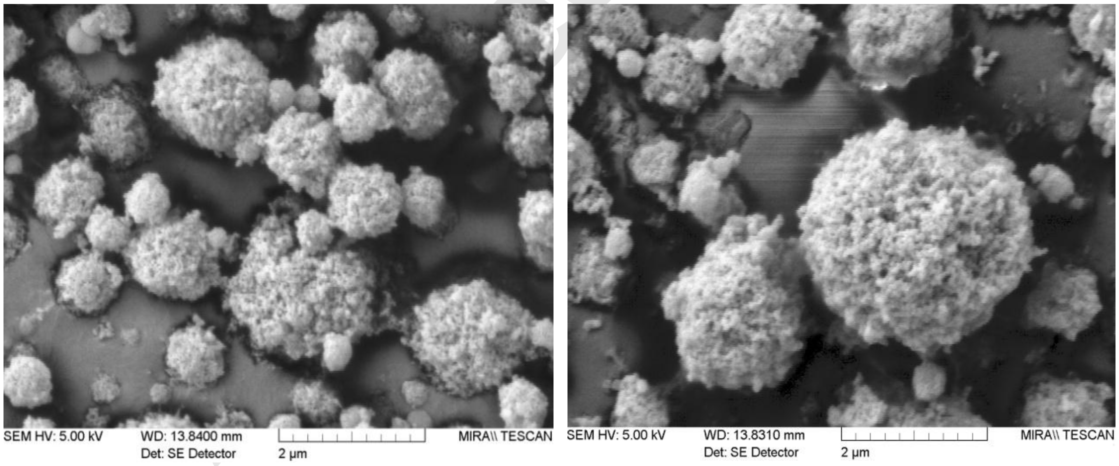
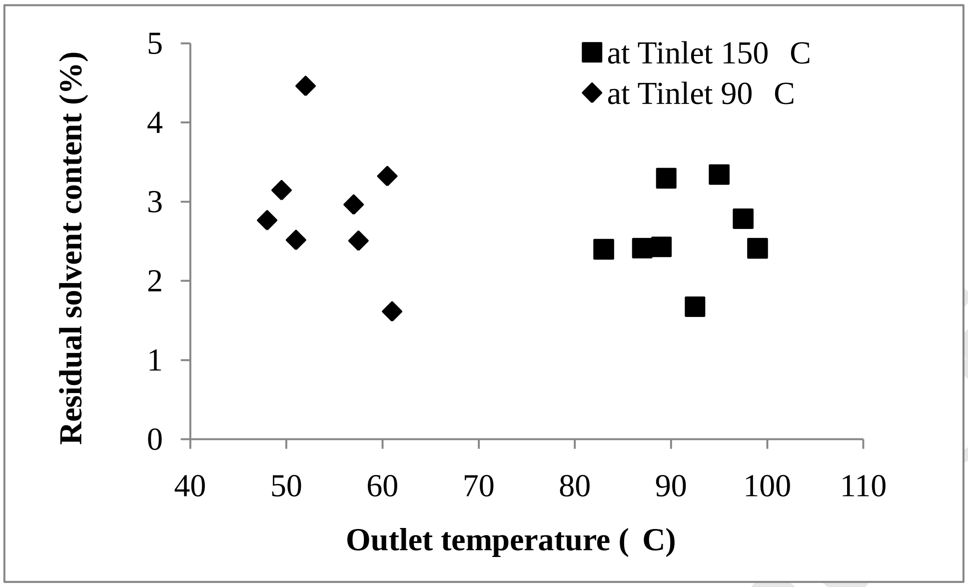


Figure 4.

a)


Residual solvent content (%)
0
1
2
3
4
5
40
50
60
70
80
90
100
110
Outlet temperature (  C)
■ at Tinlet 150   C
◆ at Tinlet 90   C

b)

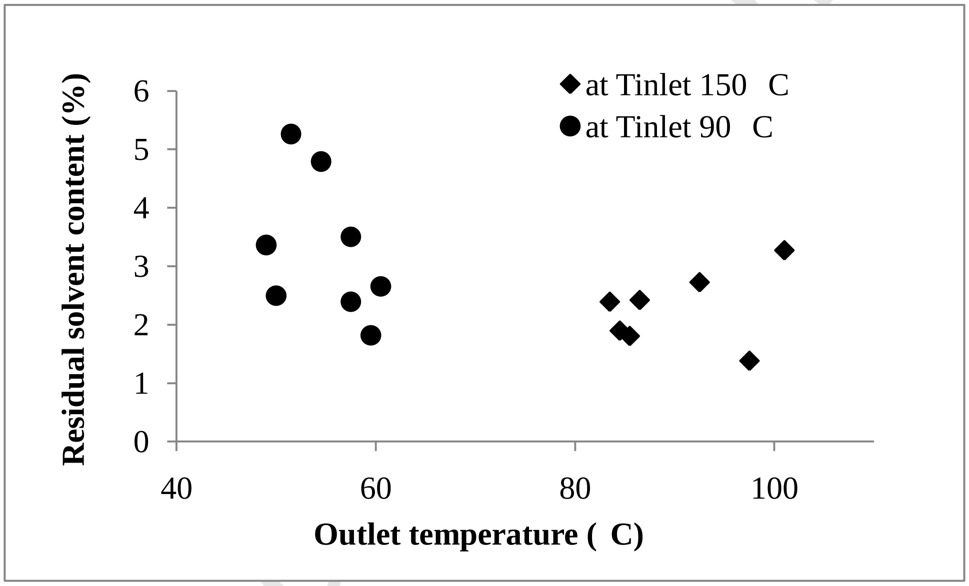

Residual solvent content (%)
0
1
2
3
4
5
6
40
60
80
100
Outlet temperature (  C)
◆ at Tinlet 150   C
● at Tinlet 90   C

Figure 5.

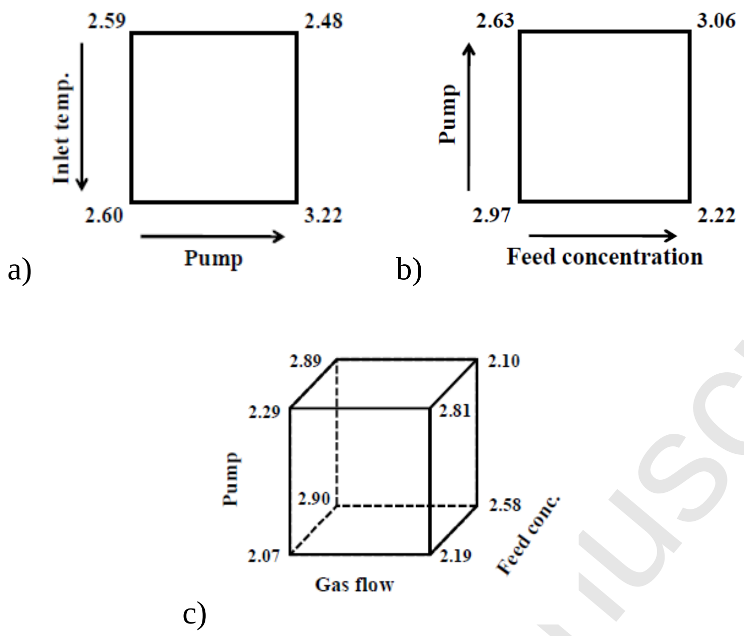


Figure 6.

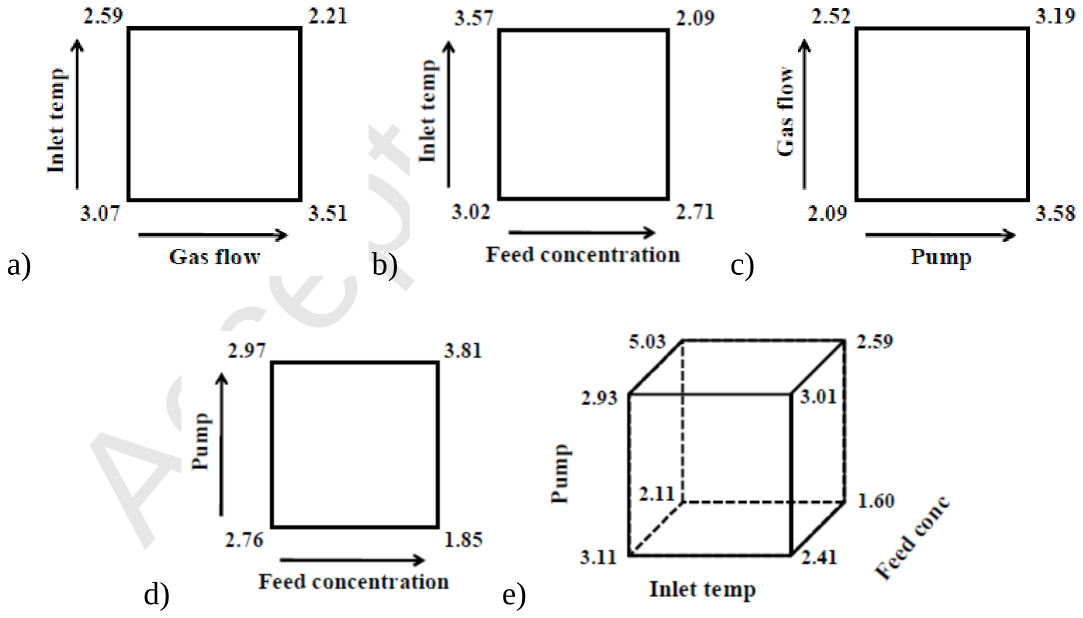


Figure 7.

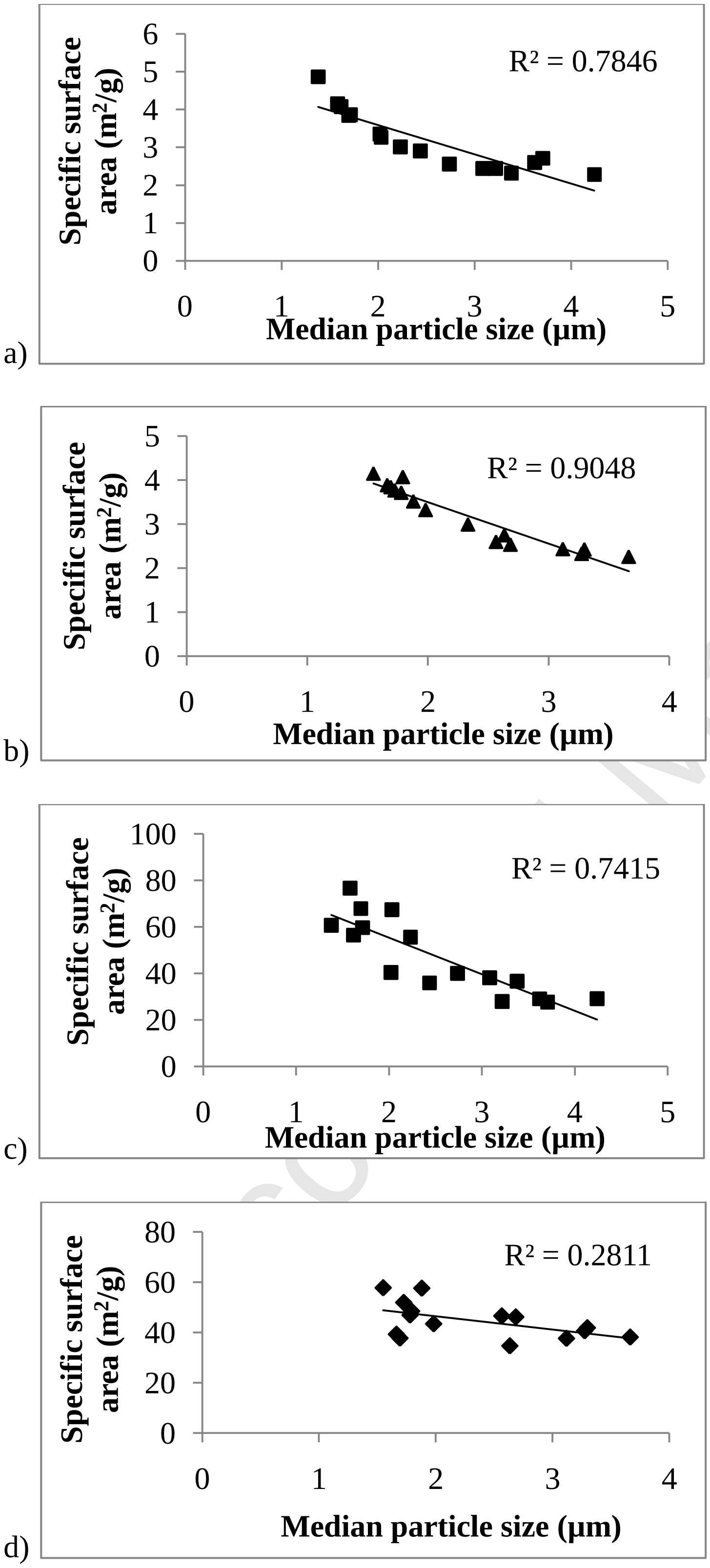

a)
Specific surface area (m²/g)
Median particle size (µm)
R² = 0.7846
0
1
2
3
4
5
6
b)
Specific surface area (m²/g)
Median particle size (µm)
R² = 0.9048
c)
Specific surface area (m²/g)
Median particle size (µm)
R² = 0.7415
0
20
40
60
80
100
d)
Specific surface area (m²/g)
Median particle size (µm)
R² = 0.2811

Figure 8.

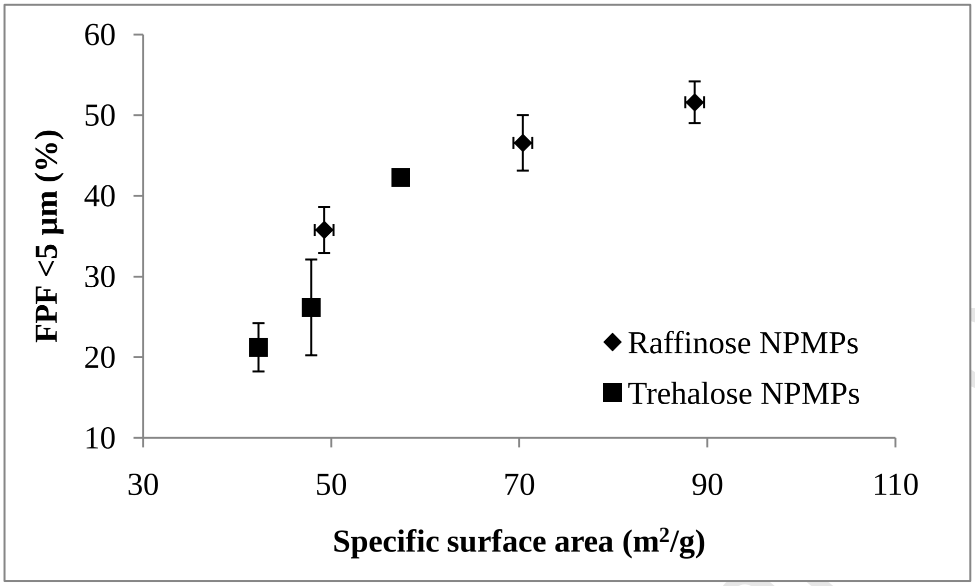

60
50
40
30
20
10
FPF <5 µm (%)
30
50
70
90
110
Specific surface area (m²/g)
◆ Raffinose NPMPs
■ Trehalose NPMPs

Figure 9.