Production and characterization of spray-dried theophylline powders prepared from fresh milk for potential use in paediatrics

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Abstract

Objective: This work evaluates the potential of using fresh milk to deliver theophylline to children.

Methods: Theophylline-fresh milk systems were prepared using different solids ratios (0:1 to 1:0) and three fat contents in commercial milks (low, medium and high), which were spray-dried at different inlet air temperatures (T_{inlet} – 105, 130 and 150°C). The process was evaluated for yield and the resulting powders for moisture content, particle size and shape, density and wettability. Theophylline-milk potential interactions (DSC and FT-IR), and chemical (theophylline content) and microbiological stability of powders (shelf and in-use) were also evaluated.

Key findings: The production yield (13.6-76.0%), moisture content (0.0-10.3%) and contact angles in water (77.29–93.51°) were significantly ($p<0.05$) affected by T_{inlet}, but no differences were found concerning the mean particle size (3.0-4.3μm) of the different powders. The milk fat content significantly ($p<0.05$) impacted on the density (1.244–1.552g/cm^3). Theophylline content remained stable after 6 months of storage, prior to extemporaneous reconstitution. After reconstitution in water, low fat milk samples (stored at 4°C) met the microbial pharmacopoeia criteria for up to 7 days. No theophylline-milk components' interaction was observed.

Conclusion: Spray-dried milk-composed powders may be used as vehicles for theophylline delivery in paediatrics following further characterization and in vivo evaluation.

Keywords:

Extemporaneous-preparation; Milk; Solid-oral-drug-delivery; Paediatrics; Spray-drying; Theophylline.

List of abbreviations

API – Active Pharmaceutical Ingredient

Θ – Contact angle

Θ_D – Contact angle in diiodomethane

Θ_w – Contact angle in water

DSC – Differential Scanning Calorimetry

EMA – European Medicines Agency

FT-IR – Fourier Transform Infrared Spectroscopy

HFM – High Fat Milk

LFM – Low Fat Milk

MC – Moisture content

MFM – Middle Fat Milk

γ_s – Surface Free Energy (Total)

γ^d – Surface free energy (Dispersive component)

γ^p – Surface free energy (Polar component)

T_g – Glass transition temperature

T_{inlet} – Inlet air temperature

W_a – Work of adhesion

W_c – Work of cohesion

WHO – World Health Organization

1. Introduction

A paediatric patient belongs to a distinct and heterogeneous group regarding pharmacotherapeutic requirements (1,2). It is well established that children are not small adults and, therefore, drug formulations for paediatrics should be adapted to suit children's age, size and physiology, as well as treatment needs. Medicines' design is therefore critical to achieve not only safe and accurate dosing of drugs, but also to enhance compliance and improve clinical outcomes in children. To tackle this problem new paediatric regulations and development programs have recently been issued by the European Medicines Agency (EMA) (3) and the World Health Organization (WHO) (4,5).

Children are, however, especially challenging (6) since they are continually growing up, thus presenting constantly evolving pharmacokinetic parameters, and many existing medicines were not designed or tested *ab initio* having these considerations in mind. Moreover, many drugs are unavailable as paediatric formulations, requiring the use of unlicensed (off-label) medicines, by manipulation of existing dosage forms designed for adults. Often, these are compounded with excipients which are toxic to children and are administered without considering that paediatric patients do not have fully developed enzymatic and immunologic systems (2,7,8).

The oral route of administration remains the preferred one and liquid dosage forms (e.g. solutions and suspensions) are widely used in paediatrics' daily clinical practice. These dosage forms are preferred among children (< 5 years old) due to their ease on swallowing and dose adjustment, but can compromise the patient's compliance (e.g. bitter taste), suffer from dose inaccuracy and lack long term chemical, physical and microbiological stabilities. Small-sized multiunit solid dosage forms, such as minitablets (9,10), pellets (11) and granules (12)**,** which do not present the disadvantages discussed for liquids, become a better option in grammar school children's age (> 6 years old) due to the ease of administration (2,13). However, as discussed before, the paediatric group encompasses so many different individual characteristics and is so diverse that it is almost impossible to develop a single formulation which is accepted across the group (2).

Compliance with drug therapy in paediatrics is related to the acceptability of the formulations by patients. In some situations (e.g. bitterness of the API) nurses, parents and even caregivers are encouraged to mix the medicines with food (e.g. juices and infant milk formulas) disregarding drug stability issues (14).

In this study, milk was chosen as a matrix for potential drug delivery in paediatrics because it is a worldwide accepted food (in fact, the primary nutritional source in the newborns) which presents good organoleptic and physicochemical properties for the intended use. As a complex system − milk is simultaneously a solution (dissolved proteins, minerals and vitamins), a suspension (whey proteins) and an emulsion (lipids and lipophilic vitamins) − it incorporate drugs with different solubilities and thus represent a good matrix for paediatric drug delivery. In fact, milk components (e.g. caseins) have already been used as vehicles for bioactives (15) but a few studies have explored its potential in paediatric drug delivery (16). Milk-based oral formulations recently described in the literature consisted of alkaline/ethanolic solutions, containing different drugs, which were mixed with fresh milk immediately before administration to patients (17) or used skimmed milk to formulate solid dispersions of poorly water solubility APIs (18,19). In these cases, extra excipients will be needed in the formulation, increasing the risk of toxicity or, simply increasing the complexity of the final product.

Milk is commercially available as powdered or fresh milk. The latter can be easily converted into powdered milk by spray-drying, which is the most common method for dehydrating milk. The process promotes the stability of the final product and, at the same time, lowers the production costs due to the very short time of heat contact and high rate of evaporation (20,21). Consequently, it is possible to hypothesize that a drug-milk system can be converted into a powdered formula by spray-drying. The final product is expected to be both more stable in the long term (particularly by comparison with liquid dosage forms) and amenable to be used in a broader age group of patients (as compared to other solid dosage forms). The use of fresh, as compared to powdered milk, to incorporate the drug presents the advantage of promoting uniformity of contents and solubilisation of poorly water soluble drugs. Theophylline (1,3-dimethyl-7H-purine-2,6-dione) was considered in the study as a model drug, based on therapeutic

relevance in the treatment of acute and chronic asthma in children (22), and the possibility of extrapolating these results to other drugs that are known for their narrow therapeutic index.

The novelty of the work resides on the use of spray-dried fresh milk as a platform to deliver drugs in paediatrics, addressing three main concerns of Health Authorities (23) regarding paediatric medicines: a) safety of excipients, b) appropriateness of formulation and dosage form according to the age group considered, and c) dosing flexibility, accuracy and practical handling.

2. Materials and Methods

2.1. Materials

Fresh commercial milks (Mimosa, Lactogal Produtos Alimentares S.A., Portugal) with different fat contents (per 250ml), Low Fat Milk (LFM − 0.3g), Middle Fat Milk (MFM − 4.0g) and High Fat Milk (HFM − 9.0g), were used in the study. Anhydrous theophylline, diiodomethane, *p*-aminobenzoic acid (PABA) and acetonitrile (HPLC grade) were all from Sigma Life Science (Germany); ammonium acetate (Merck, Germany) and methanol (Fischer Chemical, USA). Deionized water (W, 18.2MΩ.cm, Milli-Q system, Merck Millipore, USA) was used throughout the study. All other reagents were of analytical grade.

2.2. Methods

2.2.1. **Preparation of theophylline : milk systems**

A theophylline stock solution (7.5mg/ml deionized water, 900rpm, 30min) was used to incorporate known amounts of drug into constant volumes of each type of milk to obtain theophylline:milk solids ratios of 0.08:1, 0.16:1, 0.31:1, 0.62:1 and 1:1 (w/w, Table 1 and Annex 1). Controls of fresh milks and aqueous theophylline solutions (0:1 and 1:0) were considered. Altogether theophylline was present in quantities from 0 to 2.5g per 2.5g of powdered milk.

145 2.2.2. **Spray-drying**

Spray-drying of theophylline in milk systems was performed in a Mini Spray-Dryer B-191 (Büchi Labortechnik GmbH, Switzerland). The feed solutions were atomized (1mm diameter static nozzle) using a feed liquid rate of 4 ml/min (*i.e.* 20%), an atomizing airflow rate of 600L/h and a constant aspiration rate of 100%; settings were kept constant throughout the study. Three different inlet/outlet air temperatures were
150 used: 105/68°C; 130/87°C; and 150/100°C. The outlet air temperature was recorded after the beginning of the drying process, and although it depends on the instrument settings, variations were smaller than ±1°C. Theophylline controls were atomized at a single inlet/outlet air temperature (130/87°C). The solutions were fed into the spray-dryer chamber, once the desired inlet temperature was achieved. The spray-dried samples were collected and stored at room temperature (24±1°C) and controlled humidity (RH=65%).

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2.2.3. **Characterization of spray-dried powders and process**

Yield and moisture content of powders: the yield of the process was obtained from the ratio between the mass of powder collected from the outlet chamber and the sum of milk solid components and theophylline masses; moisture content of dried powders was determined by the water loss on drying of powders dried
160 in an oven (Memmert, Germany) at 75±3°C, until constant weight. *Particle size and shape analysis:* The mean particle's diameter by number was determined by optical microscopy (BX51, Olympus, Japan), after suspension of powder specimens in liquid paraffin. To guarantee a normal distribution of data more than 200 particles were measured from photographs (5 different fields; 50x magnification, Olympus Stream Essentials Software, Olympus, Japan) and classified as spherical or non-spherical (ICH Topic Q4B (24)) upon
165 observation of random fields by scanning electronic microscopy (SEM after coating with chromium; JEOL JSM-T330A, JEOL, Japan). *Density:* Helium pycnometry (AccuPyc 1330, Micromeritics, USA) was used to measure the density of spray-dried milk powders (n=3). *Contact angle and surface free energy determinations:* Contact angles were measured by tensiometry (Wilhelmy plate method, K100, Krüss GmbH, Germany) once spray-dried powders were made to adhere to a rectangular support (20x20mm).
170 The testing liquids (water and diiodomethane) were placed in a glass jar (*ca* 50cm^3) which was raised at 6

mm/min until the plate was submersed for 2mm at 25±0.5°C (n=3, Thermo Haake GmbH, Germany). Contact angles and surface free energies were calculated from the Wilhelmy equation and the works of adhesion and cohesion, and spread coefficients were calculated for both the raw materials (different fat content milks and theophylline) and dried samples according to Wu equations (LabDesk v. 3.2, Krüss GmbH, Germany), as described in the literature (25).

2.2.4. **Evaluation of potential interaction between theophylline and milk components**

Fourier Transform Infrared Spectroscopy (FT-IR): Infrared spectra were collected for raw materials and processed powders (Affinity-1 FT-IR, Shimadzu Corp., Japan) in potassium bromide disks (1mg sample in 100mg potassium bromide) prepared by gently grinding the materials in a mortar prior to compaction (40kN). Data was recorded in the range 4000–400cm^{-1}, with resolution of 4cm^{-1}, providing an average spectrum from 30 scans (OriginPro software, OriginLab, China). *Differential Scanning Calorimetry (DSC):* Calorimetric studies were performed in a differential scanning calorimeter (DSC, Q200, TA Instruments, USA) in the range of 0 to 285°C, at a heating rate of 10°C/min. Powdered samples were submitted to two heating cycles in sealed pin holed pans. Data was analyzed with the Universal Analysis 2000 v.4.7A software (TA Instruments, USA).

2.2.5. **Quantification of theophylline in the spray-dried powders**

Theophylline was quantified by high pressure liquid chromatography (HPLC, Merck®-Hitachi Lachrome, Germany) at room temperature and detection at λ=272nm. A Merck® analytical Purospher C18 column (250 x 4mm internal diameter; particle size 5µm) and a Purospher C18 guard column (4 x 4mm internal diameter; particle size 5µm) were used PABA (5µg/mL) was included in every sample as an internal standard (IS). The eluent (10mM ammonium acetate buffer: acetonitrile: methanol in 90:5:5 v/v/v) was run under isocratic conditions at 1mL/min flow rate. Appropriately diluted samples were centrifuged (2000rpm/10 min) prior to injection (20µl) and analysed in triplicate. Standard solutions of theophylline (0.2

to 75µg/ml) and PABA working solution (100µg/ml), were prepared in water. The method was previously validated for specificity, linearity, accuracy, precision and recovery, according to ICH guidelines (26).

2.2.6. **Stability studies**

Shelf stability of powders and in-use stability, after reconstitution in water, was performed in a random set of seven spray-dried samples (out of 54 possible samples, according to a complete factorial design of experiments). Powders were manufactured in the same week the study started (time 0) and were retested (theophylline content and microbial burden) after 6 months of storage (25°C / 65% of RH).

Powders (2g) were reconstituted in water (15mL), in a 20 mL amber flask and shaken for 1min and equally divided into two other containers (stored at 4°C and 25°C respectively). At specific time points (0, 1, 2, 7, 14 and 28 day) 1mL aliquot of each extemporaneous preparation, was taken for drug quantitation and microbiological assay.

Total aerobic viable microorganisms, total number of yeasts and moulds, and absence of *Escherichia coli* (both in powders and reconstituted samples) was assessed according to the European Pharmacopeia (27).

2.2.7. **Statistical Analysis**

Analysis of data was performed using SPSS v.22.0 (IBM®, USA) for descriptive analysis of categorical and scalar variables. The results were further analyzed using one-way ANOVA followed by post-hoc Bonferroni test ($p < 0.05$). Unless otherwise stated, data is represented as mean with coefficients of variation smaller than 2% for all experiments.

3. Results

3.1. Properties of the spray-dried powders and characterization of the production process

Results obtained for the different medicinal powders produced by varying the milk fat content and the amount of theophylline, as well as for samples containing only milk (LFM, MFM and HFM), or theophylline, used as controls are given in Table 1 and Annex 1 and statistical analysis in Table 2.

3.1.1. Yield and moisture content (MC): Aqueous solutions of theophylline containing the same fractions as those present in the studies with different milks (controls) have shown yields within the range of 19.18-30.04% and residual water content within the range of 0.4 – 2.0% (Annex 2). These experiments have shown acceptable moisture contents but low yields of collected theophylline, anticipating the need of optimization for industrial scaling up. The spray-dried milk powders, regardless of the variables considered, presented a yield in the range of 25.2-73.3 (Table 1). Powders atomized at 130°C presented the highest yields, which were significantly different ($p<0.05$, Table 2) from those powders produced at other temperatures, suggesting that T_{inlet} is a key factor in the manufacture of the product. In contrast, neither the theophylline fraction in the powder, nor the fat content of the fresh milk, affected the yield of the powders ($p>0.05$, Table 2). The MC of the spray-dried milk powders ranged between 0.0 – 10.3% and, as the T_{inlet} increased, the moisture content of powders decreased significantly ($p<0.05$). The absence of significance of either fat content or theophylline fraction on the yield or moisture content was anticipated due to the inexistent relationships between these variables (Table 1).

3.2.1. Particle size and shape: Microscopic evaluation of powdered samples revealed that, overall, particles acquired a spherical shape on drying (Figure 1, a-b). However, some samples have shown suspended needle shaped and crystalline particles, assumed to be of theophylline (as observed under polarized light microscopy; Figure 1, c-d), as this was particularly relevant in the samples with higher theophylline : milk ratios (0.31:1, 0.62:1, and 1:1). The particles' size were in the range of 3.0 - 4.3 µm and the span values indicated narrow size distributions (Table 1). The variability observed for the data collected from this set of

experiments was reflected on the absence of significant effect on the size of particles by each of the three factors considered (T_{inl}, fat content and theophylline fraction) (Table 2).

3.2.2. *Density:* The density of powders was in the range 1.426–1.552g/cm^3 for LFM, 1.386–1.455g/cm^3 for MFM and 1.244–1.437g/cm^3 for HFM (Table 1). This property was significantly affected by the fat content of milk ($p < 0.05$) but neither the theophylline fraction, nor T_{inlet}, significantly influenced the density of the powders (Table 2). However, although not significant, a slight increase on the density was observed with the increase in theophylline fraction, in line with the density of control samples (milk only, data not shown).

3.2.3. *Surface properties of powders:* The contact angle of theophylline in water (77.33°) was smaller than that of pure milk powders (79.76-93.51°). In average, LFM produced medicinal powders with lower contact angles in water (80.62-87.17°) than MFM (79.76-93.45°) and HFM (77.29-93.51°). Table 1 summarizes the results from contact angles, γ_s and γ^p, respectively total and polar component of surface energy, of each spray-dried powder. The two interrelated properties were significantly affected by T_{inlet} , and marginally by the fat content of milk. Only the polar component (γ^p) was also significantly affected by the milk fat content ($p < 0.05$), with the MFM samples being significantly different from the other two (Table 2). LFM powder presented the highest surface free energy range (42.35-52.44mJ/m^2) and polar component range (5.08-12.30mJ/m^2). The HFM powders atomized at 105°C presented higher γ_s and γ^p (41.33-49.76 mJ/m^2 and 7.27-10.18 mJ/m^2, respectively) than the ones atomized at the other inlet air temperatures (130 and 150°C). After resting at room temperature (25°C, overnight), these values decreased slightly (data not shown) reflecting some stabilization of the powders' surfaces immediately after processing. To anticipate the interaction between theophylline and the matrix formed by milk components in the drug-loaded dried particles, the works of cohesion (W_c) and adhesion (W_a) were calculated for the controls (theophylline and milk) (Table 3). W_c of theophylline (98.48mJ/m^2) was higher than the ones observed for the three milks (78.26-97.26mJ/m^2), particularly for LFM before drying. Once controlled solutions were dried, the particles obtained presented a similar pattern with the exception of LFM dried at 105°C (104.88mJ/m^2). This sample might have been affected by the higher moisture content in comparison to the other samples (data not shown). The W_c was consistently lower in comparison to the W_a (85.96-97.83mJ/m^2) in all samples, except

in the sample mentioned earlier (101.50mJ/m^2) anticipating a fair incorporation of theophylline molecules into the milk components. Calculated spreading coefficients in general have shown positive values of λ_{12} (one sample had a negative value, Table 3) increasing with the content of fat in the milk. These values were in line with those obtained for the dried powders and confirm the spreading of milk components on theophylline molecules.

3.3. Evaluation of drug-milk potential interaction

3.3.1. *Fourier Transform Infrared Spectroscopy (FT-IR):* The FT-IR spectra from all samples and controls were evaluated but, since the different T_{inlet} considered did not produce products with different spectra, only the spectra of the spray-dried milk powders atomized at 105°C are presented as an example (Figure 2). Theophylline (raw material) showed bands at 3120.96cm^{-1} (N-H stretching), at 1716.72cm^{-1} and 1668.50cm^{-1} (C=O stretching, amide I), at 1566.27cm^{-1} (N-H bending, amide II) and the N-H wagging at 742.63cm^{-1} (28). For the samples with milk, regardless the fat content, it was possible to identify bands at 3468.16 – 3304.20cm^{-1} (O-H and N-H stretching in the protein structure), at 1660.78 – 1643.42cm^{-1} and 1546.98 – 1545.05cm^{-1} (amides I and II, respectively, in the proteins' structure) and in the region of 1200 – 900cm^{-1}, corresponding to the so called "saccharide" bands. For the middle and high fat milk powders it was also possible to identify a region at 2924.21 – 2854.77cm^{-1} originated by the vibrational C – H stretching of the fatty acids and a single high intensity band at 1745.69cm^{-1} caused by C=O group due to the stretching of the fatty esters molecules (29). When the fraction of theophylline increased in the dried powders, the signals due to the molecule of theophylline started to be more intense than the signals due to the milk components, as expected. For the 0.08:1 and 0.16:1 solids ratio samples, the N – H stretching at 3120.96cm^{-1} was not observed. The potential formation of an imine group due to the interaction between theophylline and milk components in the dried powders, reflected by new bonds formation, would have been observed in the 1647 – 1630cm^{-1} region; but it was neither observed in samples considered immediately after spray-drying or after storage (6 months) of dried powders.

3.3.2. *Differential Scanning Calorimetry (DSC):* The thermograms obtained from calorimetric studies of the spray-dried milk samples manufactured at 150°C are shown in Figure 3. For this process temperature, theophylline presented a sharp endotherm event at 272.75°C (positive control), whereas spray-dried milk powders presented a broad band that corresponds to the dehydration of the samples (40 - 110°C) and two other small bands corresponding to milk components, such as lactose or proteins (162.59-167.04°C and 198.52-199.12°C) (30). For the powders produced from middle and high fat milks, endothermic (on heating the sample) and exothermic (on cooling) events were also observed between 30-40°C (melting followed by recrystallization of fat content). Above 0.31:1 solids' ratio samples, a broad band started to appear between 233.30-249.11°C. As the theophylline fraction increased in the formulation, a shift of this peak to higher temperatures between 257.68 and 266.88°C was observed. The endothermic events characteristic of the milk components (negative controls) were also observed in the spray-dried milk powders, although the enthalpies of fusion were higher for the latter. The glass transition temperature (T_g = 30.72°C) was distinctly observed in the LFM samples in contrast to the MFM and HFM samples. It is worth noting that no other differences in the thermograms were observed for the different temperatures. Although thermograms were complex due to the complexity of the samples, the patterns of the curves were reproducible particularly when the second heating stage was applied to samples.

3.4. Quantification of theophylline in powders

The theophylline assay in freshly prepared theophylline-milk solutions has shown a slight increase on drug content as the drug fraction increased in formulations, independently of the other factors (T_{inlet} and fat content of milks, data not shown). The quantification of theophylline in the spray-dried powders has shown that T_{inlet} and milk fat content were not significant and did not affect the recovery and quantification of theophylline from samples (Table 1). A dramatic decrease on drug content above 0.31:1 was obtained and became particularly significant ($p < 0.05$) for the 0.62:1 and 1:1 ratios, and higher processing temperature and milk fat content (Table 2) suggesting that drug recovery from samples was not complete.

Overall, theophylline content was within 2-7% variation in the different powder samples assayed (data not shown).

3.5. Stability

The stability in-use was assessed after the reconstitution of dried powders in water immediately after production. After storage at room temperature (25°C) microbial criteria were met at day 1 by all samples, regardless of fat content and processing conditions. At 4°C, microbial criteria were met by all samples up to 2 days, but only LFM samples met the criteria 7 days after reconstitution. Yeasts and *E. coli* were absent in every sample tested. Theophylline content in solution remained stable over the 28 days of test, regardless of fat content and microbial growth (not shown). It is also worth to mention that samples dried at lower inlet air temperatures presented lower microbial growth.

Regarding the medium to long term stability of spray-dried powders, microbial criteria were met only by LFM samples and theophylline quantification has shown that there was no decrease in content after 6 months of storage.

4. Discussion

It was expected that as the inlet air temperature increased, the yield also increased, but that was not verified, in contrast to some previous studies (31) in which only the effect of T_{inlet} was considered. However, the complex matrix (e.g. protein, fat and lactose) used in the present work can justify the results obtained. For instance, it is known that powdered hygroscopic lactose is sticky in the presence of residual water (20) and that the yield at high T_{inlet} (32) was diminished. Furthermore, molten milk fat concentrated at the surface of the particles (33,34) at high temperatures (e.g. 150^{o}C), increases stickiness of the product to the drier's wall, with a decrease in yield. The yield of spray-dried theophylline (control) was significantly smaller than those of drug-loaded milk powders, suggesting that during the process theophylline molecules have bound to the milk components (e.g. proteins (15)), thus reducing drug loss on drying.

The powder moisture content may have a significant impact on the long-term stability, both in terms of drug degradation and microbial growth (35). As T_{inlet} increased the residual water in the spray-dried powders decreased, as corroborated by other studies (36,37). After spray-drying lactose becomes largely amorphous and highly hygroscopic (20), as observed in LFM samples, showing a T_g (ca 30.72°C) considerably lower than the T_g of amorphous lactose (97–116°C) (20,38), emphasizing the care required to assure optimal storage conditions (RH and temperature) of the powders. This T_g was neither found in MFM, nor in HFM powders, which might have embedded the lactose or, simply overlap the thermic event due to the proximity of the melting range of fat components (38).

Sizing and shaping of particles by microscopy confirmed the expected spherical shape and narrow size distribution of dried particles (3.0 to 4.3 µm in diameter) which are appropriate for reconstitution in water (39). These properties were independent of the factors considered (T_{inlet}, theophylline fraction), in contradiction to other studies (31,37) which have concluded that particle size was influenced by different T_{inlet} because higher temperatures of atomization led to an expansion of the final particles, whereas lower temperatures promoted shrinkage of their structure. In the present case the fat component may have accommodated the impact of T_{inlet} on particles' size.

The density of spray-dried powders was only affected by the fat content of the fresh milk, with the LFM samples presenting the highest values, in contrast to the HFM samples, due to the fact that fat is less dense then other components in the powders. It was not surprising that LFM samples were more hydrophilic (lower $\underline{\theta_w}$) and presented higher wettability (better redispersibility in water), in line with what was observed before with solid dispersions of poorly water soluble drugs (19). During particle formation, it is possible that protein, either as free molecules or in micelles, deposited on the surface of the forming particle, lowering their surface energy. On the contrary, higher fat content milk, in which fat globules have the ability to disperse and migrate to the surface of the particles, produced a hydrophobic surface (40). Interestingly, some MFM samples have shown higher contact angles than HFM samples. One can hypothesize that, throughout drying, the re-arrangement of the milk components in these two types of milk was different leading to different fat / protein surface compositions. The residual water might have been

the justification of the lowering θ_w of HFM samples dried at 105°C which decreased the fraction of fat with impact on the redispersibility in water. Spreading coefficients indicate that milk components had a tendency to spread over theophylline making the properties of bulk particles independent of theophylline fraction, the latter more difficult to extract and quantify.

Data from the FT-IR spectra was sensitive to the ratio between theophylline and milk components. As theophylline fraction increased, particularly above 0.31 in MFM and HFM powders, the signal due to milk components decreased until complete replacement by the theophylline signal, particularly in the region observed for the peaks of the proteins' amides I and II in higher fat milks. This observation was harder to verify in spray-dried particles due to the fact that such particles presented a fat rich surface, particularly evident in MFM and HFM, with broad peaks overlapping those of the proteins (41–43). On the contrary, in powders with low theophylline fractions (e.g. 0.08 and 0.16) the presence of the N-H stretching in the region of 3120.96cm^{-1} was difficult to observe due to a dilution effect and overlap of the drug's output. This means that the absence of peaks is due to the fractions of components in the powders, and not to the interaction between any component of the milk and theophylline. These observations are in agreement with that of a solid dispersion made of powdered skimmed milk as carrier of valsartan, a drug molecule which presents an amine group, as theophylline does (19). The potential interaction of lactose with theophylline (e.g. Maillard's reaction) (44) was evaluated immediately after drying and 6 months later. Likely this potential interaction would have been reflected by the N-H bond of the drug and the O-H of the lactose. Data from FT-IR in the region of 1647-1630cm^{-1} revealed that no imine group was formed, thus, no reaction was deemed to have occurred. The DSC thermograms did not show any thermic events, particularly between 100-150°C or at higher temperatures (e.g. 220°C) (45) temperatures at which possible products of degradation might have been formed, as observed for other drugs and dosage forms (46,47). The presence of milk fat and protein components may have prevented the interaction between the drug and lactose (40).

The quantification of theophylline in dried powders indicated that T_{inlet} and milk fat content did not affect the quantity of theophylline in samples, in contrast to the fraction of theophylline. However, the

amount of theophylline in dried powders was not entirely reflected in powders above 0.31:1 ratio, particularly at 0.62:1 and 1:1 ratios ($p < 0.05$). However, since the analytical technique was previously validated, the partial recovery of theophylline may have been due to uncollected theophylline particles, i.e., partial loss of theophylline to the exhaust of the spray-drier, or an incomplete extraction of theophylline from the particles. This observation is supported by the low yields obtained when theophylline was dried alone from solutions. If a correlation between these observations is made, then one can say that milk decreases the loss of theophylline during the drying process by incorporation of theophylline molecules within milk components.

The microbiological evaluation of the reconstituted powder (in-use stability) has shown that the Pharmacopeia criteria were met on the 1st day for samples stored at 25°C. After 2 days of reconstitution, criteria were met by all samples at 4°C and after 7 days only LFM samples met the criteria. These results are comparable to that (13.7 days) obtained for pasteurized skimmed milk (LFM), stored at 5°C (48). Theophylline content in solution remained constant throughout the study in every extemporaneous sample. The lower microbial growth presented by samples dried at lower inlet air temperatures, suggests that the longer time taken to dry the droplets, resulted in a smaller microbial survival rate.

In powders stored for 6 months, theophylline also remained chemically stable, but microbiological specifications were no longer observed, except for LFM. However, non-compliance was not dependent on MC, since LFM with a 6.4% MC passed the test. Samples with lower milk fractions were closer to acceptability than the others, which is not surprising if one considers that milk is an excellent microbial culture media.

5. Conclusions

The study has confirmed the potential of using fresh milk to produce powders as a solid dosage form to deliver drugs to children, as such or after extemporaneous reconstitution in water (or indeed, as a the starting point for other dosage forms). The work highlights an innovative approach of using milk as a

likely alternative platform to deliver drugs to the paediatric population of different age groups. The process and product presents several advantages, namely, the fact that drug is mixed with milk only, decreasing the toxicity concerns of the final formulation and incorporation of drug in solution promotes uniformity of content. An oral solid dosage form is suggested since theophylline content remained stable after 6 months of storage of powders, as well as after reconstitution in water. In-use and shelf-life stability of powders showed that LFM samples met the microbial criteria, at 4°C for 7 days and at 25°C for 6 months, respectively. Furthermore, no drug-milk interactions were observed. The use of different fresh milks, preferably LFM, should be considered as a possible drug-powder-solution formulation that, due to its likeliness to a well known food to children, would result in increased patient's compliance. For parents, nurses and caregivers this composed pharmaceutical powder would be easy to prepare, requiring only reconstitution in water (simple due to the low contact angles exhibited), thus minimizing the problems related to dose flexibility and accuracy.

To fully establish this approach, a further characterization of the system (namely in terms of drug entrapment and interaction between low solubility / hydrophobic drugs and milk fat components) and *in vivo* studies (e.g. to investigate the influence of fat on the digestion and release of the drug) are warranted. Furthermore, since the source of milk is likely to affect the final product, special attention should be paid to the specifications of the fresh milk used, to guarantee reproducibility.

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6. References

1. Krause J, Breitkreutz J. Improving Drug Delivery in Paediatric Medicine. *Pharmaceut Med* 2012; 22(1):41–50
2. Ali AA et al. Pediatric drug development: formulation considerations. *Drug Dev Ind Pharm* 2014; 40(10):1283–99.3.
3. EMEA/CHMP/PEG/194810/2005. Formulations of choice for paediatric population. 2006.
4. World Health Organization (WHO). WHO Model List of Essential Medicines for Children. 2015.
5. World Health Organization (WHO). Promoting Safety of Medicines for Children. 2007. 1-61.
6. Ivanovska V et al. Pediatric drug formulations: a review of challenges and progress. *Pediatrics*. 2014; 134(2):361–72.
7. Walsh J et al. Delivery devices for the administration of paediatric formulations: overview of current practice, challenges and recent developments. *Int J Pharm* 2011; 415(1-2):221–31.8.
8. Richey RH et al. Manipulation of drugs to achieve the required dose is intrinsic to paediatric practice but is not supported by guidelines or evidence. *BMC Pediatr*; 2013; 13(1):81.
9. Thomson SA. Minitablets: new modality to deliver medicines to preschool -aged children. *Pediatrics* 2009; 129(3):462–9.
10. Stoltenberg I, Breitkreutz J. Orally disintegrating mini-tablets (ODMTs)-a novel solid oral dosage form for paediatric use. *Eur J Pharm Biopharm* 2011; 78(3):462–9.
11. Kayumba PC et al. Quinine sulphate pellets for flexible pediatric drug dosing: formulation development and evaluation of taste-masking efficiency using the electronic tongue. *Eur J Pharm Biopharm* 2007; 66(3):460–5.
12. Mambrini P, Kibleur Y. Successful development of an orphan drug for the pediatric population. *Int J Pharm* 2013; 457(1):350–1.
13. Lopez FL et al. Formulation approaches to pediatric oral drug delivery: benefits and limitations of current platforms. Expert Opin Drug Deliv 2015;12(11):1727–40.
14. Zajicek A et al. A report from the pediatric formulations task force: perspectives on the state of child-friendly oral dosage forms. *AAPS J* 2013; 15(4):1072–81.
15. Livney YD. Milk proteins as vehicles for bioactives. *Curr Opin Colloid Interface Sci* 2010; 15(1-2):73–83.
16. Pinto JT et al. Evaluation of the ability of powdered milk to produce minitablets containing paracetamol for the paediatric population. *Chem Eng Res Des* 2016; 110: 171–182.
17. Kytariolos J et al. Stability and physicochemical characterization of novel milk-based oral formulations. *Int J Pharm* 2013; 444(1-2):128–38.

18. Gurusamy S et al. Preparation and Evaluation of Solid Dispersion of Meloxicam with Skimmed Milk. *Pharm Soc Japan* 2006; 126(2):93–7.

19. Kumar KV et al. Preparation and in vitro characterization of valsartan solid dispersions using skimmed milk powder as carrier. *Int J PharmTech Res* 2009; 1(3):431–7.

20. Lasekan O, Khalil SK. The Significance of Glass Transition Temperature in Processing of Selected Fried Food Products : A Review. *Mod Appl Sci* 2010;4(5):3–21.

21. Nijdam JJ, Langrish T a. G. The effect of surface composition on the functional properties of milk powders. *J Food Eng* 2006; 77(4):919–25.

22. Barnes PJ. Theophylline. Am J Respir Crit Care Med 2013;188(8):901–6.

23. EMA/428172/2012. 5-year Report to the European Commission. 2012.

24. ICH, Q4 (B). Annex 12 Analytical Sieving General Chapter, in: Proceedings of International Conference on Harmonization, Geneva. 2010.

25. Rowe RC. Surface free energy and polarity effects in the granulation of a model system. *Int J Pharm* 1989;53:75–8.

26. ICH, Q2 (R1). Validation of analytical procedures, in: Proceedings of International Conference on Harmonization, Geneva. 2005.

27. European Pharmacopoeia 8th Edition. Council of Europe: European Directorate for the Quality of Medicines and Healthcare, Strasbourg. 2010.

28. Schnitzler E et al. Thermoanalytical study of purine derivatives compounds. *Eclética Químico* 2004; 29(1):71–8.

29. Mousia Z et al. Effect of water partitioning on the glass-transition behaviour of phase separated amylopectin–gelatin mixtures. *Polymer (Guildf)* 2000; 41(5):1841–8.

30. Sunooj K V et al. Thermal Degradation and Decomposition Kinetics of Freeze Dried Cow and Camel Milk as well as Their Constituents. *J Food Sci Eng* 2011; 1:77–84.

31. Tonon R V et al. Influence of process conditions on the physicochemical properties of açai (Euterpe oleraceae Mart.) powder produced by spray drying. *J Food Eng* 2008; 88(3):411–8.

32. Bhandari BR et al. Problems Associated With Spray Drying Of Sugar-Rich Foods. *Dry Technol* 1997; 15(2):671–84.

33. Vehring R. Pharmaceutical particle engineering via spray drying. *Pharm Res* 2008; 25(5):999–1022.

34. Nijdam JJ, Langrish T a. G. The effect of surface composition on the functional properties of milk powders. *J Food Eng* 2006; 77(4):919–25.

35. Gerhardt AH. Moisture Effects on Solid Dosage Forms — Formulation, Processing, and Stability. *J GXP Compliance* 2009; 13(1):58–66.

36. Phisut N. Spray drying technique of fruit juice powder: some factors influencing the properties of product. *Int Food Res J* 2012; 19(4):1297–306.

37. Koç B, Kaymak-Ertekin F. The effect of spray drying processing conditions on physical properties of spray dried maltodextrin. *Foodbalt* 2014; 243–7.

38. Vuataz G. The phase diagram of milk : a new tool for optimising the drying process. *Lait* 2002; 82(4):485–500.

39. Koç B, Kaymak-Ertekin F. The effect of Spray Drying processing conditions on physical properties of spray dried maltodextrin. *Foodbalt* 2014; 243–7.

40. Nijdam JJ, Langrish T a. G. An Investigation of Milk Powders Produced by a Laboratory-Scale Spray Dryer. *Dry Technol* 2005; 23(5):1043–56.

41. Madarász J et al. Thermal, FTIR and XRD study on some 1:1 molecular compounds of theophylline. *J Therm Anal Calorim* 2002; 69:281–90.

42. Deng Y et al. Analysis and Discrimination of Infant Powdered Milk via FTIR Spectroscopy. *Spectrosc Spectr Anal* 2006; 26(4):636–9.

43. Uhumwangho MU, Ramana MK V. In-vitro Characterization of Optimized Multi-Unit Dosage Forms of Theophylline and its Solid State Characterisation. *J Appl Sci Environ Manag* 2011; 15(4):649–55.

44. Nursten H. The Maillard Reaction: Chemistry, Biochemistry and Implications. Cambridge: Royal Society of Chemistry 2005. 1-208 .

45. Lapčík L et al. Surface energy analysis (SEA) and rheology of powder milk dairy products. *Food Chem* 2015; 174:25–30.

46. Monajjemzadeh F et al. Assessment of feasibility of maillard reaction between baclofen and lactose by liquid chromatography and tandem mass spectrometry, application to pre formulation studies. *AAPS PharmSciTech* 2009; 10(2):649–59.

47. Wirth DD et al. Maillard reaction of lactose and fluoxetine hydrochloride a secondary amine. *J Pharm Sci* 1998; 87(1):31–9.

48. Duyvesteyn WS et al. Determination of the End of Shelf-life for Milk using Weibull Hazard Method. *LWT - Food Sci Technol* 2001; 34(3):143–8.

Table 1 - Properties of the spray-dried milk powders and process characterization [1]

T_{inlet} / T_{outlet} (°C)	Fat Content (g)	Property	Theophylline : milk solids ratio (w/w)					
			0:1	0.08:1	0.16:1	0.31:1	0.62:1	1:1
105 / 68	O.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
		Assay	0.0	100.0	97.8	89.7	75.0	61.8
		d	3.3	3.1	3.5	3.5	3.6	3.7
		Span	1.25	0.90	1.17	1.20	1.44	1.26
		ρ	1.501	1.509	1.520	1.499	1.504	1.515
		Θ_W	85.14	83.74	83.53	83.94	80.62	81.23
		Θ_D	38.87	41.72	37.90	45.15	47.46	44.18
		γ^p	11.14	9.20	9.07	9.69	9.54	10.40
		γ_s	52.44	49.35	49.18	48.44	46.40	50.15
	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
		Assay	0.0	96.5	93.6	88.8	72.8	65.5
		d	3.3	3.3	3.0	3.6	3.7	3.5
		Span	1.30	1.30	1.08	1.12	1.42	1.29
		ρ	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
		Θ_D	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_s	41.38	45.47	45.28	45.69	47.92	48.09
	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
		Assay	0.0	96.5	93.6	88.8	72.8	65.5
		d	3.4	3.4	3.7	3.6	3.4	3.8
		Span	1.28	1.14	1.25	1.23	1.28	1.24
		ρ	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
		Θ_D	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_s	42.72	41.33	45.33	46.53	49.58	49.76

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Table 1 - Properties of the spray-dried milk powders and process characterization [1] (cont.)

T_{inlet} / T_{outlet} (°C)	Fat Content (g)	Property	Theophylline : milk solids ratio (w/w)					
			0:1	0.08:1	0.16:1	0.31:1	0.62:1	1:1
130 / 87	O.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
		Assay	0.0	101.4	103.8	97.7	78.3	61.2
		d	3.3	3.6	3.8	4.2	4.0	4.0
		Span	1.18	1.03	1.23	1.36	1.45	1.42
		ρ	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
		Θ_D	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_s	48.49	45.02	46.38	43.25	48.02	49.47
	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
		Assay	0.0	102.9	102.5	89.5	74.9	66.0
		d	3.7	3.5	3.7	3.6	3.5	3.4
		Span	1.15	1.18	0.93	1.26	1.06	1.15
		ρ	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
		Θ_D	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_s	43.66	46.55	47.43	44.94	46.50	47.84
	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
		Assay	0.0	94.03	98.9	102.2	79.1	64.9
		d	3.5	3.6	3.7	3.6	3.8	3.7
		Span	2.20	1.55	1.17	1.25	1.09	1.16
		ρ	1.389	1.244	1.287	1.336	1.361	1.386
		Θ_W	85.72	86.62	93.51	92.76	85.71	81.69
		Θ_D	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_s	40.82	40.69	43.73	43.66	46.84	47.76

555 ***Table 1 - Properties of the spray-dried milk powders and process characterization [1] (cont.)***

T_{inlet} / T_{outlet} (°C)	Fat Content (g)	Property	Theophylline : milk solids ratio (w/w)					
			0:1	**0.08:1**	**0.16:1**	**0.31:1**	**0.62:1**	**1:1**
150 / 100	**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
		Assay	0.0	101.8	97.3	81.6	75.8	58.6
		d	3.9	3.6	3.4	3.6	3.8	3.1
		Span	1.31	1.33	1.23	1.40	1.26	1.27
		ρ	1.426	1.552	1.515	1.501	1.486	1.476
		Θ_W	84.01	82.96	85.69	85.11	86.28	86.33
		Θ_D	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_s	48.63	47.34	42.35	46.57	45.04	44.80
	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
		Assay	0.0	102.8	93.8	89.5	75.8	61.3
		d	3.6	3.6	3.8	3.6	4.0	4.3
		Span	1.29	1.24	1.46	1.39	1.31	1.35
		ρ	1.407	1.376	1.386	1.396	1.418	1.424
		Θ_W	91.99	87.05	83.72	86.01	93.45	82.28
		Θ_D	41.88	43.35	37.69	38.30	32.85	37.10
		γ^p	5.17	4.70	8.67	4.67	4.49	8.72
		γ_s	45.13	45.72	48.63	46.37	46.66	50.43
	9 (HFM)	**Yield**	31.2	34.0	50.4	52.5	53.3	51.6
		MC	0.0	1.2	0.0	1.2	2.8	0.4
		Assay	0.0	100.7	87.1	88.7	76.6	47.6
		d	3.7	3.9	3.5	3.7	3.5	4.0
		Span	1.30	1.43	1.43	1.34	1.27	1.31
		ρ	1.296	1.298	1.309	1.326	1.367	1.384
		Θ_W	84.07	92.72	92.66	87.61	93.36	86.85
		Θ_D	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_s	39.13	40.68	40.01	46.20	42.35	41.82

API	Θ_W	Θ_D	γ^p	γ_s
Theophylline Raw Material	77.33	48.46	11.64	49.24

[1] Coefficients of variation (CV) for the means and for all experiments were smaller than 2%.

560 Yield - %; MC – Moisture Content (%); Assay – Theophylline content assayed by HPLC (%); d – Particle size (μm); ρ – Particle density (g/cm^3); Θ_W (^{o}) - Contact angle in water; Θ_D – Contact angle in diiodomethane; Υ^p (mJ/m^2) – Polar part; Υ_s (mJ/m^2) – Surface Free Energy.

Table 2 - Evaluation of the effect of the T_{inlet}, fat content of milk and theophylline fraction in the properties of the spray-dried milk powders by one-way ANOVA.

Factor	Yield (%)			Moisture Content (%)			Particle Size (μm)			Density (gcm^{-3})			Polar Part (γ^p /mJ/m^2)			Surface Free Energy (γ /mJ/m^2)			Theophylline Assay (%)		
	Effect	MSq[a]	p [1]	Effect	MSq	p [1]	Effect	MSq	p [1]	Effect	MSq	p [1]	Effect	MSq	p [1]	Effect	MSq	p [1]	Effect	MSq	p [1]
T_{inlet}	14.44	139.3	**0.000**	29.37	3.226	**0.000**	5.340	0.056	**0.548**	1.182	0.005	**0.315**	4.826	5.891	**0.012**	5.607	8.873	**0.006**	0.064	84.40	**0.938**
Fat content of milk	0.043	217.8	**0.957**	0.700	6.758	**0.501**	0.135	0.067	**0.874**	84.71	0.001	**0.000**	4.924	5.872	**0.011**	2.564	9.835	**0.087**	0.004	5.521	**0.996**
Theophylline Fraction	1.227	205.5	**0.311**	0.516	7.001	**0.763**	1.218	0.064	**0.315**	0.342	0.005	**0.885**	0.507	7.069	**0.769**	1.367	10.07	**0.253**	577.9	22.78	**0.000**

[a] MSq – Mean Square

[1] statistically significant result at $p < 0.05$

Table 3 – Works of adhesion and cohesion and spreading coefficients of samples containing milk and theophylline.

Samples	T_{inlet} (°C)	W_c (mJ/m^2)	W_a (mJ/m^2)	λ_{12}	λ_{21}
LFM	**105**	104.88	101.50	-3.38	3.02
	130	96.98	97.05	0.07	-1.43
	150	97.26	97.83	0.57	-0.65
MFM	**105**	82.76	86.67	3.91	-11.81
	130	87.32	90.39	3.07	-8.09
	150	90.26	91.81	1.55	-6.67
HFM	**105**	85.44	93.69	8.25	-4.79
	130	81.64	87.72	6.08	-10.76
	150	78.26	85.96	7.70	-12.52
Theophylline		98.48	-	-	-

Annex 1: Preparation of theophylline : milk systems.

Theophylline : Milk solid components ratio (w/w)	Mass of theophylline in 2.5g of powdered milk (g)	Volume of theophylline solution [1] (mL)	Volume of milk containing 2.5g of solid content (mL)		
			LFM	MFM	HFM
0:1	0.00	0.00	27.30	23.78	20.12
0.08:1	0.20	26.67			
0.16:1	0.45	59.73			
0.31:1	0.78	103.33			
0.62:1	1.55	206.67			
1:1	2.50	333.33			

[1] volume withdrawn from a solution of theophylline (7.5 g/l)

LFM – Low fat milk; MFM – Medium fat milk; HFM – High fat milk

Annex 2: Yield and moisture content of spray-dried theophylline solutions[1]

Theophylline in aqueous solution (w/v)	Yield (%)	Moisture content (%)
0.08:1	20.59	2.0
0.16:1	21.59	0.5
0.31:1	19.18	0.9
0.62:1	19.79	1.8
1:1	30.04	0.4

[1] Operating conditions: T_{inlet} = 130°C; airflow rate = 600 l/h; aspirator rate = 100%; and feed liquid rate = 4 mL/min).