Preparation and characterization of a powder manufactured by spray drying milk based formulations for the delivery of theophylline for pediatric use

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Abstract

The study considered different fat content cow milks to deliver theophylline orally. Powders were obtained by spray drying theophylline dispersed in fresh milk according to a full factorial design of experiments. The correlation of the independent (milk fat content, skimmed to whole milk, theophylline fraction, and drying temperature) with the dependent (yield of the process and residual moisture content of the powder, particle size and distribution, density, surface polarity and theophylline content) variables enabled the construction of a mathematical model and a desirability function to predict the optimized levels of the variables. Good predictability was achieved for density, fairly good for yield, moisture content, surface polarity and yield whereas theophylline content and particle size were poorly predicted. Powders with up to 60% theophylline presented spherical (3.7µm) and narrow sized distribution particles, with high density (1.6 g/cm^{-3}) in high yields (>70%), stable for 6 month (25°C/65%RH) in a closed container and for no longer than 2 day, after reconstitution in water due to bacteria growth (no pathogens) **without signs of crystallinity**. Preparations obtained with low fat milk were less stable than high fat content milk. Therefore, fresh milk can be transformed into stable powder compositions to prepare oral solid/liquid dosage forms to deliver individualized doses of theophylline.

Highlights

- Inclusion of theophylline in fresh milk delivers a composite powder made of milk solid components and theophylline;
- A full factorial design of experiments, combined with a desirability function, identified the main formulation and process parameters and levels to manufacture a powder with optimal properties;
- Spray drying a mixture of fresh milk with different fractions of theophylline produced a powder with elegant properties and high dosing flexibility;
- The powdered material was physical, chemical and microbiologically stable for 6 month in dried containers and stable for 2 day at room temperature after reconstitution. Lack of stability of the reconstituted product was due to microbiological growth and not to physical or chemical properties.

Keywords

Composite milk; fresh milk; factorial design; pediatric delivery system; spray dried powder; theophylline

1. Introduction

The delivery of drugs in pediatrics poses a major challenge for researchers because it must take into account many variables related to the physiology of a children. Regulatory agencies require manufacturers to take this fact into consideration when developing new medicines and providing data to support new marketing authorization [EMA, 2013]. New regulations and development programs have been issued by the Committee for Medicinal Products for Human use (CHMP) of the European Medicines Agency as a reflection paper on formulations of choice for the pediatric population [EMA, 2006] and a note for clinical investigation of medicinal products for the pediatric population [EMA, 2017]. The issue of medicines for pediatric use has also attracted the attention of the World Health Organization (WHO) experts that periodically updates the model list of essential medicines for children [WHO, 2015]. This list presents a small number of solid dosage forms administered orally, despite being the preferable route of administration compared to parenteral or inhalation routes regarding compliance and costs or, oral liquid dosage forms regarding stability issues [WHO, 2015, Ivanovska et al., 2014]. To overcome this shortage the current practice pharmacists are forced to manipulate or rearrange dosage forms designed and available for adults, to meet the different pediatric needs [O'Hara, 2016]. Subject of interest in drug development is finding new drug delivery systems to overcome the problem of lack of "*child-friendly*" and "*user friendly*" preparations, particularly when considering the highly heterogeneous nature of the patient group, ranging from newborns to adolescents [Krause and Breitkreutz, 2008]

The compliance of a medicinal product for children is closely related to the route of administration and to the type of pharmaceutical dosage form and, therefore, to the formulation, the toxicity of excipients and the sensorial characteristics of the dosage form (e.g. palatability, smell or texture). Frequently, when there is a particularly bitter or bad taste active ingredient, nurses, parents or care-takers mix the medicine with food or drinks to improve or disguise the taste, neglecting stability issues derived from possible interactions between the drug and food or drink components, besides the ability to deliver the prescribed dose of the drug in an accurate and precise fashion [Wals et al., 2011]. Mixing a chemically identifiable drug as a weak base with an acidic drink, such as a fruit juice, can cause chemical instability, undermining the therapeutic efficacy and the safety of the medicine [Ali et al., 2014]. Similarly, the physical stability can be affected by incorporating a granulate conveying a thermolabile drug in a hot drink, likely to modify the physical and chemical characteristics of the drug. Many drugs (e.g. cardiovascular drugs, drugs acting on the central nervous system, on the gastro-intestinal tract or broad spectrum antibiotics) are not available in formulations for pediatrics and must be used "off-label" requiring adaptation of existing medicines designed for adults to children, or, in those cases where

transformation is possible, are presented as adapted galenic preparations. Often, the medicines containing drugs used "off-label" designed for the adult population, may contain potentially toxic excipients for children, without taking into consideration that the physiological, immunological and enzymatic developments of the latter, are not complete. The percentage of side effects and adverse reactions derived from this misuse of medicines for these reasons is unacceptable high [Tuleu and Breitkreutz, 2013].

Milk is a complex aqueous system made of different combinations of many chemically different substances that make the potential use in pharmaceutics difficult although it is one of the most consumed food in the world. Consequently, any study considering the use of milk needs to take into account a large number of variables related to the composition and complexity of this liquid system prior to its use. Milk is a natural, fairly abundant and inexpensive natural product with carrier potential for the oral administration of drugs. Several reports in the literature suggest that the solubility and rate of dissolution of lipophilic drugs in milk is much higher than in aqueous media [Kamal et al., 2016]. It has also been shown that the dissolution and absorption of some drugs is higher from a drug-fresh milk dispersed system than from a drug-powdered milk physical mixture [Sunooj et al., 2011]. The broad acceptance of milk by different communities worldwide, regardless religion and social backgrounds of consumers, suggests its use in the production of medicines for children. Some components of milk (e.g. casein) have already been used as vehicles for bioactive molecules, but there have been few developments in the field of pediatric medicine although they are included in the list of substances generally recognized as safe (GRAS) (FDA [Muehlhoff et al., 2013, Livney, 2010]). Milk is both marketed as a liquid product and as a powder, the latter being generally obtained by spray-drying, the most effective method used to dehydrate milk.

The transformation of liquid systems into powders can be achieved by different technologies, namely spray drying. Spray-drying is a drying technique widely used because of the many advantages it offers: drying occurs in a very short time, the technique is appropriated for processing thermolabile substances such as those contained in milk, ensuring the chemical integrity of the substances present in its composition and, once reconstituted with water, the sensorial properties resemble the ones presented by the fresh milk. The process encompasses different phases namely the dispersion of the fed solution into small droplets, contact of the droplets with a heated gas (often air), that promotes both heat and mass transfer between the liquid system and the gas, promoting drying with the removal of the solvent, ending up in the formation of solid particles with unique properties, conveyed pneumatically by the wet gas to a cyclone where they are collected [Sunooj, 2011]. The technology has been considered for decades to manufacture powdered milk because it is gentle process of drying. To optimize the process different parameters must be considered (e.g. the inlet

temperature, solution/suspension feed rate, spray air flow and aspirator flow) impacting on the product's properties (e.g. powder moisture content, particles size and size distribution, yield and morphology) [Muehlhoff et al., 2013].

Theophylline (Figure S1, supplementary material) was selected as a model drug commonly used in therapy for respiratory diseases, namely chronic obstructive pulmonary disease (COPD) or asthma. It causes a general effect of relaxing the bronchial muscles, which is why it is used against respiratory diseases and improving the data from spirometry (FVC, FEV1 and VC, respectively the forced vital capacity or the total volume of air expelled in a forced expiration from an inspiration at maximum capacity, the forced expiratory volume in one second and the Tidal volume of air mobilized in a breath). It is particularly suitable for administration to children due to its low toxicity and high safety [Molfino and Zhang, 2006, Barnes, 2013].

The aim of the present work was the evaluation of the potential of milk **based powders as** vehicle for safe and effective delivery of drugs (e.g. theophylline) in medicines for children. Moreover, solid dosage forms are the most stable and well accepted ones by patients and manufacturers [Kumar and Mishra, 2006], thus the inclusion of a drug (e.g. theophylline) into fresh milk and converting the new system into a powdered solid, that, after storage, can be reconstituted again into a liquid system to deliver the drug. In this respect, the product combines advantages of both solid (e.g. stability, handling, dose flexibility) and liquid (e.g. easy administration and dose flexibility) dosage forms. Given the complexity of the study and manufacturing technology and to accommodate recent guidance on product development (International Conference on Harmonization, ICH Q8, [EMA, 2017]) a Quality by Design (QbD) approach was considered based on a Design of Experiments (DoE) with the aim of maximizing the quantity and quality of information collected from raw data. In this way, mathematical models were constructed and their ability to correlate formulation and process variables with powder's properties was evaluated.

2. Materials and Methods

2.1 Materials

Anhydrous theophylline (Lusifar, Lisboa, Portugal), deionized water (W, 18.2 MΩ * cm, produced by a Milli-Q system, Merck Millipore, MA, USA) and fresh cow milk with different fat contents (UHT, Mimosa, Lactogal Produtos Alimentares, Porto, Portugal) were considered in the studies (Table S1 and Table S2, supplementary material). Di-

iodomethane, p-aminobenzoic acid (PABA), acetonitrile (HPLC grade) (all supplied by Sigma Life Science, Sintra, Portugal), methanol (HPLC grade, Fischer Chemicals, Zurich, Switzerland) and ammonium acetate (Merck, Darmstadt, Germany). Other reagents were analytical grade.

2.2. Methods

2.2.1. Preparation of powdered materials

2.2.1.1. Preparation of samples

A stock solution of theophylline (7.5 mg/mL) was prepared in a low concentration to prevent crystallization (stirring at 700-900 rpm at 40°C). This concentration was selected to allow the desired proportion of theophylline to the solid content of the milk, allowing the concentration of theophylline in solution to be different according to the different percentages of fat in each type of milk (Table S3, supplementary material). 400 mL samples were prepared in a beaker at room temperature (24°C) the same day they were processed by spray drying, as follows: first, a known volume taken from the theophylline stock solution was incorporated into a known volume of milk, based on the fraction of its solid content; second, deionized water was added up to 400 mL In this way, an increasing volume of theophylline solution had to be considered to keep constant the proportion of theophylline to milk solid components in every sample (2.5 g). For each fat content in the milk raw material, six samples were prepared.

2.2.1.2. Spray drying

Samples were prepared with three commercial fresh milks with different contents of fat, as stated before: low fat milk (LFM), middle fat milk (MFM) and high fat milk (HFM). The feed solutions of each type of milk with theophylline at different concentrations, based on milk solid content (8, 16, 31, 62 and 100%), were atomized using a Mini Spray-Dryer B-191 (Büchi Labortechik, Flawil, Switzerland) at three different inlet/ outlet air temperatures, respectively, 105/ 68 ± 2°C, 130/ 87 ± 1°C and 150/ 100 ± 2°C to allow the comparison between different processing conditions. The other instrument settings were kept constant: 1 mm nozzle diameter, aspiration air flow rate 100%, feed liquid rate 4 mL/min (20 %) and spray flow rate at 600 L/h in the chamber. The liquid systems were placed under magnetic stirring during the entire process of drying that lasted a total of 40 to 60 min, before collection of the powdered composite material and storage in a dark place at room temperature (24°C) and controlled humidity (RH=65%) for up to 6 months.

2.2.2. Characterization of powdered materials

2.2.2.1. Yield and moisture content of spray-dried milk powders

The powder collected from the cyclone and collector was weighted to obtain the yield of the process, based on the relationship between the expected weight (amount of theophylline and milk present in the liquid systems, taking into account protein and fat contents, before spray-drying) and the observed weight. The residual moisture content (MC) of the powders defined as the loss mass on drying. Powders were stored in an oven (Heraus, Hanau, Germany) at 74 ± 3°C (temperature selected based on stability of samples) until constant weight was observed. The relative decrease between the initial and the final weights of samples provided the moisture content.

2.2.2.2. Particle size and shape analysis

The powders from the different formulations were analyzed by optical microscopy (Olympus BX51, Olympus, Tokyo, Japan) at magnifications of 50x and 100x. Samples were prepared in a slide containing a droplet of liquid paraffin. Observations were recorded with Live Stream Essential software (JEOL JSM-T330A; JEOL, Peabody, MA, USA). The morphology of theophylline particles was investigated using polarized light, to take advantage of diffraction of light caused by the particles, in 5 different fields of each one of 5 samples (more than 250 observations in 25 locations).

2.2.2.3. Density

True densities of powder samples (about 2 g) were determined by helium pycnometry (AccuPyc 1330, Micromeritics, Norcross, GA, USA) in triplicates where each sample was measured 20 times. The samples were dried in the oven (74 ± 3°C for 3 h) and then placed in a desiccator for 30 min to cool. Density was calculated as the ratio between the powder samples mass and the volume found.

2.2.2.4. Surface energetics (contact angle and surface free energy)

Triplicates of samples were prepared by gentle milling aggregates of each powder in a quartz mortar prior to sticking their particles to a double-side tape several times to assure complete coverage of the tape. Then the tape was stuck to a lamella placed in the tensiometer holder (Tensiometer K100, Kruss, Hamburg, Germany) to perform the analysis according to the Wilhelmy plate method. Deionized water and di-iodomethane (Sigma-Aldrich, Munich, Germany) were considered as probing liquids (about 50 mL). The plate was submersed in the liquid at a distance of 2 mm at controlled temperature 25±0.5°C, (Thermo Haake, Karlsruhe, Germany). LabDesk v. 3.2 (Krüss, Hamburg, Germany) enabled the calculation of the contact angles and surface free energy values of different samples, based on the Wu equation [Wu, 1971]. Once the surface polarity of the powders has been obtained, the Van Oss-Chaudhury-Good (VCG) theory was applied to calculate the percentage of surface polarity of the solid to that of the liquid anticipating the powder's ability to be wetted and to re-dispersed in water - the closer the percentage of surface polarity of the solid to that of the liquid, the more it was keen to be wetted and re-dispersed [Zenkiewicz, 2007].

2.2.2.5. Quantification of theophylline

The content of theophylline in dried samples was found by high pressure liquid chromatography (HPLC; Merck-Hitachi Lachrome, Darmstadt, Germany), at room temperature (22°C) and detection at a wavelength equal to λ = 272 nm. A Merck Purospher C_{18} analytical column (250x4 mm internal diameter; 5 µm grain size) and a Purospher C_{18} protection column (4x4 mm internal diameter; 5 µm grain size) were used in the analysis. An internal standard (p-aminobenzoic acid, PABA, 5 µg/mL) was incorporated in each sample. The eluent [ammonium acetate buffer (10 mM): acetonitrile: methanol in 90: 5: 5 v/v/v] was run in an isocratic condition at 1 mL/min flow rate. The samples, appropriately diluted, were centrifuged before injection (20 µL) and analyzed in triplicates. Standard theophylline solutions in water (0.2-75 µg/mL) and PABA (100 µg/mL) were prepared. The method was previously validated for specificity, linearity, accuracy, precision and reproducibility, according to the ICH guidelines [EMA, 1995].

2.2.2.6. Stability studies

The powders were tested immediately after production, after 7 days of production and retested after 6 months of storage (24 °C, or 8 °C when stated, and RH = 65%) for theophylline **content** and bacterial count. **Samples were prepared by dispersing 2 g of powders in 15 mL of water in 20 mL amber flask and stirred magnetically for 5 min**. The stability of the extemporaneous preparations of each powder was investigated immediately after the preparation (t_0) and after a period of storage, by reconstituting in water 2 g of powder with 15 mL of water, in 20 mL amber flask kept under constant magnetic stirring at 25 °C and 65% RH for 5 min. At time points (0, 1, 2, 7, 14 and 28 days), a 1 mL sample of each extemporaneous preparation was taken and tested for theophylline content and microbiological load [**2.6.12. Microbiological examination of non-sterile products: microbial enumeration tests, Eur. Pharm. 10.0**].

2.2.3. Design of experiments (DoE) and statistical analysis

Experiments were run according to a factorial design (Table 1). The independent variables were fat content in milk, theophylline content and inlet /outlet drying air temperatures, whereas the dependent variables were the yield and moisture content of dried powders, particles size and shape, density, surface energetics and quantification of theophylline. Table 2 presents all the combinations of experiments that were performed in random manner. The data collected from the characterization of the products obtained according to a full factorial design of experiments was analyzed by multiple linear regression and analysis of variance, to calculate mathematical models that correlate the factors chosen as independent variables and the properties of the dried powders [Armstrong, 2006]. Calculations were performed with the Minitab software (GSML, Nerviano, Italy).

3. Results and Discussion

The experiments run according to the experimental design (54 tests, Table 2) provided satisfactory results that were considered in the calculation of mathematical model. Although some difficulties were encountered in various stages of the production process they did not prevent individual runs to be performed. Formulations underwent the process of spray-drying in a random order and it was observed that samples with high-fat milk (HFM) have adhered to the wall of the cyclone in high fractions of the dried powder with a negative impact on the yield of the respective batch. Also in HFM formulations, fat has increased the difficulty of drying the powders at low temperatures, while at high temperatures the likely higher mobility of fat component molecules has allowed a better drying of the forming powders. The dissolution of theophylline in the milk was often difficult requiring the moderate use of heat (up to 40°C). Another complication was the fast microbial growth in powdered samples, particularly the ones with low-fat milk (LFM), for all the amounts of theophylline, when drying was conducted at 105°C and 130°C, requiring storage of all samples at a low temperature (8^{o}C) and controlled humidity (65%).

3.1. Factorial design (DoE)

3.1.1. Selection of independent variables and their levels in experiments

The experimental space, i.e. the all possible combinations of factors levels set was chosen to cover the widest range of possibilities providing products with sufficient quality grade, according to studies [Habtegebriel et al., 2018] (Table 1).Three independent variables, milk fat content (LFM-0.3 g, MFM-4.0 g and HFM-9.0 g), inlet air drying temperature (T_{inlet} = 105, 130 and 150^{o}C), and fraction of theophylline in formulations (0, 8, 16, 31, 62 and 100%), expressed as a percentage (w/w) of the fraction of solid content of the milk were found to be the most relevant in the design. The central T_{inlet} point was set at 130^{o}C based on previous reports [Birchal et al., 2005], whereas 105°C was chosen as the minimum temperature at which it was possible to have a fast and effective drying of the material and a maximum value of 150°C as the maximum temperature at which the process could be conducted, to prevent the risk of degradation of proteins contained in the different samples [Habtegebriel et al., 2018]). The levels of theophylline in the experiments were defined as the quantities of drug that could accurately be conveyed by the solid part of the milk. Preliminary tests have shown that theophylline crystals were found in some dried powders reflecting an upper limit of the capacity of milk components to incorporate theophylline.

3.1.2 Choice of the dependent variables

The experiments were designed in agreement with the Quality by Design (QbD) approach applied for medicines. In order to accomplish with this, the dependent variables, or responses were chosen to better reflect the Critical Quality Attributes (CQA) [Dumoulin and Bimbenet, 1998] of the products enabling a better description of the overall quality of the product, i.e., the Quality Target Product Profile (QTPP) of pharmaceutical products [EMA, 2017]. Thus, the yield of the process, the moisture content, the size and size distribution of particles of the powders, the true density, the contact angle with water, the total surface energy, the quantity of theophylline an the stability of the dried powders were taken into consideration to allow the setting of the QTPP for the dried milk formulations.

3.2. Computational model proposed for the different properties of the powders

3.2.1. Setting the full factorial design of experiments (DoE)

The mathematical model chosen to describe the relationship among each response and factors contains linear, interaction and quadratic terms that could be described as follows:

$$Y_n = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \varepsilon$$

where, Y_n is the dependent variable, X_1, X_2 and X_3 are the independent variables or factors (milk fat content, spry-drying temperature, amount of theophylline, respectively), β_0 is the intercept, β_1, β_2 and β_3 are the first order linear coefficients of the three main factors, β_{12}, β_{13}, β_{23} are the interaction coefficients between the three factors, β_{11}, β_{22}, β_{33} are the quadratic coefficients of the three factors and ε is the value of the experimental error.

In order to calculate these coefficients of the model that describe how X_1, X_2 and X_3 influence the Y answers, it was necessary to perform at least 10 experimental tests, as many as the coefficients to be calculated. At the end, it was decided to perform a full factorial experimental design with 54 experimental tests corresponding to all possible combinations of the variables and levels (Table 2) to obtain higher precise results and considering the fact that the time and amount of material requested were reasonable.

3.2.2. Evaluation of the dried powders (dependent variables)

3.2.2.1. Yield and moisture content: Regardless the fat content of milk and theophylline fraction, the yield of the powders atomized at 105, 130 and 150°C ranged between 28.4 – 56.6%, 31.0 – 76.0% and 13.6 – 68.3%, respectively (Table 3). The yields of the powders atomized at 130°C were generally the highest ones considering also the other two parameters (fat content of milk and theophylline fraction).

From the intercept value (Table 4) it can be stated that, at the center of experimental space, the yield of the process could be considered good (62.9%), particularly if one takes into consideration the small operational scale used for the process of drying. The selected intervals of the three independent variables were well selected to obtaining the desired product. As for first order effects, the influence of the fraction of theophylline was significant with a positive effect, i.e., as the fraction of theophylline in the formulation increased, the yield also increased. It could be hypothesized that this effect was a consequence of the fact that, throughout the process of drying, the decrease on the weight of the powder was not directly related to the quantity of liquid used, and when expressed in a percentage ratio, they tend to decrease. The quadratic effects of T_{inlet} on the yield was significant: the parabola, which represents the influence of the temperature of the incoming air, presenting a negative coefficient for the quadratic term, has the concavity downwards. It follows that decreasing values for the yield were obtained when moving away from the values of temperature of the incoming air at the vertex of the parabola, presenting a coordinate on the axis of the independent variable 0.62, corresponding to a T_{inlet} value close to 142°C. This shows that the range of temperature values chosen contained the drying temperature (142^{o}C) which allowed the maximum yield. Following the convexity of the parabola towards higher values of T_{inlet} approaching 150°C, the yield decreased, probably due to the melting of the fat component which therefore increased the tendency of the dried particles to adhere to the walls of the collecting chamber. In fact, at the end of processes involving higher temperatures, a larger quantity of material remained adhered to the walls of the cyclone, promoting a decrease on the yield. On the other hand, for minimum values of T_{inlet}, close to 105°C, the yield was even lower than the process carried out with the maximum temperature value, for reasons that have not been fully explained but likely related to higher moisture contents of particles. Between content of fat and T_{inlet} there was a significant positive interaction, reinforcing the hypothesis that an increase in fat content in the dispersion to be dried delivered different yields based on the temperature at which one operates, due to the influence on the adhesion of the material to the walls of the equipment's chamber. From a visual inspection of what happened during the series of processes, it has been noted that the quantity of material that remained adhered to the walls of the cyclone increased when operating with incoming air at higher temperatures and with larger quantities of fat, because the possibility of melting or softening the part of the material made up of fats increased. There was also a synergistic effect, with low significance between the temperature of the incoming air and the fraction of theophylline in the modified milks. As the amount of theophylline increased, the slope of the branches of the parabola increased, which represented the trend of the yield as a function of temperature and that, with increasing T_{inlet} and increasing the fraction of theophylline, the effect of the latter becoming more evident on the yield. It can be deduced that, as the fraction of theophylline increased, the temperature became a more critical

parameter. When higher values were reached, the energy within the system increased and therefore the possibility of interaction between the milk components and theophylline also increased. Furthermore, it has been observed that, at higher drug fractions, theophylline crystals were observed under the microscope. Thus, it can be deduced that the spray-drying process, in these conditions, was not able to amorphisize the all quantity of theophylline in the powder, leaving some part as crystals. The value of R^2_{adj} indicates that the mathematical model did not fully describe the trend of the yield due to the varying factors considered. However, bearing in mind the complexity of the system and the technology used, the value obtained still could be considered acceptable, as observed in the work by Habtegebriel et al. [2018].

The moisture contents of the powders atomized at 105, 130 and 150°C ranged between 4.1 – 10.3%, 1.0 – 8.2% and 0 – 5.9%, respectively. As the T_{inlet} increased the moisture content of powders increased, as anticipated, but no significant relationship was observed with the increase of theophylline fraction and fat content. This suggests that neither theophylline nor fat have played a major role in the evaporation of water. As the T_{inlet} increased it was expected an increase of the yield, but it did not happen, likely due to the fluidity of fat components and rubber state of proteins [Dumoulin and Bimbenet, 1998]. The absence of a clear relationship between moisture content and fat content revealed the complexity of drying aqueous systems with high fractions of fat materials and proteins (Table 2).

The multiple linear regression analysis of the data related to the influence of the three independent variables on the moisture content of the powders, revealed an intercept of 4.1%. This result falls fully within the range of expected values for the moisture content of powders obtained by spray-drying, which was expected to be between 0.4 and 5% [Nuzzo et al., 2017]. As far as the influence of the variables is concerned, only the linear effect of the temperature was inversely proportional, i.e., as T_{inlet} increased the moisture content decreased, by virtue of the larger amount of heat, conveyed by the air to the materials promoting a more efficacious drying. There was a negative interaction, although not very significant, between fat content and T_{inlet}. This interaction suggests that, as one variable increased, the effect of the other decreased. In this case, the influence of T_{inlet} on the moisture content decreased with increasing fat content in the milk. By changing the type of milk, low-fat milk (LFM) to semi-skimmed milk (MFM) or, even to whole milk (HFM), the effect of the temperature of the incoming air on the moisture content decreased, suggesting that incoming heated air became less efficient. The value of R^2_{adj} can be considered acceptable, always taking into consideration the complexity of the process and given the small scale of operation.

3.2.2.2. Particle size, size distribution and morphology: From the microscopic analysis the mean diameter of the particles was found to be roughly the same for all samples obtained with different fractions of theophylline and processing temperatures (Table 3). These results were in line with the ones found in the literature when the same working

conditions were considered [Nuzzo et al., 2017, EMA, 2006]. Spherical particles with a mean particle size ranging from 3.0-4.3 µm, with a narrow size distribution were always obtained regardless of the T_{inlet} considered. The small differences between batches seem mostly due to the processing conditions and not to the theophylline fraction in the formulations. Initially, the microscopic observation revealed that theophylline raw material was present as crystals (needle shape, Figure 1a) or in suspension (31%) for different fat content milks (Figure 1b). After drying the crystals disappeared and the particles failed to reveal crystallinity (Figure 1c) which is supported by the fact that spray-drying promotes amorphization of materials [Birchal et al., 2005]. However, when the fraction of theophylline was high (62 and 100%), crystals of theophylline could still be observed. This suggests that up to 62% theophylline was embedded in milk components but, above this value, saturation was reached. Dried particles revealed a spherical shape that was in line with the process of atomization and drying. These particles also revealed a tendency to agglomerate. It is also demonstrated by the fact that the lactose, the predominant solid part of the milk, was almost 100% in the amorphous state, which facilitated the agglomeration of the particles after spray drying (Figure 1d).

The intercept value, i.e., the value calculated at the central point of the experimental space (Table 4), indicates that particles have shown a mean diameter of 3.7 µm, in line with the parameters set for the powders obtained by the process of spray-drying. The linear effects of the drying air temperature and the fraction of theophylline in the samples were found as positive. Consequently, an increase in each of these factors would increase the particle size. Regarding the effect of the T_{inlet}, it was conceivable that, at the highest temperature, the process of evaporation was the fastest and the particles did not have time to recover into less porous structures. The presence of a larger fraction of theophylline in the formulation entailed a larger quantity of material to be dried, or a larger concentration than the initial dispersion. This resulted in a larger particle size for the same set parameters of the samples with larger fractions of theophylline, particularly when prepared with high fat milk. In any case, the values of these effects were quite limited, proving that they did not have an important effect on the particle size, confirming the hypothesis of a good robustness of the technique for this answer. Regarding the variability of the mean diameters it can be seen that the only significant effect observed was the antagonistic interaction between the fat content and the temperature of the incoming air. This effect, considering also the non-significance of the linear effects of the two factors, was indeed very limited. This also indicated the robustness of the method. The low values of the correlation indexes indicated a poor ability of the models to explain the relationships between the factors and the outputs and could be due to the good robustness of the results obtained.

3.2.2.3. Density: The densities of the particles measured with gas pycnometry seems to increase when the fraction of theophylline increased, while remaining in a range of similar values comparable for those particles obtained by the

process performed on the controls. i.e., samples containing only milk (LFM, MFM or HFM, Table 3). As anticipated, when fat content increased in the formulations, the density of the particles decreased. It was also observed the large variability between the three different fat content milks, regardless the inlet air temperature.

The true density of the powders calculated at central point of experimental space was 1.6 gcm^{-3}. It can be observed (Table 4) that all factors, with the exception of the theophylline content, have significant influence on the density of the powder. In particular, the effect of the fat content can be described by a convex parabola with a vertex corresponding to a fat content of 3.7 g/100 g for which the maximum density of particles would be obtained. Moving away from this value, either to higher or lower fat contents, there was a reduction in the response considered. The protein component fraction was present in a relatively large proportion in low fat milk, could affect the increasing volume, with the reduction of its density. Higher lipid content, given their reduced specific weight, decreased the value of the density. It must be pointed out that fat components can migrate to the surface of the particles delivering a non-uniform particle and a coat to the particle promoting a decrease on the density of particles [Nijdam and Langris, 2006].

The density trend as a function of the T_{inlet} values could also be described by a convex parabola with the maximum at 140°C. The density decreased when moving towards either the minimum or the maximum values in a non-symmetrical manner. It could be hypothesized that at higher temperatures a more marked and robust drying occurred and did not allow the particles to be rearranged. In this way the globules produced in the drying chamber had no way of coming into intimate contact with each other, leaving more substantial intra-particle spaces that were not measurable by the pycnometer, since the helium only fills the inter-space voids in particulates, thus providing a lower density datum than that of samples obtained from processes performed with lower inlet air temperatures. The reduced drying that occurred at lower temperatures left residual water in the material providing a lower density than the raw materials and, therefore, reduced the overall value for the density. The interaction observed was negative with opposing effects between the fat content and the temperature of the air entering the drying chamber. When high fat milk was used, the applied temperature had less influence on the density. The high value of R^2_{adj} indicated that the model described well the trend of the property under examination.

3.2.2.4. Contact angle and surface free energy: The results obtained from the tests carried out for the assessment of wettability have shown that an increase in the content of fat in the samples corresponded to a poorer water wettability, due to the greater hydrophobic nature of the surface of the powder particles obtained (Table 3). The values observed for the contact angle with water were close or, marginally smaller than 90°, reflecting residual water wettability. The results obtained with iodomethane have shown good wettability likely due to the lipidic component. The processing temperature

did not affect the results, likely because the impact of the residual amount of water in the products and the transformations of the raw materials due to the process were pareil between experiments.

The powders produced with LFM were more hydrophilic and more wettable. During the formation of particles, it was possible that proteins, that for this type of milk constitute a larger portion of the solid content of milk than in HFM, both as free molecules or in micelles, were deposited on the surface of the formed particles, lowering their surface energy. In contrast, HFM, in which fat globules formed in contact with water have the ability to disperse and migrate to the surface of particles have produced a more hydrophobic surface [Nijdam and Langris, 2006]. Some MFM based particles have shown higher contact angles with water than low-fat content particles. It is conceivable that, throughout drying, the reorganization of the milk components in these two types was strictly dependent on the different fat / protein surface composition. The residual water could be the justification of the lowering of the contact angle of the dried LFM samples at 105°C, which reduced the fat fraction with impact on the reconstitution in water.

The two components of the surface free energy of the powders (polar and total), that can be added to allow the calculation of the respective polarity, are dependent on the arrangement of the internal structure of each particle. These parameters were calculated according to the Wu equation and reflect the surface composition of the powder, dependent on the compositions of the dispersions from which they were produced. From the analysis of the linear regression of the data it can be hypothesized, given the respective coefficients, that the surface of the powders mainly provided dispersive type interactions and only a minimal part of polar interactions. The coefficients were influenced by both types of energy based on linear effects of all three independent variables, although the polar surface free energy component was less important. During the formation of the particles, it was conceivable that the proteins of the milk, either as free molecules or as organized structures (e.g. micelles) were placed on the surface of the formed particles, contributing to the increase of the dispersive component of the surface free energy and thus making the surface hydrophobic. Parallel to what has been verified for the contact angle with the polar solvent, the free energy concerning the lipophilic surface interactions had a better correlation coefficient than the one related to the polar surface energy, demonstrating the larger influence of the factors taken into consideration for this property.

The calculation of surface free energy mathematical model, has shown that the increase in fat content caused a reduction of the surface energy. This could be caused by the fact that fat tends to accumulate on the surface of the particles increasing their hydrophobicity (Table 3) impacting negatively on the dissolution of the active ingredient and on the physical stability of the powder.

The analysis of the effects of the three variables on the responses considered have shown that their effects were significant but of little importance on the trend of the values of the dependent variables (Table 4). The contact angles, measured with the two test liquids for all samples were compared and related to a decrease on the wettability of theophylline. However, the solubility increased resulting from the likely incorporation of theophylline in the solid fraction of the milk. The fat content and the T_{inlet} have shown significant negative effects on of the surface free energy of the particles. The analysis for first order effects, both of the fat content and of the T_{inlet}, has shown a significant and positive interaction, i.e. considering the progress of the process starting from the lower values up to the highest values set for the two independent variables, the contact angle in water increased, implying a decrease on the wettability of the powders produced. Overall, the fat content and the T_{inlet} have shown significant negative effects on the surface free energy.

The cause of these effects could be found in the fact that, by increasing the fat fraction of the milk composition, the lipophilic component of the material grows becoming less suitable for reconstitution in water. Similarly, by increasing the temperature in the chamber to a maximum of 150 °C, one should have observed a lower water content in samples due to the more extreme drying conditions, leading to a less hydrophilic product and, therefore, less prone to reconstitution in water. Important, but not significant, was the quadratic effect of the influence of the amount of theophylline loaded with respect to the trend of the contact angle in water which produces a convex parabola, whose vertex is -0.39 which corresponds to 40.5% of loaded theophylline. For higher and lower values there were no important differences in the response values. The synergistic effect between the fat content and the temperature of the drying air on the contact angle value in water was also highlighted. The R^2_{adj} value was not plenty satisfactory, however, given the minimal levels of data variability it would be difficult to find higher values. This could be, as explained for other answers, an index of the robustness of the process.

The data relating to the contact angle measurements enabled the establishment of a relationship in which the intercept presents a contact angle of 38.17° in diiodomethane and 86.6° in water. The fat content had an influence on the values of the measure property that can be described by a concave parabola with vertex next to values of zero fat content. Consequently, increasing the amount of lipids present, the contact angle in iodomethane increased marcably. A higher fat content, as one could easily hypothesize, increased the lipophilic component and consequently an increase on the wettability by a nonpolar solvent. However, the presence of other components in milk (e.g. proteins), a different organization of lipid molecules when present in higher fractions or the presence of higher theophylline contents, may explain the relationship observed. The fraction of theophylline also influenced the value of the contact angle, but in a negative manner: as the amount of theophylline increased, the decrease in the angle value in iodomethane indicates a

lower lipophilicity of the product. In particular, it can be hypothesized that theophylline, when added to the solid component of milk, can be embedded into micelles consisting mainly of fat and proteins, reducing their lipophilicity. From the arguments reported it was evident that, there can therefore be a synergy between the fat content and the amount of theophylline effects: to an increase in the first variability and, there may be an increase in the influence of the loaded amount of drug in the dispersion, both of which affect the degree of hydrophilicity / lipophilic of the particles. In any case, considering also the value of R^2_{adj} equal to 0.33, it can be said that although significant effects were observed with low ability to describe the relationship.

3.2.2.5. Theophylline content: The analyzes conducted by HPLC on the theophylline content in the samples showed that there was a correlation between the fraction of theophylline in the formulations and the percentage of material lost after spray-drying. There was a progressive reduction in the theophylline fraction in the final product when its fraction was increased from 8 to 100% (Table 3). The larger the fraction of the drug loaded, the larger the incorporation into the milk dispersion, but not in the same proportion, resulting in a progressive large exposure to the environment of the theophylline molecules not incorporated in fat or proteins of milk.

From the intercept value (Table 4) there was an average percent title of theophylline of 103.6% indicating that there was no loss of theophylline due to the process, particularly no degradation such as to affect the amount of theophylline initially loaded in the powders. Although there was not a linear effect of any of the three independent variables, a significant quadratic effect of the amount of theophylline was observed. The parabola defined by this effect is convex and has the vertex at -0.13 corresponding to a value content of theophylline equal to 43.5%. The drug content decreased for both lower and higher fractions than this initial loaded quantity, but the variations produced were not important. This confirms the robustness of the process. The low value of R^2_{adj} indicates a low correlation between variables, likely because the only significant effect was the amount of theophylline.

3.2.2.6. Assessment of the stability of the different powders: The stability of the powdered milks was investigated after production and storage at room temperature (24°C) and controlled humidity (65% RH) for 6 months. Powders were reconstituted with water and the aqueous systems obtained and stored at 4°C have shown that the limits for the microbiological tests were met by all samples up to 2 days, but only samples with LFM passed the test 7 days after reconstitution [Eur. Pharm., **2020**]. In all cases the content of theophylline remained constant throughout the 28 days of storage upon reconstitution regardless the fat content of the milk used and independently of the ongoing microbial growth. Yeasts and *E. coli* were absent in all tested samples. It is also worth mentioning that the products dried at low temperatures showed lower microbial growth and higher moisture contents, suggesting that heat due to processing could

not prevent the growth of bacteria that carried out over storage suggesting that, the medium-long term stability of these powders was compromised, because the limits for the microbiological tests were exceeded only by the samples produced with low-fat milk, while the quantification of theophylline showed that there was no decrease in the content after 6 months of storage. This means that theophylline was kept stable from the environment and milk components did neither promote degradation neither prevented theophylline from quantification. Thus, milk fat has also contributed to the stability of samples.

3.2.3. Optimization of the spray drying process: application of the desirability function

The results of the multivariate analysis based on the data collected from the characterization of the products have allowed the evaluation of the influence of the factors studied on the values of the individual responses. The next step was the investigation on how the independent variables have influenced the overall quality of the products to enable the persecution of the study for the search of the formative and operative conditions suitable to reach the desired Quality Target Product Profile, made of the set of optimal quality and sturdiness characteristics to proceed with the production of powders carrying a model drug with reduced risk of failure. For this purpose, it was decided to use "*the desirability function*" [Derringer and Suich, 1980], a tool capable of simultaneously consider all the answers studied (Table 5). The first step for the application of this tool is the definition of a series of functions of "*individual desirability*", d_j, for each answer considered, which allows the transformation of the value of the "*response variable*" (Y_j) into a value of desirability based on criteria chosen by the researcher. In particular, the values of each dependent variable are normalized on a common dimensionless scale to allow direct and reciprocate comparison between the outputs of the experiments. The scale used ranged between the lower limit 0, when the value of the response is unacceptable and therefore the desirability for this is 0, and the upper limit 1, when the value is completely satisfactory or optimal and the desirability turns out to be maximum {S4}:

$$0 < D_j < 1$$

The individual desirability function was constructed for each of the responses considered, which were in accordance with the specific requirements for each parameter. The responses taken into consideration were the process yield, moisture content, mean particle size and size distribution, true density of powdered milks, contact angles in either water or in iodomethane and theophylline content (Table 5).

The overall desirability function (D) is the geometric weight average of the combination of individual desirability functions, according to the following equation:

$$D = \sqrt[8]{(d_{Yield} * d_{moisture\ content} * d_{mean\ particle\ size} * d_{mean\ span} * d_{theophylline\ content} * d_{true\ density} * d_{contact\ angle\ water} * d_{contact\ angle\ diodomethane})}$$

In this work the relevances of the variables were assumed to be the same, therefore the same weight was given to each one of the 8 individual desirability functions. The trend of the overall desirability can be described, as presented in Figure 2, in which the fourth column represents the eight functions of individual desirabilities. The last line shows the trend of the overall desirability function as the level of each one of the 3 factors varies, keeping the other 2 at the optimal level. For each of the first 8 rows of the first 3 columns the trends of the 8 responses are shown as the 3 factors described by the respective individual desirability functions vary. The optimal combination of levels is shown by the black dashed lines (Figure 2). The trends for each dependent variable [yield, moisture content (MC), average diameter and diameter distribution, density, contact angle in water and diiodomethane and theophylline content] with varying levels of each independent variable (fat content, T_{inlet} and fraction of theophylline) are reported in the first 8 lines, keeping the other 7 variables fixed at the optimal level. The trends of the overall desirability function are shown in the last line: they vary with the level of each factor, while the level that determines the best desirability is kept fixed for all others. Taking into consideration the trends observed and by maximizing the overall desirability function, it was possible to calculate the combination of levels for the factors studied assuring a product with the best compromise between the values of the observations (Table 6, upper part).

The maximum desirability value can be obtained from the combination of values which foresees the production of powders starting from the dispersion of milk by choosing the one with the highest fat content and a quantity of theophylline loaded with 64.5%. The spray-drying process is maximized if conducted with an inlet air temperature of 135°C. These conditions are very close to those applied for the DoE test number 23. The overall desirability value obtained under the conditions found as optimal is equal to 0.63, which indicates a good capacity of the system to obtain a product with a good quality profile. The desirability always remains at good levels also varying the levels of the factors as can also be deduced from the low slope of the curves of the last line of the graphs in Figure 2. In particular, the variation is minimal for the different contents of fat and the values remain close to the maximum even when the other two independent variables vary. As for the fraction of theophylline used, it has already been highlighted by calorimetry (DSC) and from the images collected under the microscope that have suggested that values higher than 62% did not guarantee the absence of crystals of theophylline, suggesting a limit in the interaction capacity of milk components with theophylline. For this reason, it was imposed a maximum value of 50% theophylline content to ensure a good

probability for obtaining an amorphous product. Consequently, the values for the other variables were recalculated and are presented in Table 6 (lower part). As observed there was no significant difference between the optimal levels for the other two factors studied. This result also demonstrates the robustness of the technology applied to the system under study, confirming the goodness of the initial choices. Indeed, the Overall (or Composite) Desirability is approximately constant through the Design Space confirming the robustness of process.

4. Conclusions

The study confirmed the ability of commercialized cow milk to deliver a drug (e.g. theophylline) orally as a composite powder that was easily reconstituted to obtain a liquid system. The powder, manufactured with milks with different fat contents and fractions of theophylline, was stable after storage. Chemical and microbiological stability studies have shown that dried powders were stable for at least 6 months but once reconstituted bacteria started to grow decreasing the stability in use from 2 to 7 days, for skimmed or whole milks. No pathogenic microorganisms were observed. When theophylline was present above 60% (w/w fat content) crystals were observed in the final product by opposition to the powders with lower fractions in which materials in the amorphous were collected. The moisture content decreased with increasing drying temperature (about 5.9%) but no relationship with fat and theophylline contents was obtained. Neither theophylline nor fat contents affected significantly the size (3.7 μm) and size distribution of particles. The density of particles (about 1.6 gcm^{-3}) was dependent on the fat content of the milk. As anticipated, an increase on fat content in the formulations translated into less wettable particles whereas an increase on theophylline content turned the particles more hydrophilic. The content of theophylline measured was more dependent on the fat content and less on the drying temperature.

The factorial design of experiments enabled the establishment of correlations between the independent variables (fat content, theophylline fraction and inlet spray drying temperature) and the dependent properties of the powders (yield, moisture content, particle size and size distribution, density, surface polarity and theophylline content). The use of the overall desirability function has shown a good capacity of the system to obtain a product with high quality

profile. Desirability has always remained at high levels (0.63) regardless changes of the levels of the independent variables: the variation was minimal for the different fat contents and remained at values close to the maximum even when the other two independent variables varied. The complexity of milk has impaired the construction of a better model but it was possible to confirm that fresh milk could be transformed into a composite powder containing theophylline to be delivered orally in a flexible dosage pattern. Overall the desirability function based on the models proposed managed to predict the outcome of the process, particularly for the density, fairly for the moisture content and for the nonpolar component of the surface free energy and yield and it was unable to predict the value for the size of particles and for the content of theophylline.

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Figure Captions:

Figure 1: Microscopic evaluation of samples of theophylline raw material (a), or in suspension (31%) for different fat content in milk (b), powdered milk after drying (c and d) and likely crystallized theophylline in milk (e).

Figure 2: Graphical representation of individual desirability trends and overall desirability.

Apolar E – non polar surface free energy component; Polar E – polar surface free energy component; Angle D – contact angle in diiodomethane; Angle W – contact angle in water; SPAN – particle size distribution; MPS – Median particle size; Theo – theophylline content; MC – moisture content; Yield – yield of the spry dried product.

***Conflict of Interest**

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

none

Credit Author Statement

Carlotta Nesse – investigation, writing original draft.

Luca Palugan – writing review and editing, data curation.

Matteo Cerea – conceptualization, methodology, validation, analysis, resources, writing review and editing, visualization, supervision,

João F Pinto – conceptualization, methodology, validation, analysis, resources, writing review and editing, visualization, supervision, project administration.

Table(s)

Table 1: Variables and levels of variables considered in the study, expressed as real and coded values.

Variable	Codification	Non Coded Variable	Coded variable
Fat content (g/250mL)	LFM	0.3	-0.74
	MFM	4.0	0
	HFM	9.0	+1
T_{inlet} (^oC)	Low	105	-1
	Mean	130	0
	High	150	+0.8
Fraction of theophylline (%)		0	-1
		8	-0.84
		16	-068
		31	-0.38
		62	0.22
		100	+1

LFM – low fat content milk; MFM – medium fat content milk; HFM – high fat content milk

Table 2: Matrix of the full factorial Design of Experiments.

Experiment	Non Coded Variables			Coded Variables		
	Fat Content	T_{inlet}	Theophylline fraction	Fat Content	T_{inlet}	Theophylline fraction
1	0.3	105	0	-0.74	-1	-1
2	0.3	105	8	-0.74	-1	-0.84
3	0.3	105	16	-0.74	-1	-0.68
4	0.3	105	31	-0.74	-1	-0.38
5	0.3	105	62	-0.74	-1	0.22
6	0.3	105	100	-0.74	-1	1
7	4	105	0	0	-1	-1
8	4	105	8	0	-1	-0.84
9	4	105	16	0	-1	-0.68
10	4	105	31	0	-1	-0.38
11	4	105	62	0	-1	0.22
12	4	105	100	0	-1	1
13	9	105	0	1	-1	-1
14	9	105	8	1	-1	-0.84
15	9	105	16	1	-1	-0.68
16	9	105	31	1	-1	-0.38
17	9	105	62	1	-1	0.22
18	9	105	100	1	-1	1
19	0.3	130	0	-0.74	0	-1
20	0.3	130	8	-0.74	0	-0.84
21	0.3	130	16	-0.74	0	-0.68
22	0.3	130	31	-0.74	0	-0.38
23	0.3	130	62	-0.74	0	0.22
24	0.3	130	100	-0.74	0	1
25	4	130	0	0	0	-1
26	4	130	8	0	0	-0.84
27	4	130	16	0	0	-0.68
28	4	130	31	0	0	-0.38
29	4	130	62	0	0	0.22
30	4	130	100	0	0	1
31	9	130	0	1	0	-1
32	9	130	8	1	0	-0.84
33	9	130	16	1	0	-0.68
34	9	130	31	1	0	-0.38
35	9	130	62	1	0	0.22
36	9	130	100	1	0	1
37	0.3	150	0	-0.74	0.8	-1
38	0.3	150	8	-0.74	0.8	-0.84
39	0.3	150	16	-0.74	0.8	-0.68
40	0.3	150	31	-0.74	0.8	-0.38
41	0.3	150	62	-0.74	0.8	0.22
42	0.3	150	100	-0.74	0.8	1
43	4	150	0	0	0.8	-1
44	4	150	8	0	0.8	-0.84
45	4	150	16	0	0.8	-0.68
46	4	150	31	0	0.8	-0.38
47	4	150	62	0	0.8	0.22
48	4	150	100	0	0.8	1
49	9	150	0	1	0.8	-1
50	9	150	8	1	0.8	-0.84
51	9	150	16	1	0.8	-0.68
52	9	150	31	1	0.8	-0.38
53	9	150	62	1	0.8	0.22
54	9	150	100	1	0.8	1

Table 3: Properties of the powders obtained from each type of milk, fraction of theophylline and drying temperature.

T_{inlet}/T_{outlet} (°C)	Fat Content (g)	Property	Theophylline:milk solids ratio					
			0:1	0.08:1	0.16:1	0.31:1	0.62:1	1:1
105 / 68-70	0.3 (LFM)	Yield (%)	42.0	33.7	45.8	52.7	49.9	53.6
		MC (%)	4.9	6.4	4.7	5.7	5.5	6.4
		Particle Size (µm)	3.3	3.1	3.5	3.5	3.6	3.7
		ρ ($g.cm^{-3}$)	1.501	1.509	1.520	1.499	1.504	1.515
		θ_w (°)	85.14	83.74	85.53	83.94	80.62	81.23
		$\theta_{iodomethane}$ (°)	38.87	41.72	37.90	45.15	47.46	44.18
		γ^P	11.14	9.20	9.07	9.69	9.54	10.4
		γ	52.44	49.35	49.18	48.44	46.40	50.15
		Polarity (%)	21.24	18.64	18.44	20,00	20.56	20.74
		Theophylline (%)	0	100.0	97.8	89.7	75.0	61.8
	4 (MFM)	Yield (%)	39.2	56.6	54.2	36.6	38.5	54.1
		MC (%)	6.1	6.5	8.5	4.5	5.3	4.1
		Particle Size (µm)	3.3	3.3	3.0	3.6	3.7	3.5
		ρ ($g.cm^{-3}$)	1.426	1.401	1.413	1.446	1.449	1.455
		θ_w (°)	86.70	85.21	85.33	93.32	84.63	83.55
		$\theta_{iodomethane}$ (°)	45.47	43.10	43.50	32.05	43.52	37.74
		γ^P	3.82	6.90	7.40	4.56	7.55	8.66
		γ	41.38	45.47	45.28	45.69	47.92	48.09
		Polarity (%)	9.23	15.17	16.34	9.98	15.76	18.01
		Theophylline (%)	0	96.5	93.6	88.8	72.8	65.5
	9 (HFM)	Yield (%)	28.4	38.9	36.6	46.6	33.8	37.4
		MC (%)	7.1	4.8	10.3	5.9	9.4	4.2
		Particle Size (µm)	3.4	3.4	3.7	3.6	3.4	3.8
		ρ ($g.cm^{-3}$)	1.382	1.382	1.385	1.357	1.412	1.437
		θ_w (°)	84.79	86.64	83.18	81.53	82.70	81.31
		$\theta_{iodomethane}$ (°)	56.11	48.00	47.63	51.88	47.46	44.18
		γ^P	9.18	7.27	8.73	10.18	8.00	9.56
		γ	42.72	41.33	45.33	46.53	49.58	49.76
		Polarity (%)	21.49	17.59	19.26	21.88	16.14	19.21
		Theophylline (%)	0	96.5	93.6	88.8	72.8	65.5
130 / 87-88	0.3 (LFM)	Yield (%)	76.0	73.3	68.5	65.3	43.0	70.0
		MC (%)	1.6	1.1	1.0	1.0	2.5	1.1
		Particle Size (µm)	3.3	3.6	3.8	4.2	4.0	4.0
		ρ ($g.cm^{-3}$)	1.493	1.491	1.498	1.501	1.504	1.498
		θ_w (°)	85.50	84.73	85.36	86. 29	87.17	85.23
		$\theta_{iodomethane}$ (°)	40.32	31.77	30.65	39.11	47.95	44.78
		γ^P	8.23	8.78	9.04	5.08	9.73	11.33
		γ	48.49	45.02	46.38	43.25	48.02	49.47
		Polarity (%)	16.97	19.50	19.49	11.75	20.26	22.90
		Theophylline (%)	0	101.4	103.8	97.7	78.3	61.2
	4 (MFM)	Yield (%)	59.6	31.0	57.0	58.3	71.0	56.3
		MC (%)	5.1	6.3	4.8	4.1	3.6	3.0
		Particle Size (µm)	3.7	3.5	3.7	3.6	3.5	3.4
		ρ ($g.cm^{-3}$)	1.386	1.431	1.409	1.397	1.417	1.433
		θ_w (°)	92.55	83.95	85.54	83.38	85.31	79.76
		$\theta_{iodomethane}$ (°)	45.42	42.54	42.67	44.55	41.36	41.96

		γ^P	5.16	8.37	9.45	4.98	8.07	8.77
		γ	43.66	46.55	47.43	44.94	46.5	47.84
		Polarity (%)	11.82	17.98	19.92	11.08	17.35	18.33
		Theophylline (%)	0	102.9	102.5	89.5	74.9	66.0
	9 (HFM)	**Yield (%)**	47.2	72.1	72.6	45.7	64.6	68.9
		MC (%)	6.1	2.6	1.0	2.8	8.2	4.1
		Particle Size (µm)	3.5	3.6	3.7	3.6	3.8	3.7
		ρ (g.cm^{-3})	1.389	1.244	1.287	1.336	1.361	1.386
		θ_w (º)	85.72	86.62	93.51	92.76	85.71	81.69
		$\theta_{iodomethane}$ (º)	49.17	47.58	40.97	43.90	42.67	38.83
		γ^P	5.42	5.37	4.64	5.61	9.10	8.70
		γ	40.82	40.69	43.73	43.66	46.84	47.76
		Polarity (%)	13.28	13.20	10.61	12.85	19.43	18.22
		Theophylline (%)	0	94.03	98.9	102.2	79.1	64.9
150/100-102	**0.3 (LFM)**	**Yield (%)**	13.6	25.2	32.5	36.0	44.9	61.9
		MC (%)	0.7	5.9	2.1	2.7	1.7	0.6
		Particle Size (µm)	3.9	3.6	3.4	3.6	3.8	3.1
		ρ (g.cm^{-3})	1.426	1.552	1.515	1.501	1.486	1.476
		θ_w (º)	84.01	82.96	85.69	85.11	86.28	86.33
		$\theta_{iodomethane}$ (º)	47.54	49.35	47.98	47.07	33.29	43.95
		γ^P	12.30	12.25	5.63	9.02	8.00	7.16
		γ	48.63	47.34	42.35	46.57	45.04	44.80
		Polarity (%)	25.29	25.88	13.29	19.37	17.76	15.98
		Theophylline (%)	0	101.8	9.3	81.6	75.8	58.6
	4 (MFM)	**Yield (%)**	28.4	44.1	38.3	68.3	47.4	50.0
		MC (%)	2.8	1.9	0.3	1.9	1.8	0.4
		Particle Size (µm)	3.6	3.6	3.8	3.6	4.0	4.3
		ρ (g.cm^{-3})	1.407	1.376	1.386	1.396	1.418	1.424
		θ_w (º)	91.99	87.05	83.72	86.01	93.45	82.28
		$\theta_{iodomethane}$ (º)	41.88	43.35	37.69	38.30	32.85	37.10
		γ^P	5.17	4.7	8.67	4.67	4.49	8.72
		γ	45.13	45.72	48.63	46.37	46.66	50.43
		Polarity (%)	11.46	10.28	17.83	10.07	9.62	17.29
		Theophylline (%)	0	102.8	93.8	89.5	75.8	61.3
	9 (HFM)	**Yield (%)**	31.2	34.0	50.4	52.5	53.3	51.6
		MC (%)	0	1.2	0	1.2	2.8	0.4
		Particle Size (µm)	3.7	3.9	3.5	3.7	3.5	4.0
		ρ (g.cm^{-3})	1.296	1.298	1.309	1.326	1.367	1.384
		θ_w (º)	84.07	92.72	92.66	87.61	93.36	86.85
		$\theta_{iodomethane}$ (º)	53.63	48.81	51.10	49.14	43.71	47.92
		γ^P	5.51	5.44	5.68	8.91	4.98	5.11
		γ	39.13	40.68	40.01	46.20	42.35	41.82
		Polarity (%)	14.08	13.37	14.20	19.29	11.76	12.22
		Theophylline (%)	0	100.7	87.1	88.7	76.6	47.6

MC – moisture content (%);
ρ – density (pycnometry);
θ_w – contact angle in water [surface tension of water: γ = 72.8 mN/m; polar component (γ^P) = 51.0 mN/m; polarity = 70.05 %];
θ_D – contact angle in diiodomethane [surface tension of diiodomethane: γ_t = 50.8 mN/m; polar component (γ^P) = 0.02 mN/m; polarity = 0.02 %];

Table 4: Outputs for the model equations for each property studied.

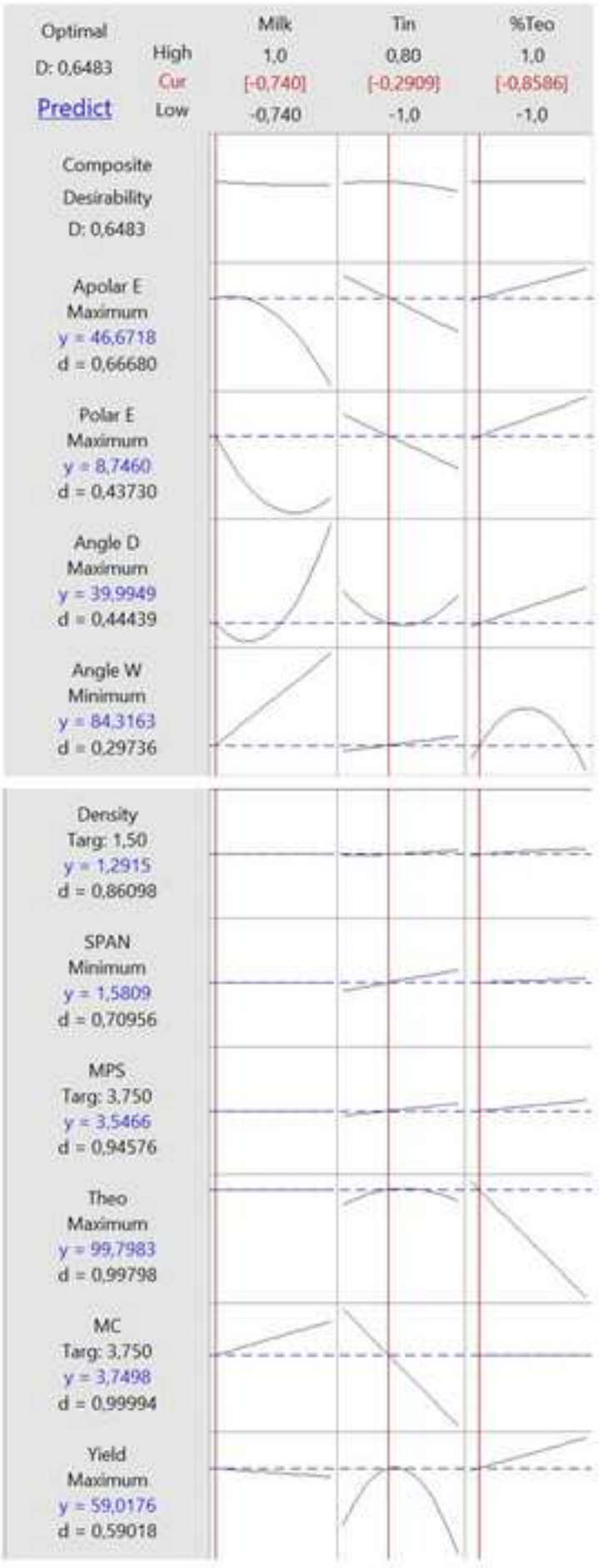
	Intercept	β_1	β_{11}	β_2	β_{22}	β_3	β_{33}	β_{12}	β_{13}	β_{23}	R^2_{adj}
					Yield						
coeff	62.9	-0.8		1.0	-18.6	5.5		4.7		4.4	0.453
0.593	0.000	0.649			0.000	0.012		0.037		0.096	
					Moisture content						
coeff	3.79	0.46		-2.32				-0.63			0.554
p	0.000	0.116		0.000				0.078			
					Mean Size						
coeff	3.64			0.12		0.11					0.212
p	0.000			0.003		0.016					
					Size distribution (span)						
coeff	1.28	0.01		0.05		0.01			-0.13		0.130
p	0.000	0.839		0.092		0.864			0.010		
					True density						
coeff	1.34			0.03	0.04	0.04					0.627
p	0.000			0.000	0.000	0.000					
					Surface free energy (polar component)						
coeff	6.67	-1.04	1.66	-0.71		0.61		-0.56			0.324
p	0.000	0.001	0.003	0.023		0.094		0.134			
					Surface free energy (non-polar component)						
coeff	46.2	-1.3		-1.0		1.6			1.5		0.490
p	0.000	0.001		0.006		0.000			0.005		
					Contact angle (water)						
coeff	86.6	1.0		1.54		-1.47	-1.89	1.18			0.275
p	0.000	0.056		0.004		0.018	0.094	0.062			
					Contact angle (iodomethane)						
coeff	39.2	1.9	3.6	-0.0	2.2	-1.5			-2.6		0.328
p	0.000	0.025	0.010	0.963	0.105	0.089			0.023		
					Theophylline Content						
coeff	101.0					4.3	-52.0				0.306
p	0.000					0.453	0.000				

Table 5: Individual desirability functions for each dependent variable considered in the study.

Independent Variable (D_j)	Dependent Variable (Y_j)	Level of Y_j	Desirable value
1	Yield	$Y_j = 0$ $0 < Y_j \leq 100$	0 $Y_j / 100$
2	Moisture content	$0 < Y_j \leq 3.75$ $3.75 < Y_j \leq 12.5$	$Y_j / 3.75$ $(12.5 - Y_j) / (12.5 - 3.75)$
3	Mean Size	$Y_j \leq 3.75$ $3.75 \leq Y_j \leq 10$	$Y_j / 3.75$ $(10 - Y_j) / (10 - 3.75)$
4	Size Span	$Y_j < 1$ $1 < Y_j < 3$ $Y_j > 3$	1 $(3 - Y_j)/(3 - 1)$ 0
5	True density	$0 < Y_j < 1.5$ $1.5 < Y_j < 4$ $Y_j > 4$	$Y_j / 1.5$ $(4 - Y_j)/(4 - 1.5)$ 0
6	Contact angle (water)	$0 < Y_j < 120$ $Y_j > 120$	$(120 - Y_j) / 120$ 0
7	Contact angle (iodomethane)	$0 < Y_j < 90$ $Y_j > 90$	$Y_j / 90$ 1
8	Surface free energy (γ^p – polar component)	$0 \leq Y_j \leq 20$	$Y_j / 20$
9	Surface free energy (γ – total free energy)	$20 \leq Y_j \leq 60$	$(Y_j - 20) / 40$
10	Theophylline content	$0 \leq Y_j \leq 100$	$Y_j / 100$

Table 6: Optimal combination of the levels of the independent variables considered calculated from the optimization of the overall desirability function and after setting the fraction of theophylline.

Independent variable	Coded Level	Real level
Overall desirability function		
Fat content	-0.74	LFM
T_{inlet}	-0.29	128°C
Fraction of theophylline	-0.86	7%
Overall desirability		0.648
Desirability function set to the fraction of theophylline at 50% corresponding to the coded value 0		
Fat content	-0.74	LFM
T_{inlet}	-0.29	128°C
Fraction of theophylline	0	50.0%
Overall desirability		0.647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Predict
High
Cur
Low
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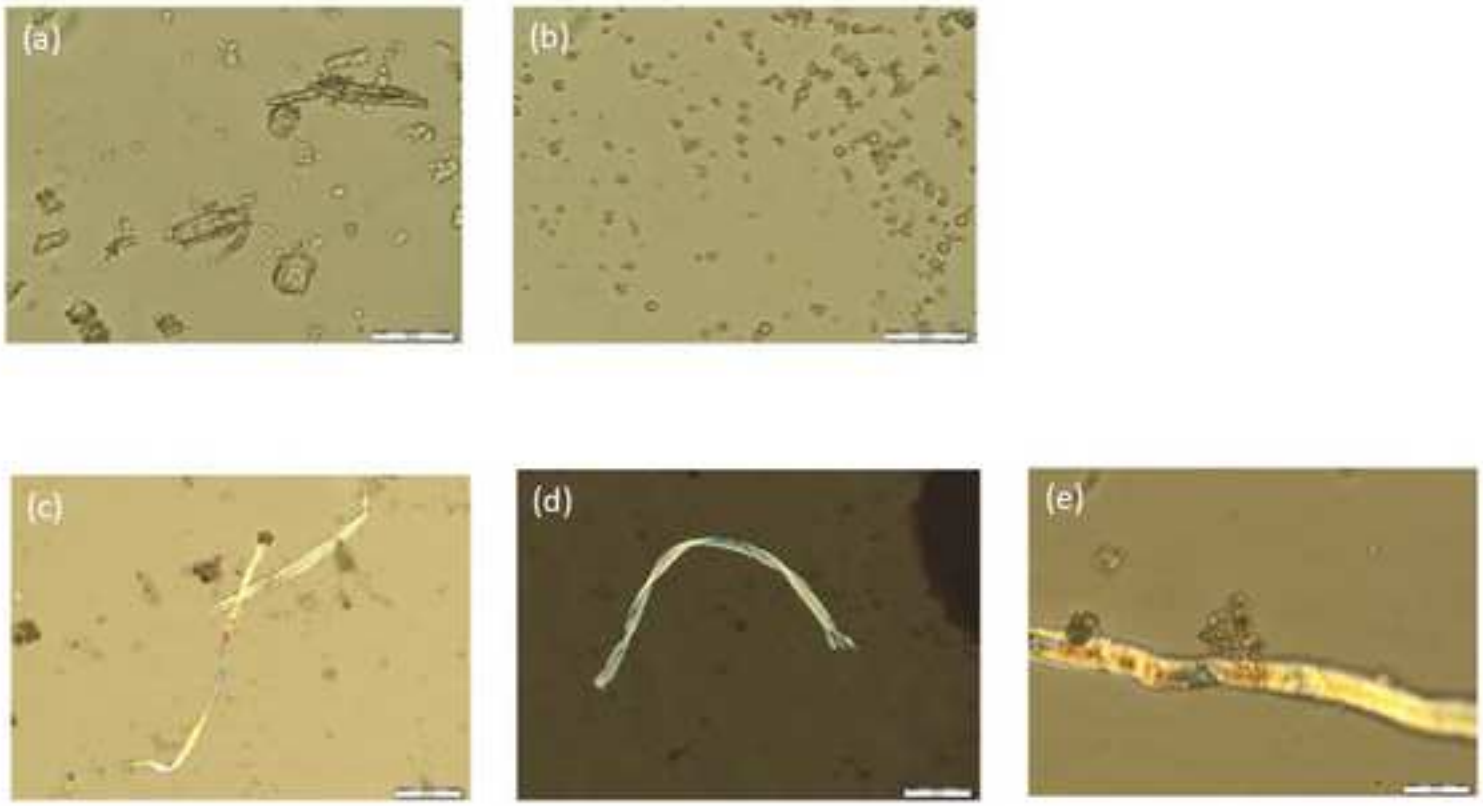
-0,740
Tin
0,80
[-0,2909]
-1,0
%Teo
1,0
[-0,8586]
-1,0
Composite Desirability
D: 0,6483
Apolar E
Maximum
y = 46,6718
d = 0,66680
Polar E
Maximum
y = 8,7460
d = 0,43730
Angle D
Maximum
y = 39,9949
d = 0,44439
Angle W
Minimum
y = 84,3163
d = 0,29736
Density
Targ: 1,50
y = 1,2915
d = 0,86098
SPAN
Minimum
y = 1,5809
d = 0,70956
MPS
Targ: 3,750
y = 3,5466
d = 0,94576
Theo
Maximum
y = 99,7983
d = 0,99798
MC
Targ: 3,750
y = 3,7498
d = 0,99994
Yield
Maximum
y = 59,0176
d = 0,59018

Figure 1

Figure 2



Supplementary Material

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