



MYKAELLA PRISCILLA PIRES MESTRE

BSc in Chemical and Biochemical Engineering

LEVERAGING ATOMIZATION STRATEGIES TOWARDS THE PRODUCTION OF COMPOSITE PARTICLES

MASTER IN CHEMICAL AND BIOCHEMICAL ENGINEERING

NOVA University Lisbon
September, 2023

LEVERAGING ATOMIZATION STRATEGIES TOWARDS THE PRODUCTION OF COMPOSITE PARTICLES

MYKAELLA PRISCILLA PIRES MESTRE

BSc in Chemical and Biochemical Engineering

Adviser: Doctor Rui Churro

Senior Scientist, Hovione

Co-adviser: Prof. Doctor Ana Aguiar Ricardo

Cathedratic Professor, NOVA University Lisbon

Examination Committee

Chair: Prof. Doctor Mário Fernando José Eusébio

Assistant Professor, FCT-NOVA

Rapporteur: Doctor José Filipe Oliveira Granjo

Modelling and PAT Scientist, Hovione

Co-adviser: Prof. Doctor Ana Isabel Nobre Martins Aguiar de Oliveira
Ricardo

Cathedratic Professor, FCT-NOVA

MASTER IN CHEMICAL AND BIOCHEMICAL ENGINEERING

NOVA University Lisbon

September, 2023

Leveraging atomization strategies towards the production of composite particles

Copyright © Mykaella Priscilla Pires Mestre, NOVA School of Science and Technology, NOVA University Lisbon.

The NOVA School of Science and Technology and the NOVA University Lisbon have the right, perpetual and without geographical boundaries, to file and publish this dissertation through printed copies reproduced on paper or on digital form, or by any other means known or that may be invented, and to disseminate through scientific repositories and admit its copying and distribution for non-commercial, educational or research purposes, as long as credit is given to the author and editor.

ACKNOWLEDGEMENTS

I would like to express my gratitude to Hovione Farmacênciã S.A. for the great opportunity to develop this dissertation in their facilities, it was pleasure working at company with such good values and enriching environment.

Thank you to my supervisors, Dr. Rui Churro, Dr. LÍdia Santos and Prof. Dr. Ana Aguiar-Ricardo for their continuous help and support, guidance and valuable advice. Thank you for believing and trusting me.

I would also like to demonstrate my appreciation for the IADD team, from PhD students to lab technicians to scientists, for always making me feel welcome, motivating, and helping me, making my experience at Hovione even more fulfilling.

Thank you to my college friends, who also endured these last few years and with whom I shared many good and bad moments, making this a memorable journey.

I also want to thank João for all his love, comprehension and care throughout this journey and for always supporting me in everything.

Finally, I want to thank my parents, Fátima and Jacinto, for their endless love and support, for always being there for me and always encouraging me to reach my goal.

Thank you.

ABSTRACT

Encapsulated composite particles designed for inhalation are commonly prepared by spray drying. However, a challenge arises as common inhalation excipients are water soluble whereas most commercial active pharmaceutical ingredients (APIs) are not. This creates an obstacle for inhalation formulations manufactured by spray drying, wherein the traditional approach requires excipients to be dissolved in the same solvent, leading to limitations such as incompatibilities between the API/ excipient and the solvent and limited solubility. The utilization of a three-fluid nozzle (3FN) as atomizing tool arises as an interesting alternative to overcome the mentioned challenges, enabling the use of two independent solutions. Therefore, the present work aims at preparing encapsulated composite particles using the 3FN enabling the definition of a process development methodology from lab scale to manufacturing scale.

The use of a 3FN was extensively investigated using a model inhalation formulation containing Leucine as shell forming agent. Aligning with a quality-by-design approach, this study comprised a design of experiments combined with empirical model development, to determine the potential critical process parameters and link them to the investigated critical quality attributes. It was demonstrated that the 3FN is an efficient atomization strategy to prepare particles within the inhalable range ($D_{v50} < 5 \mu\text{m}$) and with fine particle fraction between 38 and 53%. The thorough study of process parameters revealed that atomization flow rate is of paramount importance in defining the particle size and consequently aerosol performance. Furthermore, this methodology is demonstrated to be adaptable for encapsulating different APIs, with Leucine emerging as the hydrophobic amino acid with most potential for encapsulation.

This work established the feasibility of using the 3FN towards the manufacture of encapsulated particles and advanced process knowledge within this specific context. Furthermore, the methodology whereby followed constitutes a robust process development tool with applicability across multiple phases of pharmaceutical development.

Keywords: Spray drying, 3-fluid nozzle, Composite particles, Encapsulation, Empirical modeling

RESUMO

A secagem por atomização é amplamente utilizada para preparar partículas compósitas encapsuladas para inalação. No entanto a maioria dos excipientes para inalação são solúveis em água, enquanto os ingredientes ativos API não são. Tal é um desafio no desenvolvimento de formulações para inalação, já que é necessário dissolver o IA e excipientes no mesmo solvente, potencialmente causando incompatibilidades entre API/excipientes e solvente e também solubilidade limitada do API. Um atomizador de três fluidos (3FN) é uma alternativa interessante para este desafio, ao permitir utilizar duas soluções independentes. Assim, este estudo visa produzir partículas compósitas encapsuladas utilizando o 3FN, estabelecendo assim uma metodologia de desenvolvimento de processo aplicável desde a escala laboratorial à industrial. A utilização do 3FN foi extensamente estudada empregando uma formulação modelo com Leucina, como agente de encapsulação. Adotando uma abordagem de qualidade por design, o trabalho incluiu um desenho de experiências combinado com desenvolvimento de modelos empíricos. Assim é possível determinar os potenciais parâmetros críticos de processo e correlacioná-los com os atributos críticos de qualidade. Foi demonstrado que o 3FN é uma estratégia de atomização eficiente para produzir partículas inaláveis ($D_{v50} < 5 \mu\text{m}$) e fração de finos entre 38 e 53 %. A análise detalhada dos parâmetros de processo revelou que o caudal de atomização desempenha um papel fundamental na determinação do tamanho de partícula e consequentemente fração de finos. Para além disso, a metodologia aplicada é adaptável para encapsular outros API, tendo sido determinado que a Leucina é o aminoácido hidrofóbico que confere um processo de encapsulação mais simples. Este trabalho comprovou a aplicabilidade do 3FN para a produção de partículas compósitas encapsuladas bem como permitiu aumentar o conhecimento sobre o processo, dentro do contexto estudado. Para além disso, a metodologia seguida constitui uma ferramenta robusta para desenvolvimento de processo com aplicabilidade em diversas fases do desenvolvimento farmacêutico.

Palavras-chave: Secagem por atomização, atomizador de três fluídos, partículas compósitas, modelação, encapsulação

CONTENTS

List of Figures	viii
List of Tables	x
Acronyms	xi
1 Context and Motivation	1
2 Theoretical Introduction	3
2.1 Pulmonary drug delivery	3
2.2 Types of formulations for dry powders inhalers	5
2.3 Composite particles	6
2.4 Spray Drying	9
2.5 Three-fluid nozzle	11
2.6 Process development methodologies	13
3 Materials and Methods	18
3.1 Materials	18
3.2 Methods	18
3.2.1 Spray Drying	18
3.2.2 Spray-dried intermediate Characterization	20
3.2.3 Drug product characterization	23
3.2.4 Modelling Strategy	24
4 Results and discussion	26
4.1 Formulation screening	26
4.2 Three-fluid nozzle: Comprehensive study	30
4.2.1 Exploratory data analysis	30
4.2.2 Empirical modeling	38
4.2.3 Proof of concept: poorly water-soluble API	44
4.2.4 Proof of concept: hydrophobic amino acid shell	46

4.2.5 Proof of concept: Template-assisted SD methodology	49
5 Conclusions and Future Perspectives	54
Bibliography	57
Appendices	
A Supplementary UV-Vis results	69
B XPS Spectra	70
C Excitation-Emission Matrixes	72
D Supplementary SEM micrographs	74
E Empirical modeling - Outliers and residuals analysis	75

LIST OF FIGURES

2.1	Lung deposition mechanisms [8]	4
2.2	Spray drying process [66]	10
2.3	Atomization strategies, (A) three-fluid nozzle, (B) two-fluid nozzle	11
3.1	Design space according to the upper and lower level of the factors T _{out} , F _{atom} and M _{ratio}	20
4.1	Data projection in the principal component space according to the first two PCs	29
4.2	SEM analysis of trial #5: A - EDS (green and red colors correspond to Cl and C elements, respectively) and B - SEM micrograph of FIB cross-section of a spherical particle	30
4.3	D_{v50} across DoE combinations, the high level of the factors is denoted as + and the low level as -.	32
4.4	Influence of the DoE factors on the D_{v50} response, A - F _{atom} , B - M _{ratio}	33
4.5	ED across DoE combinations, the high level of the factors is denoted as + and the low level as -.	33
4.6	Influence of the DoE factors on the ED response, A - F _{atom} , B - M _{ratio} , C - RS _{out}	34
4.7	FPF across DoE combinations, the high level of the factors is denoted as + and the low level as -.	35
4.8	Influence of the DoE factors on the FPF response, A - F _{atom} , B - M _{ratio} , C - RS _{out}	36
4.9	SEM micrographs of the prepared SDI at a x5000 magnification, A - trial #1 ITZ, B - trial #2 Leu, C - trial #10, D - trial #13, E - trial #14 and F - trial #17	37
4.10	Actual vs Predicted plots for the developed models, A - D_{v50} and B - FPF.	39
4.11	Observed tendencies for the validation dataset, A - D_{v50} as a function of F _{atom} , B - D_{v50} as a function of M _{ratio} , C - FPF as a function of F _{atom} and D - FPF as a function of M _{ratio}	42

4.12	Actual vs Predicted plots for the developed models including the validation data set, A - D_{v50} and B - FPF.	43
4.13	Sensitivity analysis plots of the developed models for the operating conditions A - F_{atom} , B - M_{ratio} , C - T_{out}	44
4.14	Particle surface morphology of RIF composite particles with Leu as SEM micrographs, A - 3000x magnification and B - 10000x magnification	45
4.15	SEM micrographs of the SDIs containing Val as a shell former, A - 1500x magnification, B - 10000x magnification and C - SEM-EDS.	47
4.16	Actual vs Predicted plots including trial #24 and #25, A - D_{v50} and B - FPF	48
4.17	SEM micrographs of SDIs obtained with $NaHCO_3$ in solution, A - trial #22A 1500x magnification, B - trial #22A 10000x magnification, c - trial #22B 1500x magnification, D - trial #22B 5000x magnification and E - SEM-EDS	51
4.18	SEM micrographs of SDIs obtained with $NaHCO_3$ in suspension, A - trial #23A 1500x magnification, B - trial #23A 3000x magnification, c - trial #23B 1500x magnification, D - trial #23B 5000x magnification and E - SEM-EDS	53
A.1	UV-Vis results, A - API and AA spectral scans, B - ITZ calibration curve at 260 nm, C- RIF calibration curve at 240 nm	69
B.1	XPS of single components, A - Itraconazole, B - Leucine, C- Lysine, D - Glycine	70
B.2	XPS of formulations, A - trial#5, B - trial#6, C - trial#7, D - trial#8, E- trial#9	71
C.1	EEMs of single components, A - Itraconazole, B - Leucine, C- Lysine, D - Glycine	72
C.2	EEMs of formulations, A - Trial #5, B - Trial #7, C - Trial #8	73
D.1	SEM micrographs at 5000x magnification, A trial#12, B- trial#13, C-trial#15, D-trial#16, E-trial#18	74
E.1	Residuals analysis for the D_{v50} , A - residuals vs predicted, B- residuals vs row number C - normality plot	75
E.2	Residuals analysis for the FPF, A - residuals vs predicted, B- residuals vs row number C - normality plot	76

LIST OF TABLES

2.1	Summary of particle engineering techniques	8
2.2	Theoretical influence of SD parameters on final powder properties, adapted from [12]	11
3.1	Spray drying conditions for the formulation screening trials.	19
3.2	Spray drying conditions of the DoE trials	20
3.3	Spray drying conditions of the empirical model external validation and template-assisted optimization trials.	21
4.1	Results of the formulation screening regarding API load, a summary of surface composition by XPS, particle size (n=3), and aerosol performance (n=3). Results expressed as mean $\pm$ standard deviation	26
4.2	API load (n=4), particle size (n=3) and aerosolization performance (n=4) results according to the investigated DoE levels. Results are expressed as mean $\pm$ standard deviation.	31
4.3	Empirical modeling results: Model expression, fit and parameters' VIFs	39
4.4	ANOVA results of the developed models.	40
4.5	Particle size distribution (n=3) and aerosol performance (n=3) for the validation data set. Results are expressed as mean $\pm$ standard deviation.	41
4.6	API load (n = 4), particle size (n =3) and aerosol performance (n =3) of the formulation with RIF as the API and comparison with ITZ-based formulation. Results are expressed as mean $\pm$ standard deviation.	45
4.7	API load (n =4), particle size (n =3) and aerosol performance (n =3) of the formulation with Val as a shell former and comparison with Leu-based formulation. Results are expressed as mean $\pm$ standard deviation.	46
4.8	Particle characterization results of the trials containing the pore-forming agent and comparison with the formulations without a pore-forming agent. Results are expressed as mean $\pm$ standard deviation (n=3).	50
E.1	Validation dataset actual results, model prediction and relative error	75

ACRONYMS

D_{ae}	aerodynamic diameter
2FN	two-fluid nozzle
3FN	three-fluid nozzle
AA	amino acid
AIC	Akaike Information Criterion
ANOVA	analysis of variance
API	active pharmaceutical ingredient
CPP	critical process parameter
CQA	critical quality attribute
DCM	dichloromethane
DoE	design of experiences
DPI	dry powder inhaler
ED	emitted dose
EDA	exploratory data analysis
EDS	energy dispersive X-ray spectroscopy
EEM	excitation emission matrix
EMA	European Medicines Agency
FDA	Food and Drug Administration
FPF	fine particle fraction
Gly	Glycine
ITZ	Itraconazole

Leu	L-Leucine
Lys	L-Lysine
MLR	multivariate Linear Regression
PC	principal component
PCA	principal component analysis
pCPP	potential critical process parameter
pCQA	potential critical quality attribute
Ph. Eur	European Pharmacopeia
PSD	particle size distribution
QbD	quality by design
RIF	Rifampicin
SD	spray drying
SDI	spray dried intermediate
SEM	scanning electron microscopy
Tre	D-(+)-Trehalose dihydrate
USP	United States Pharmacopeia
Val	(S)-(+)-Valine
VIF	variation inflation factor
XPS	X-Ray photoelectron spectroscopy

CONTEXT AND MOTIVATION

The preparation of composite particles intended for inhalation purposes commonly involves spray drying, wherein one or more active pharmaceutical ingredients (APIs) are combined with suitable excipients within a single solid phase. The excipients are selected based on their properties to enhance powders' properties, particularly aerosolization. However, a challenge arises when generally accepted inhalation excipients are water soluble whereas most commercial APIs for inhalation and new chemical entities under development possess poor water solubility [2]. This poses a great challenge for inhalation formulations manufactured by spray drying (SD), wherein the traditional approach requires API and excipients to be dissolved in the same solvent system. This setting leads to further complications, namely finding the adequate solvent system, ensuring there are no incompatibilities between the API/ excipient and the solvents. Additionally, the API solubility might limit the maximum quantity to be solubilized, negatively influencing process throughput.

The utilization of a three-fluid nozzle (3FN) as an atomization tool, offers a solution to these challenges as it enables the use of two independent solutions. Hovione already holds intellectual property on the utilization of this nozzle for the production of encapsulated composite particles. Therefore, the present work aims at preparing encapsulated composite particles via SD utilizing the 3FN as the atomization strategy, as proposed by Hovione. The developed project works as a proof of concept of the implementation of the 3FN towards the preparation of this specific class of inhalable particles. Furthermore, the methodology developed during the project serves as a tool for process development.

To achieve this objective, an extensive study of the 3FN will be conducted using a model inhalation formulation, selected through an initial excipient screening. To adhere to quality by design (QbD) approach this study comprises the utilization of a design of experiments methodology complemented with the development of empirical models of the investigated critical quality attributes (CQAs). Thus, this comprehensive approach seeks to establish a QbD framework for employing the 3FN as a novel atomization strategy for efficiently preparing encapsulated composite particles for pulmonary drug delivery.

The knowledge accumulated throughout this work will not only contribute to establishing the viability of the 3FN towards the production of encapsulated particles but also enhance process understanding within this specific context. Additionally, the following methodology serves as a robust process development tool applicable to other processes and to formulation design.

THEORETICAL INTRODUCTION

2.1 Pulmonary drug delivery

According to the World Health Organization, infectious pulmonary diseases have been a major health issue at a global level. Statistics reveal that chronic obstructive pulmonary disease and lower respiratory infections are ranked third and fourth leading causes of death, with a growing prevalence [3–6] as they are challenging to treat with conventional drug delivery approaches [7].

Drugs can be delivered through a multitude of routes, such as parenteral, oral, topical, ocular, and pulmonary [5, 8]. Inhalation-based pulmonary drug delivery is a promising alternative approach [9–11], compared to traditional drug delivery systems, as it can deliver APIs directly to the lungs as an aerosol [10, 11]. This strategy is effective in treating local lung diseases [12–14] such as asthma, chronic obstructive pulmonary disease and cystic fibrosis [11, 15–17], yielding an immediate response [11]. Moreover, it has also shown potential for the treatment of systemic diseases [12, 14, 18, 19] such as diabetes, Parkinson’s disease [5, 11, 14, 15, 20, 21], and schizophrenia [11, 22].

The global market of pulmonary delivery systems was valued at 49782.8 million USD in 2020 and is projected to grow at a compound annual growth rate of 6.1% from 2021 to 2030, reaching 91128.6 million USD by then [13]. Market growth is driven by increased air pollution and the prevalence of pulmonary diseases [13].

Pulmonary drug delivery presents several advantages when compared to the typical routes of administration. These benefits are closely linked to the physiological properties of the lungs [15, 23]. They have a large surface area ($>100\text{ m}^2$ [9, 14]), are highly vascularized and have high permeability due to the thin epithelial membrane [5, 6, 8, 9, 14, 18], resulting in fast absorption [6] and systemic distribution [8, 10, 15, 18]. Moreover, this delivery route bypasses the first-pass metabolism and has low enzymatic activity [6, 14, 15, 18], enabling the use of lower doses [8, 16] and reducing the occurrence of systemic side effects [15, 24]. Therefore, this delivery method promotes a fast drug onset [8, 10] and increases drug bioavailability [11, 15], being particularly beneficial for APIs that are easily metabolized [11].

Orally-inhaled drugs can be delivered to the lungs, in a non-invasive way [14, 18], through a nebulizer, metered dose inhaler, soft mist inhaler, and dry powder inhaler (DPI) [6, 15, 25]. Nebulizers, metered dose inhalers, and soft mist inhalers deliver drugs in the form of solutions or suspensions [15], whilst DPIs are in the solid state [15, 25]. Notably, DPIs have been gaining increased attention to treat infectious lung diseases [6], due to their distinct advantages over the other inhalers [11, 19, 24]. These advantages include being propellant-free, breath-actuated, having improved chemical stability, linked to the API's solid-state, portable, and not requiring breath and device coordination [5, 6, 12, 14, 15, 17, 24, 25]. Moreover, they are an alternative for the administration of high doses, instead of nebulization which requires long administration times and cleaning procedures [11, 26].

The human pulmonary airway system comprises three distinct regions: extrathoracic, tracheobronchial, and alveolar [5, 27]. Each of these regions has a different particle deposition extent [18] as particles deposit according to their aerodynamic diameter (D_{ae}), as is shown in Figure 2.1. The D_{ae} of a particle refers to the diameter of a sphere with a unit density, possessing the same settling velocity in the air as the particle under study [28].

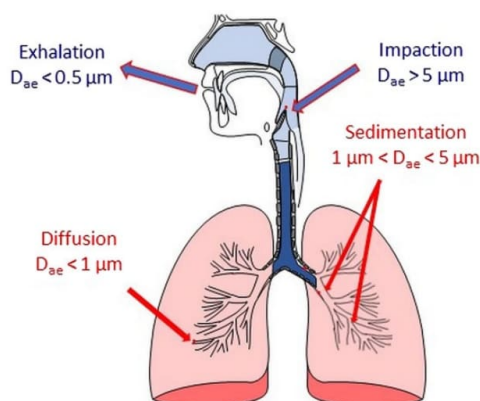


Figure 2.1: Lung deposition mechanisms [8]

It is widely recognized that, for optimum lung delivery, particles should have a D_{ae} between 1 and 5 μm [6, 9, 24, 29], depositing in the bronchial and alveolar region due to gravitational sedimentation [6, 8, 15, 18]. Larger particles ($D_{ae} > 5 \mu\text{m}$), deposit on the upper airways due to inertial impaction [5, 6, 8, 15, 18]. Lastly, particles under 1 μm are deposited in the deep alveoli by Brownian diffusion, while even smaller particles ($< 0.5 \mu\text{m}$) remain in the air stream and are exhaled [15, 18].

However, pulmonary drug delivery has some drawbacks and challenges. The respiratory system has a low tolerance of foreign particles [18], and they can be removed by two clearance mechanisms, (i) the mucociliary response and (ii) alveolar macrophage activity, that specifically target particles between 1 to 3 μm [6, 15, 18, 28]. This quick clearance of the site of deposition decreases drug concentration in the lungs [15], thus compromising the therapeutic effect [18]. Another challenge is tied to the DPI's breath-activated

nature, which requires forceful inhalation to fluidize the drug powder and generate the aerosol, posing difficulties for children and patients with severe lung impairments [11, 30]. Although devices do not influence bioavailability their proper handling determines it [18]. Additionally, the drug formulation should avoid causing local irritation, to maintain patient compliance [18] and the formulation should be optimized to avoid particle deposition in the oropharynx instead of the lungs, due to ineffective deagglomeration [5].

Therefore, the successful deposition of the drug particles in the lung is governed by i) the deposition site, which is dictated by the physical properties of the powder, linked to the formulation and the device [11, 31]; ii) inhalation conditions, such as the inspiratory flow and volume, linked to the individuals breathing pattern and lung health [4, 6, 27, 31]; and iii) respiratory tract factors, such as its geometry [6, 27, 31] and the occurrence of clearance mechanisms [31].

2.2 Types of formulations for dry powders inhalers

To assure correct lung deposition and increased bioavailability, dry powders must be designed with specific physicochemical properties, particularly enhanced aerosolization performance and dispersibility [6, 11, 18, 31]. However, this can be challenging, considering that dry powder particles, of low micron size, have high surface energy, exhibiting high cohesion and adhesion forces, that result in poor flowability, dispersion, and aerosolization [19, 28, 32].

Consequently, the most usual form of dry powders consists of carrier-based formulations [11, 18, 28, 33]. These formulations involve blending the fine API particles with a coarse carrier particle to improve flowability, handling, dispensing, and metering [11, 12, 28, 32]. Carrier-based formulations require the micronization (size reduction) of the API followed by blending with a coarse carrier particle, commonly lactose monohydrate, enabling the adhesion of the API to the carrier surface [23, 24, 32, 34, 35].

However, this approach exerts poor control over particle size, shape, and morphology [24]. Hence carrier-based formulations present challenges such as poor blend uniformity [12, 34] and inadequate aerosolization [11, 12, 34], linked to the need for optimal adhesion forces, which must assure, simultaneously, homogeneity, powder stability and API detachment during inhalation [11, 12, 23, 31]. Additionally, carrier-based blends are not suitable for high doses, as the ratio between carrier and API is high and there is a limited amount of powder that can be inhaled, as usually drug loads up to 5% w/w are used in this type of formulation strategy [28, 32, 36].

A novel solution to these challenges is carrier-free composite particles, where one or more APIs and possibly excipients are embedded in a single solid phase [2, 12, 32, 34, 37]. This approach improves uniformity and dispersibility, yielding increased therapeutic activity [12, 31]. Such particles can present different morphologies, such as spheroids, porous particles, and encapsulated particles, containing a solid core surrounded by an

outer layer [12]. Additionally, this approach is suitable for high dosages, as there is a limited amount of excipients in the formulation [19, 31, 32].

Preparation of composite encapsulated particles is particularly interesting for pulmonary drug delivery as encapsulation grants API protection from ambient conditions [38–40], can prevent moisture uptake [19, 24], potentiates controlled release [38], enhances stability [39, 40], and can improve powder dispersibility [24, 38, 40] depending on the selected formulation.

Among others, one of the potential critical quality attributes (pCQAs) of dry powders for respiratory drug delivery, is the particle size distribution (PSD) [6, 28, 29]. This pCQA has been shown to directly affect the aerosol performance and site deposition within the lungs [6, 11, 18, 36]. The PSD can be expressed by D_{v10} , D_{v50} , and D_{v90} , which are the geometric particle sizes that correspond to 10%, 50% and 90% of the volumetric size distribution, with D_{v50} being the most reported value as it divides the PSD into two halves [8, 18]. The span, (Eq.2.1), reveals the degree of heterogeneity in the PSD [18].

$$Span = \frac{D_{v90} - D_{v10}}{D_{v50}} \quad (2.1)$$

The aerosolization performance is another crucial property of dry powders, and it can be expressed by multiple pCQAs such as D_{ae} , the emitted dose (ED) and the fine particle fraction (FPF) [8, 18, 41]. The ED is the percentage of powder that is released from the device and the FPF corresponds to the percentage of the ED that exhibits a D_{ae} under 5 μm [8, 18].

Particle shape, morphology and surface roughness also play a crucial role in aerosol performance [6, 11, 18, 28, 31]. These parameters dictate agglomerate formation, surface area, and friction between particles, thereby influencing powder flowability and dispersibility [28]. Moreover, they have been shown to impact the D_{ae} , by altering the shape factor and density. [18, 28]. For instance, wrinkled/corrugated and porous particles have surface irregularities that prevent close contact between particles, reducing interparticle cohesive forces and agglomeration, resulting in powder with enhanced dispersibility [8, 18, 28].

Furthermore, properties such as particle charge, density, moisture content, and hygroscopicity also impact the effectiveness of dry powders. For optimal lung delivery, the particles should have a density below 1 g/cm^3 and exhibit both low hygroscopicity and moisture content [11, 18, 27, 31]. The latter is also beneficial in terms of powder stability [18].

2.3 Composite particles

The formulations can include excipients, which are pharmacologically inactive compounds, that improve the properties of drug products and must be chosen based on their precise functions [12, 32] For inhalation purposes the excipients should reduce particle cohesiveness, thus enabling good aerosolization [2, 12].

The use of excipients is regulated by the authority that approves the pharmaceutical product, for example, the Food and Drug Administration (FDA) or the European Medicines Agency (EMA) [32]. Although there is a limited number of approved excipients for pulmonary delivery [31], the FDA has a list of substances generally recognized as safe, and the choice of excipients often is based on this list [32].

The two classes of excipients mostly used for pulmonary applications, namely the preparation of encapsulated particles, often have the function of glass stabilizers and/or shell formers [40]. Upon SD the APIs can be dried into amorphous or crystalline solids. Amorphous solids can display poor long-term stability due to recrystallization, therefore they are commonly combined with glass stabilizers [40]. This class of excipients, positively influences stability, increasing storage time as they have the ability to increase the formulation's glass transition temperature [19, 40, 42]. Saccharides, and polymers such as trehalose, sucrose, mannitol, and inulin are examples of commonly used glass stabilizers [5, 19, 40].

Amino acids (AAs) or lipids are being proposed in the literature as shell formers, to promote the formation of an encapsulating shell around the API, assuring its protection and therefore stability, and enabling aerodynamic performance [32, 43–48]. Among these, AAs are by far the most common shell-forming agents, currently being proposed in literature [12, 19, 49]. They improve aerosolization due to their potential to increase dispersibility, reduce moisture sorption, and decrease surface tension [12, 19, 24, 49]. Leucine and glycine are among the AAs that have been extensively reported as shell-forming agents and have been approved by the FDA for injectables and for Exubera® (a DPI commercial product containing glycine) [32]. Moreover, they are endogenous substances [32], with a high potential to be biocompatible [14] and their *in vitro* safety has been proven in a lung cell line [50].

Leucine is a hydrophobic AA, and it is generally regarded as the most suitable AA for increased dispersibility and aerosolization [12, 19, 40, 49, 51, 52]. It yields hollow-shell type morphologies [41, 51–53], with increased surface rugosity and reduced surface energy [28, 40, 54]. These properties enable a reduced contact area between particles and external surfaces, leading to decreased interparticle forces and improved aerosol flowability [28, 40, 52]. Furthermore, the hydrophobic nature of the shell reduces cohesive forces and provides moisture protection, thus safeguarding the inner core and improving formulation stability [19, 51, 53, 54].

Composite formulations, namely encapsulated particles, can be prepared recurring to particle engineering techniques [31, 32]. The most well-established techniques in the pharmaceutical industry are spray drying (SD), lyophilization, emulsion-based systems, and coacervation [2, 39, 55–58]. Additionally, there are new encapsulation technologies under development such as interfacial solvent exchange, electrospray drying, and supercritical fluid-assisted spray drying, although these newer processes are still overly complex and pose several scale-up challenges [2, 56, 58].

Emulsion-based methods are usually employed for the development of controlled

Table 2.1: Summary of particle engineering techniques

Technique	Process	Disadvantages
Emulsion-based	Emulsion preparation followed by oil removal	Complexity Large waste generation Slow progress rate
Coacervation	Phase separation induced by temperature, pH, composition	Complexity Multi-step separation
Liophilization	Emulsion freezing followed by moisture sublimation	High energy consumption Slow progress rate

release formulations often in the form of injectables [58] and require at least two immiscible liquids [58, 59]. Emulsions can be classified as oil-in-water or water-in-oil (O/W, W/O), which are simple emulsions, or multiphase emulsions (W/O/W, O/W/O) [57–60]. One of the phases is dispersed in the other as small droplets, with the core material usually being in the dispersed phase with the shell material in the continuous phase [59]. Hydrophilic APIs are commonly encapsulated with W/O/W emulsions, conversely hydrophobic APIs require O/W or O/W/O [57, 61]. The emulsion preparation is followed by oil removal, either by evaporation, non-solvent extraction (anti-solvent, solvent exchange), or solvent dilution [2, 58, 62].

These methods can be challenging due to their complexity, slow progress rate, and issues with emulsion stability and uniformity. Another drawback is linked to the utilization of multiple solvents, leading to large waste generation and complicated full solvent removal, which compromises efficacy and toxicity. Furthermore, this process requires additional manufacturing steps that increase the manufacturing burden, risk of non-quality or non-compliance, and are of difficult scale-up [58, 62].

In coacervation a change in temperature, solution composition, or pH induces a phase separation in a solution between two liquid phases, differing only in the composition of solutes (polymer-rich and solvent-rich phase). More complex systems are designed to lead to the deposition of a shell material around the core of the API to be encapsulated [58, 60, 62]. However, this process requires multi-step particle separation and the system’s physical chemistry is highly complex [58, 62].

Lyophilization enables the entrapment of an active compound by freezing an emulsion containing a core material and a coating material [60]. It simply consists of moisture sublimation, involving three steps [57, 63]. The initial phase comprises freezing the product, thereby moisture is frozen to ice. Afterward, the product is dried under a high vacuum, to sublime the ice, followed by a secondary drying step [59, 63]. Even though this process enables milder conditions than SD [59], it has a long processing time coupled with high consumption of energy and high costs [59, 60, 63].

The above-mentioned techniques are summarized in Table 2.1. They require specific solvent systems, high energy operations, are time-constrained, and require multiple steps to yield the final drug product intermediate [58], thus exhibiting increased difficulties

when compared to the simplicity and straightforwardness of the SD process [2].

Hence, presently, SD is the most commonly used process to yield encapsulated particles [2, 39, 57, 58, 63–65]. Furthermore, SD is known for its suitability to produce composite particles, specifically for inhalation purposes [2, 12, 18, 52, 57], as a particle engineering method, that enables fine-tuning of powder properties [19, 28, 32, 36, 51].

2.4 Spray Drying

The first patent reporting SD technology dates to the early 1870s. Ever since SD has evolved and currently it is a well-established process in the pharmaceutical industry [66, 67].

There is great interest in SD as the process is single-step, simple, flexible, reproducible, cost-effective, and easily scalable [18, 25, 40, 66–68]. Moreover, it enables particle engineering, by fine-tuning the physicochemical properties of the particles, namely size distribution, surface morphology, density, flowability, and moisture content [14, 18, 19, 25, 33, 36, 51, 69].

The SD market share in the pharmaceutical industry is predicted to experience a stable 7.1% growth from 2023 until 2028, linked to the economic growth share of the pharmaceutical market and the development of innovative technologies [70, 71].

SD is amongst the most widely used process to manufacture inhalable dry powders [18, 19, 28]. Some examples of approved spray-dried formulations suitable for inhalation currently available in the market are the Tobipodhaler® (tobramycin, Mylan) and Bronchiol® (mannitol, Pharmaxis), both used for the treatment and maintenance of cystic fibrosis, as well as Inbrija® (levodopa, Acorda Therapeutics), used for the treatment of off episodes in patients with Parkinson's disease [14, 23, 26, 67, 69]. A spray-dried insulin inhalation powder, Exubera® (insulin, Pfizer) was also approved by the FDA and EMA, but it was removed from the market due to poor acceptance [32, 72].

SD can incorporate different components in a formulation, originating composite particles with different morphologies. Moreover, it has been recognized as a pivotal tool for microencapsulation as mentioned previously.

The spray drying process, depicted in Figure 2.2, consists of four main processing steps i) liquid feed atomization, ii) droplet drying, iii) powder formation, and iv) powder collection [12, 66].

Firstly, a liquid feed (solution, suspension, or emulsion [18, 40, 67]) containing the desired compounds is prepared. This liquid feed is then pumped and atomized creating a fine spray with a specific droplet size [25]. The atomization step can be conducted with different nozzles, such as rotary, single-fluid, multi-fluid, and ultrasonic [25, 66]. Multi-fluid nozzles are commonly employed in the pharmaceutical industry [12, 14, 40], wherein a gas feed is used to break up the liquid. The liquid and gas flow separately inside the nozzle, meeting at the tip, where droplets are created [68].

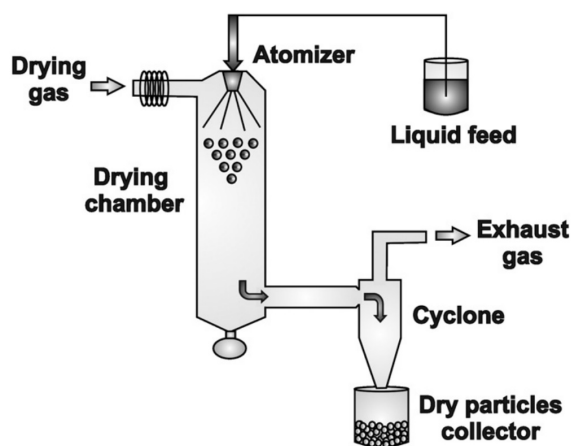


Figure 2.2: Spray drying process [66]

Atomization plays a pivotal role in SD, as it affects both the drying time, droplet size distribution, and density, thus greatly influencing the particle properties, namely the final particle size [12, 18, 25, 29, 68]. Additionally, the droplet size distribution can be estimated according to the atomization process using empirical correlations [68, 73]

In the drying chamber, the droplets contact with heated drying gas, typically nitrogen, enabling heat and mass transfer between the gas and droplets [25, 40, 68]. The contact between phases in the drying chamber is influenced by the flow pattern in the SD, which can be co-current, counter-current, or mixed flow. In the co-current setting, gas and droplets flow from the top of the chamber to the bottom, whereas in the counter-current setting, gas flows upward against the droplets [25, 29, 66, 68]. Therefore, in co-current flow, the powder is in contact with the coolest gas. Conversely, in counter-current flow, the powder contacts with the hottest gas, thus this setting is not suitable for heat-sensitive materials but desirable for thermal efficiency [25, 29, 66]. In the mixed flow setting, the nozzle is in the middle of the chamber while the gas flows downward [25, 66].

Rapid solvent evaporation results in the formation of solid particles, that are carried with the gas into the cyclone [25, 40]. The cyclone's cylindrical design enables the generation of a vortex flow as the gas enters tangentially and spirals down, separating the solid particles due to centrifugal forces [25, 40, 68]. Cyclone design is crucial as its performance influences particle separation, namely, the capture of finer particles under $2\text{ }\mu\text{m}$, affecting the yield and final PSD [68].

Regarding the exhaust gas, the SD process can be conducted in two settings, open loop, where the drying gas is not recirculated and closed loop, where the gas is recirculated, passing through a condenser to remove the evaporated solvent and re-establish the humidity levels [66].

Depending on the process conditions, product specifications, and physical stability, the collected powder might undergo a secondary drying step to remove residual solvent [68].

Numerous operating parameters can be optimized to fine-tune particle properties

[12, 18, 25, 32, 40, 67], the influence of different process conditions on the final powder properties are summarized in Table 2.2

Table 2.2: Theoretical influence of SD parameters on final powder properties, adapted from [12]

Process parameters	Particle properties
Liquid feed concentration (C_{solids})	Particle size
Feed rate (F_{feed})	Particle size Particle morphology Water content
Atomization feed rate (F_{atom})	Particle size
Outlet temperature (T_{out})	Particle morphology Water content

Furthermore, other input parameters such as the atomization strategy, solvent type, and overall formulation play a significant role in determining the powder properties [14, 18, 32, 40].

2.5 Three-fluid nozzle

A 3FN, schematically present in Figure 2.3A is a novel type of multi-fluid nozzle that features a three-layered concentric structure. In this design, two liquid feeds flow through independent channels and the atomization gas flows through the third outermost channel [2, 17, 38, 64, 74]. This atomization strategy contrasts with the conventional approach of a two-fluid nozzle (2FN), pictured in Figure 2.3B, which only has one liquid channel.

Additionally, since the 3FN has external mixing, the different feeds meet at the nozzle tip, where they are co-spray dried, with no prior mixing [64, 74], originating multilayered droplets [39, 41]. It is proposed that the solvent sprayed via the outer channel may coat the solvent of the inner channel during a brief atomization process [38].

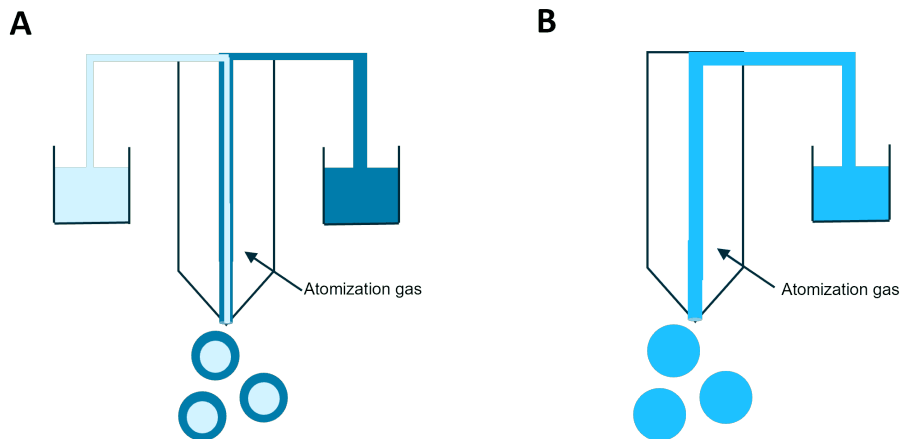


Figure 2.3: Atomization strategies, (A) three-fluid nozzle, (B) two-fluid nozzle

The 3FN offers several advantages for dry powder production. It can help overcome solubility and solvent incompatibility issues, by allowing the APIs to be solubilized in one solvent and the excipients in another [2, 7, 17, 64, 74], thus, eliminating the need for co-solvent systems that solubilize all components in a single solution (2FN) [7, 64]. Furthermore, this system enables the use of wider ranges of solids concentrations [17, 74, 75], which is advantageous as it reduces solvent usage [17, 74, 75] and increases process throughput [2, 7], thereby enhancing economic viability [2].

The 3FN has been widely studied for various applications, namely, it has been used to successfully encapsulate APIs in different matrixes for diverse purposes. Protein-loaded PLGA microparticles with varying characteristics have been prepared with a 3FN, demonstrating its suitability to encapsulate biopharmaceuticals [76]. Also, the encapsulation of diclofenac sodium with ethyl cellulose resulted in a functionalized API with the potential for enhancing delivery [39].

Encapsulation has also been investigated for sustained release of ethezenamide from microcapsules coated with ethyl cellulose, prepared from a suspension. The 3FN resulted in particles with a clear shell structure and a smaller particle size than those obtained from a 2FN. A main draw from this study is the crucial role of the arrangement of the feeds in the nozzle, whereby the shell-forming agent must be fed on the outer channel to ensure encapsulation [38].

Furthermore, the 3FN has been used to overcome incompatibility challenges between API and excipients, enabling the production of encapsulated omeprazole sodium with Eudragit® L100, without the typical API degradation observed when drying with a 2FN [64].

Besides encapsulation, solid dispersions with improved dissolution were prepared with a 3FN, showing it can improve the dissolution behavior of lipophilic drugs in a hydrophilic matrix, specifically of diazepam in mannitol [74].

This atomization strategy has also been studied regarding the preparation of amorphous solid dispersions of carbamazepine, using polymeric excipients. Additionally, the 3FN performance was compared with the conventional 2FN. The 3FN yielded particles with the desired drug load and it was concluded that solution placement on the channels substantially affects crystallization. Regarding PSD, drug content, and residual solvent content the results were similar between the 3FN and 2FN. A main takeaway of the study is the advantage rendered by the separate feed rates which enables drug load to be tuned as desired [77].

Regarding respiratory drug delivery purposes, published articles are mainly focused on the production of combination dosage formulations, where two APIs are co-spray dried. A combination of salbutamol sulfate and theophylline was prepared using the 3FN to overcome the low solubility of theophylline. The results demonstrated that the 3FN is suitable to produce homogenous particles for dry powders, although the aerosol performance required optimization to be adequate for pulmonary delivery [17].

Other combination formulations such as tobramycin with diclofenac [75] and colistin

and azithromycin [7] have also been investigated. These reports assess the effect of process parameters and compare the 3FN with the standard 2FN, regarding powder properties such as surface composition, aerosol performance, stability, and overall powder physicochemical properties. The results show that the 3FN yields larger particles, which resulted in a lower FPF when compared to the 2FN [7, 75]. Furthermore, both studies observed surface enrichment with the hydrophobic API which prevented moisture uptake in the case of colistin [7] and enabled sustained release of tobramycin [75]. Although the 3FN resulted in larger particles, it provided flexibility in terms of solvent choice and enabled drying at higher feed concentrations [7].

Additionally, the performance of the 3FN was investigated and compared to the 2FN, regarding the production of budesonide particles with mannitol. For the 2FN, budesonide was suspended in an aqueous solution, while with the 3FN the API was in a solution. The authors concluded that the API remained amorphous when spray-dried with the 3FN, but the 2FN was superior in yield and aerosol performance [35].

The currently available literature suggests that the utilization of a 3FN is mainly concerned with either the encapsulation of an API for protection and sustained release, amongst others, or with the preparation of combination dosages for pulmonary delivery.

However, there is a lack of information/research on the simultaneous use of the 3FN to overcome solubility limitations, incompatibilities, and purposeful encapsulation of the API, while yielding composite particles suitable for respiratory drug delivery. Additionally, there is a clear bottleneck in formulation development, since widely accepted inhalation excipients (sugars, salts, amino acids, alcohols, and polymers) are usually water soluble and poorly soluble in organic solvents [2], whilst most commercial APIs for inhalation and other APIs in development are poorly soluble in water [74, 78]

Hovione's patent WO 2017064481 [2] addresses this gap as it proposes the utilization of a 3FN with external mixing as an encapsulation strategy. In this setting one aqueous solution containing a shell-forming excipient is fed to the outer channel of the nozzle while an organic solution of the API is fed to the innermost channel, with the condition that the solution fed to the outer channel has a higher feed rate, to ensure encapsulation [2].

The use of a three-fluid external mixing nozzle is fundamental, as it keeps the two feed solutions apart until they are co-spray-dried. Furthermore, the microencapsulation degree can also be controlled with this technique, due to the flexibility in handling separately the concentrations of API and excipient and the relative ratios at which they are fed to the SD [2].

2.6 Process development methodologies

The success of dry powder formulations is tightly linked to formulation properties such as PSD, aerosol performance, amongst other [6, 11, 18, 28, 29, 31]. However, manufacturing via SD is a combination of two categories, the feed solution containing the formulation

and the process parameters. Therefore, the interrelations of these factors render the SD process optimization to the various product attributes a challenging task [29, 79]. In these situations, QbD approaches, based on statistical thinking and knowledge management, can be highly valuable [79, 80], especially considering that the pharmaceutical sector is process-based and quality-oriented [9].

QbD is a well-established tool [9], and it is defined in the ICH Q8(R2) as a systematic approach to product development that begins with predefined objectives and emphasizes product and process understanding and process control, based on the scientific method and quality risk management [81]. Simply put, the process shapes the quality of the final product [80], thus quality must be built into the design of the process [82]. Thus, applying a QbD framework is essential and has a significant impact on industry performance as it enables formulation and process optimization [83].

QbD requires the identification of the quality target product profile, the product's CQAs, and the critical process parameters (CPPs) [79, 82]. The CQAs are product properties or characteristics required to be within an appropriate range to ensure product quality [81, 84]. In dry powders, these would be the particle size attributes, such as the Dv_{50} , aerosol performance indicators, such as the D_{ae} , ED and FPF, and other powder properties such as the residual solvent and water content, and bulk density. The CPPs are the factors in the process, whose variability has the greatest impact on the CQAs [81, 84]. In the SD process, the operating parameters with the greatest influence on powder properties generally are T_{out} , F_{atom} , F_{feed} , and C_{solids} [12].

The implementation of a QbD framework requires the execution of an design of experiences (DoE) [9, 84], which consists in a structured and simple approach that meets the requirements of pharmaceutical development [80] and fits into regulatory requirements [80, 82].

A DoE is a rational experimental planning, that mathematically explains how input factors influence the selected responses [9, 80]. It consists of a series of tests where input variables of a process are deliberately and simultaneously changed, according to a predefined matrix of factor level combinations [82, 85], allowing for a study of the effects that those changes had on the process outputs [85]. Therefore, with a DoE it is possible to investigate multiple factors and the influence of their interactions [9, 79], having full control of the total number of experimental runs [86].

Developing a DoE enables a thorough understanding of process factors and their effects on product quality attributes [9]. Therefore, it leads to the identification of the actual CPPs and links their effects with the CQAs through mathematical functions [79, 80, 82, 84]. Moreover, the DoE helps to establish a design space where the product has the required attributes [79, 80, 84].

There are different statistical approaches when performing a DoE, namely full-factorial, fractional factorial, response surface, and mixture design approaches [87].

In a factorial design, factors are adjusted independently from one another and the design can be a full factorial, where all combinations of factors are studied or it can be a

fractional factorial, where only a subset of a full factorial design is tested [82]. However, this type of design only detects first and second-order interactions [78]. Responses surface designs are more complex and can detect and quantify non-linear behaviors, requiring at least three levels of factors [87, 88]. Mixture design is applied when the factors are interdependent and each component in the mixture depends upon the settings of another component [88].

Experimental design has several advantages when compared to the one factor at the time approach, where only one variable is changed while the remaining are held constant [82, 86]. A DoE is cost-effective as it maximizes process knowledge with minimum resources [9, 82, 84–86], provides accurate data, identifies factor interactions and their joint influence on the outcomes (which is not possible with the one factor at the time approach [79, 82, 86])[85], quantifies factor significance, establishes solid cause-and-effect relationships between CPPs and CQAs and enables CQA prediction within the design space [82].

A common extension of a DoE includes statistical estimates and process modeling [89, 90]. These complement the experimental design [91], offer predictive power and are based on statistical and multivariate analysis tools [92].

Process modeling can enhance development [89] by aiding process design [93, 94], supporting optimization, control [89, 90], scale-up [94] and conducting risks assessments [91, 93]. Therefore, they constitute a quantitative framework used to better understand the process, predict its outcomes, and control its behavior [93].

Process models are a simplified representation of a complex reality based on mathematical terms [93] and often three different modeling strategies based on diverse types of knowledge are employed: (i) mechanistic/ fundamental models, (ii) empirical/ data-driven models, and (iii) semi-empirical models [89, 93].

Mechanistic models are differential / algebraic relationships derived from physical and chemical phenomena that govern the behavior of a process [89, 95]. Even though these models enable extended process understanding, flexibility and allow for extrapolation [94], their development requires a large investment of time, effort, and resources [90, 94]. Thus, they have limited applicability and are used less often, due to their complexity and high computing times [89, 96].

Empirical models are solely based on experimental data fitted with multivariate modeling techniques [93, 96]. They can be applied to processes that are too complex to be modeled solely on scientific theory [89, 91, 94], being reliable within their validated range and reflecting the physics of the process and its variability [94]. Additionally, this approach requires minimum information and is developed quickly, due to the high availability of data from both offline and online measurements [89, 94]. Even though no mechanistic knowledge of the process can be derived from these models [89, 94], they are suitable to mathematically describe pharmaceutical processes [96] and align with the latest trends in industry 4.0 [89].

Lastly, semi-empirical models couple equations based on fundamental process knowledge with empirical parameters, that are determined experimentally [91].

Empirical modeling of linear data can be performed by multivariate Linear Regression (MLR) and latent variable methods, such as principal component analysis (PCA) and projection of latent structures [91, 94, 96]. These approaches are used extensively [94], with latent variable methods being applied when the data sets are highly correlated and no true independent input variables are identified [91, 96].

For non-linear datasets, modeling can be performed with response surface models and artificial neural networks [91, 94]. Non-linear approaches require multiple experimental runs, namely on response surface models, where multiple levels are required to account for curvatures in the response [92]. Regardless of being able to establish highly non-linear models, artificial neural networks require multiple parameters [96] and are not easily interpreted [91]. Moreover, unlike MLR and response surface models, which can be analyzed with an analysis of variance (ANOVA), there is no systematic statistical-based tool to evaluate model accuracy of artificial neural networks [94].

The selection of the most suitable modeling technique depends on the nature and complexity of the observed relationships between variables and should be based on an exploratory data analysis (EDA)). Doing an EDA enables the identification of relationships and patterns in the data as well as the detection of outliers [89].

Additionally, model simplicity is crucial, thus only predictors that explain reasonable amounts of variance should be included, which can be attained with variable selection [89, 95]. Variable selection enables the deletion of redundant predictors, therefore reducing noise, collinearity, and costs [95]. It can be performed by hierarchical removal criteria, forward selection, backward selection, or stepwise selection [89, 95]. Regardless, variables not chosen for the model are still linked to the response variable, but they do not add significant knowledge.

Model accuracy must be evaluated with an ANOVA [96] and, when applicable, a hypothesis test, whereby rejection of the null hypotheses implies that at least one predictor contributes significantly to the model [95]. Furthermore, models should be subject to validation, which aims at proving that the developed model can recreate the behavior of the system under study [96]. A common validation method is based on comparing the predictive model results with experimental data using either an internal or external dataset [96].

In this work, the 3FN will be implemented to produce encapsulated composite particles as outlined in Hovione's patent [2] adhering to a QbD approach. This approach offers a well-structured methodology for process development, which can significantly expedite both scale-up and optimization efforts. Itraconazole (ITZ), an antifungal agent, was chosen as a model of a poorly water-soluble drug (solubility in water of 1 ng/ml at pH 7 [69, 97]). Additionally, the pulmonary delivery of antifungal agents for the prevention of invasive pulmonary fungal infections has gained traction recently, owing to its ability to administer high doses locally with minimized systemic effects. Hence, the development of inhalable

formulations containing ITZ holds significant potential [69, 97]. Amino acids were used as encapsulating agents of ITZ due to their increased potential for biocompatibility [14].

DoE and multivariate data analysis techniques have been increasingly adopted in the pharmaceutical industry [83] as they enhance statistical interpretation [86]. Therefore, to comply with a QbD approach, the 3FN and its impact on powder properties will be investigated with a full factorial design. The SD conditions T_{out} , F_{atom} , and M_{ratio} (mass ratio between the outer and inner feed of the 3FN) will be examined as potential critical process parameters (pCPPs), and the powder properties D_{v50} , ED, and FPF will be considered as the pCQAs. Furthermore, the outcomes of the DoE will be utilized to develop an empirical model, fitted with MLR. The model will be subject to external validation to ensure its reliability and effectiveness within the design space.

This comprehensive work supports the feasibility of utilizing the 3FN towards the encapsulation of hydrophobic APIs with AAs, while also increasing process understanding, through the established relationships between process parameters and powder properties.

MATERIALS AND METHODS

3.1 Materials

L-Leucine (Leu), Glycine (Gly), (S)-(+)-Valine (Val) and D-(+)-Trehalose dihydrate (Tre) were purchased from EMD Milipore Corporation (Billerica MA, USA). L-Lysine (Lys) was purchased from Thermo Scientific™ (Massachusetts, USA), Itraconazole (ITZ) from Fagron (Rotterdam, Netherlands), Rifampicin (RIF) from Shenyang Antibiotic Manufacturer (Shenyang, China). Water was obtained from a Milli-Q® Water purification system and the solvents dichloromethane (DCM), acetone, isopropyl alcohol, and methanol were purchased from Drogas Vigo.

3.2 Methods

3.2.1 Spray Drying

Spray dried intermediates (SDIs) were prepared using a B-290 mini spray dryer (Büchi Labortechnik AG, Switzerland) coupled with an inert loop B-295 (Büchi Labortechnik AG, Switzerland) to process organic solvents. During trial #1 to #4 the spray dryer was equipped with a conventional 2FN, cap diameter of 1.4 mm and orifice of 0.7 mm (Büchi Labortechnik AG, Switzerland). While for trial #5 across #25 a 3FN with a cap diameter of 1.4 mm and orifice of 0.7 mm (Büchi Labortechnik AG, Switzerland) was employed. The drying was performed with nitrogen in a co-current open-loop setup for all trials. SDI particles were separated from the drying gas using a high-performance cyclone (Büchi Labortechnik AG, Switzerland), collected in glass vials, weighed, and then stored in capped HDPE containers.

In the experimental set-up with the 3FN during the formulation screening, a laboratory peristaltic pump (Watson Marlow 120U, United Kingdom) was used to pump the aqueous feed to the external nozzle liquid channel, whereas the B-290 mini spray dryer pump was used to pump the organic feed into the nozzle's inner channel. For trial #10 across #25 two laboratory peristaltic pumps (Watson Marlow 120U, United Kingdom) were used to feed the aqueous and the organic solutions.

The total solid concentration in the solution (C_{solids}) was kept constant at 3% (w/w) for all trials. The only exception was Trial #2, where this concentration was not achievable due to Leu's water solubility limitations (24.3 g/L at 25°C [98]) hence for this trial a $C_{\text{solids}} = 1.5\%$ (w/w) was used. To produce encapsulated particles the aqueous outer feed must be higher than the organic inner feed [2], thus, the aqueous/organic solution mass ratio (M_{ratio}) was kept above 1 for all trials. The organic API solutions were prepared using DCM, while the aqueous solutions containing the shell-forming excipients were prepared using Mili-Q water. Due to the 3FN setup, the organic phase solid concentration (C_{feed_o}) and aqueous phase solid concentration (C_{feed_a}) are different and were adjusted to maintain the established C_{solids} and simultaneously satisfy the M_{ratio} . The tested conditions for the formulation screening tests can be found in Table 3.1, during these trials the drying gas flow rate, F_{drying} was kept constant at 35 kg/h.

Table 3.1: Spray drying conditions for the formulation screening trials.

Process parameters	Trial #1	Trial #2	Trial #3	Trial #4	Trial #5	Trial #6	Trial #7	Trial #8	Trial #9
Formulation	ITZ	Leu	Lys	Gly	ITZ:Leu	ITZ:Leu:Tre	ITZ:Lys	ITZ:Gly	ITZ:Leu
T_{in} (°C)	50	162	155	155	138	120	120	120	65
T_{out} (°C)	35	80	80	80	62	62	62	62	40
C_{feed_o} (w/w%)	3.0	-	-	-	5.7	5.7	5.7	5.7	5.7
C_{feed_a} (w/w%)	-	1.5	3.0	3.0	2.0	2.0	2.0	2.0	2.0
F_{feed_o} (g/min)	7.6	-	-	-	2.3	2.3	2.3	2.3	0.9
F_{feed_a} (g/min)	-	7.6	7.6	7.6	6.3	6.3	6.3	6.3	2.5
$F_{\text{feed}_\text{total}}$ (g/min)	7.6	7.6	7.6	7.6	8.6	8.6	8.6	8.6	3.4
M_{ratio} (-)	-	-	-	-	2.8	2.8	2.8	2.8	2.8
F_{atom} (kg/h)	1.4	2.1	1.4	1.4	3.0	3.0	3.0	3.0	3.0
RS_{out} (%)	0.5	4.1	4.0	4.0	6.6	6.6	6.6	6.6	8.7

To study the impact of the 3FN and SD operating parameters on particle properties, a DoE was performed using a 2^3 full factorial design. Additionally, a central point was also included in the design. The selected DoE factors were: (i) T_{out} , (ii) F_{atom} , and (iii) M_{ratio} , considered to be the pCPPs. The design space, featuring the experimental points and factor levels is schematically represented in Figure 3.1, while the correspondent tested SD conditions can be found in Table 3.2.

T_{out} was selected by evaluating the expected drying gas residual solvent content (RS_{out}). The RS_{out} is a result of the heat and mass balance of the spray drying process at the outlet conditions. The F_{atom} lower-level threshold of the tested design space was set at 2.1 kg/h because preliminary tests demonstrated that this was the minimum atomization flow rate that promoted atomization by means of visual assessment of the spray pattern. The M_{ratio} was evaluated between 2.8 and 3.8, being the lower end of the range limited by Leu's water solubility, while for the upper end of the range, higher ratios would imply higher feeding rates, increasing the RS_{out} and increasing the risk for high residual water content, therefore, this limit was defined to meet with an RS_{out} threshold of approximately 20%. In these trials, F_{drying} was kept constant at 30 kg/h.

The DoE was followed by the development of empirical models, which must be validated with a new set of independent experiments. The SD conditions used for these

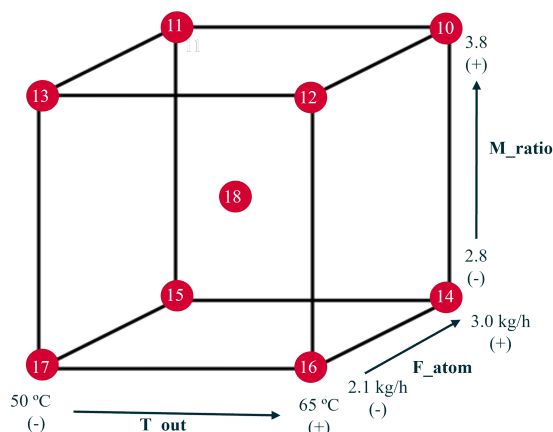


Figure 3.1: Design space according to the upper and lower level of the factors T_{out} , F_{atom} and M_{ratio}

Table 3.2: Spray drying conditions of the DoE trials

Process parameters	Trial #10	Trial #11	Trial #12	Trial #13	Trial #14	Trial #15	Trial #16	Trial #17	Trial #18
Formulation	ITZ:Leu								
T_{in} (°C)	141	114	147	117	128	103	128	100	119
T_{out} (°C)	65	50	68	53	65	50	65	50	58
C_{feed_o} (w/w%)	7.2	7.2	7.2	7.2	5.7	5.7	5.7	5.7	6.4
C_{feed_a} (w/w%)	1.9	1.9	1.9	1.9	2.0	2.0	2.0	2.0	2.0
F_{feed_o} (g/min)	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.2
F_{feed_a} (g/min)	8.5	8.5	8.5	8.5	6.4	6.3	6.3	6.3	7.3
$F_{feed_{total}}$ (g/min)	10.7	10.7	10.7	10.7	8.6	8.6	8.6	8.6	9.5
M_{ratio} (-)	3.8	3.8	3.8	3.8	2.8	2.8	2.8	2.8	3.3
F_{atom} (kg/h)	3.0	3.0	2.1	2.1	3.0	3.0	2.1	2.1	2.5
RS_{out} (%)	10.1	20.5	10.1	20.5	7.5	15.2	7.5	15.2	12.1

trials (#19 across #21) can be found in Table 3.3.

Additionally, a formulation with a second shell-forming agent, Val (trial #24), and a second poorly water-soluble API, namely RIF (trial #25) were studied to assess methodology suitability. Template-assisted optimization trials were performed using ITZ as a model drug and Leu as a shell-forming excipient (trial #22 and #23). A template agent ($NaHCO_3$) composition of 1% w/w was kept constant across these trials. Tested conditions for these trials can also be found in Table 3.3. DCM was kept as the organic solvent for all trials except #25, where the organic solution comprised DCM:methanol (80:20 w/w%) due to the different API being evaluated. In trial #22 and #23 the outlet temperature had to be adjusted to ensure salt sublimation.

3.2.2 Spray-dried intermediate Characterization

Quantification

For all trials, the API was quantified using UV-visible spectroscopy. Sample absorbance was measured using a Biotek Synergy HTX multimode reader (Agilent, USA) with a quartz 24-well plate. Spectral scans of ITZ, Leu, Lys, Gly, and Val were acquired from 200 nm to 700 nm by dissolving the SDI in the dissolution medium (80:20 (w/w) isopropyl alcohol:

Table 3.3: Spray drying conditions of the empirical model external validation and template-assisted optimization trials.

Process parameters	Trial #19	Trial #20	Trial #21	Trial #22A	Trial #22B	Trial #23A	Trial #23B	Trial #24	Trial #25
Formulation	ITZ:Leu			ITZ:Leu:NaHCO ₃			ITZ:Val		RIF:Leu
T _{in} (°C)	107	129	168	163	163	158	158	126	133
T _{out} (°C)	54	62	75	85	85	85	85	65	65
C _{feed_o} (w/w%)	6.0	6.8	7.3	5.7	5.7	5.7	5.7	5.7	5.7
C _{feed_a} (w/w%)	2.0	1.9	1.8	2.0	2.0	2.0	2.0	2.0	2.0
F _{feed_o} (g/min)	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
F _{feed_a} (g/min)	6.8	7.9	10.2	6.2	6.2	6.2	6.2	6.2	6.2
F _{feed_total} (g/min)	9.1	10.2	12.5	8.5	8.5	8.5	8.5	8.5	8.5
M _{ratio} (-)	3.0	3.5	4.5	2.8	2.8	2.8	2.8	2.8	2.8
F _{atom} (kg/h)	3.0	2.5	3.0	3.0	2.1	3.0	2.1	3.0	3.0
RS _{out} (%)	13.5	10.7	7.8	3.2	3.2	3.2	3.2	7.4	7.6

water) and adding 2 ml to the microplate wells. A calibration curve was developed starting from a stock solution at 600 µg/mL and diluting in the range of 6 to 60 µg/mL. On the reader, 1 ml of each standard was measured in duplicates against a blank consisting of the dissolution medium. To quantify the API in the spray-dried samples two dilutions were prepared from a mother solution containing 30mg of each sample dissolved in a 25ml volumetric flask with the dissolution medium. The measurements were performed in duplicate, each compared against a blank sample, and the API load was determined using the calibration curve.

Water content

The water content of the spray-dried samples was quantified using an 831 Karl Fischer Coulometer coupled with an 874 Oven Sample Processor and 728 Stirrer (Metrohm AG, Switzerland). Samples of 75 mg of SDI were weighed in a headspace vial and closed with a septum cap. Additionally, three blanks were prepared for each run, to capture the humidity conditions of the room where the samples were prepared. The analysis was performed with nitrogen at 150 °C with a flow of 40 mL/min. The water content was determined by the Tiamo Software.

Residual solvent content

SDI solvent content was determined using Hovione's proprietary method.

Particle size distribution

The PSD of the SDIs was determined with a HELOS series laser diffraction equipment (Sympatec GmbH, Germany), coupled with a RODOS M dry dispersing unit (Sympatec GmbH, Germany) and ASPIROS feeder (Sympatec GmbH, Germany). Round glass cuvettes were filled with about 1/3 in volume and the samples were evaluated with either an R2 (0.45-87.5 µm) or R4 (1.8-350 µm) lens, with an injector width of 4 mm and a probe slider speed of 50 mm/s. Reference measurements were performed with a duration of 10 s and samples were considered valid if the optical concentration was between 5% and 15%. The PSD was determined with the Fraunhofer Enhanced Evaluation theory, considering

the standard conditions of density equal to 1 g/cm³ and Heywood shape factor of 1. Primary pressure curves, ranging from 0.5 bar to 4 bar, were developed for each sample to determine the appropriate de-agglomeration pressure, at which the samples were then evaluated in three replicates. The PSD is characterized by the D_{v10} , D_{v50} , D_{v90} , and the span.

Surface composition

To assess surface composition and confirm shell formation samples were analyzed with X-Ray photoelectron spectroscopy (XPS). The analysis was conducted with an AXIS Supra+ (Kratos Analytical, UK). Photoelectrons were excited with a monochromatic Aluminum K alpha X-ray source. Samples were scanned widely for a complete spectrum and in more detail for the elements C, N, O, and Cl. The applied X-ray power was 150 W, and the analyzer pass energy was 80 eV and 10 eV for the wide and detailed scans, respectively. The samples were analyzed as tablets of compressed powder, prepared using a press, to prevent powder from dispersing away inside the equipment. Fragments of each sample were placed inside aluminum pans and loaded into a sample cartridge to be analyzed.

Surface morphology

The SDIs surface morphology was evaluated by scanning electron microscopy (SEM), some samples were also evaluated with energy dispersive X-ray spectroscopy (EDS) and FIB (focused ion beam). This analysis was performed with a Regulus 8220 (Hitachi, Japan). The powder samples were placed in a metal support with carbon tape and sputter-coated with a 20 nm layer of gold and palladium. For the micrographs the equipment was set at 10 μ A current, 5 kV accelerating voltage. The SEM-EDS samples required different conditions, namely a 15 μ A current and 20 kV accelerating voltage. Micrographs were taken at multiple magnifications.

Nitrogen adsorption

Trial #14, #22 and #23 were characterized in terms of surface area and porosity. This analysis was conducted with nitrogen adsorption with an ASAP 2000 (Micromeritics, USA). Surface area and average pore diameter were calculated by the Brunauer, Emmet and Teller (BET) method and pore volume by Barret, Joyner and Halenda (BJH) method.

Helium picnometry

The true density of the SDI obtained from trial #14, #22 and #23 was evaluated with helium picnometry using an AccuPyc 1330 (Micromeritics, USA). Samples were evaluated with helium using a cell with a standard volume of 12.2 cm³.

2D Fluorescence

SDIs were analyzed with a Varian Cary Eclipse Fluorescence Spectrophotometer (Agilent, USA), equipped with excitation and emission monochromators, and coupled to a fluorescence optical fiber bundle probe. The data was acquired on an excitation wavelength

range of 200 nm to 640 nm and an emission wavelength range between 210 nm and 650 nm, with a slit width of 5 nm and incrementing step of 5 nm for both excitation and emission. The SDIs were placed inside a glass vial large enough to fit the probe and measurements were taken always with the same distance between the probe and the SDI surface. All samples were evaluated in duplicates. The resulting excitation emission matrixes (EEMs) are very complex and difficult to interpret, therefore data deconvolution strategies were employed to simplify the results and extract the most relevant information. The collected data was analyzed with a PCA using a MATLAB script.

3.2.3 Drug product characterization

Capsule filling

To evaluate the in vitro aerosolization performance of the SDIs, hydroxypropyl methylcellulose capsules (Size 3; Capsugel, Australia) were hand-filled with 20 mg (± 2 mg) of the material produced from each spray drying trial.

In vitro aerosolization

Aerodynamic performance was studied with a fast-screening impactor (Copley Scientific, UK). Samples were evaluated with a low-resistance inhaler device (Plastiap, Italy) with the previously filled capsules. The inhaler device was connected to a 3D mouthpiece adapter and a United States Pharmacopeia (USP) induction port. The European Pharmacopeia (Ph. Eur) <2.9.18> and USP <601> suggest a pressure drop of 4 kPa in the inhaler [99–101], which was achieved by adjusting the airflow rate using a pump and checked with a DFM 2000 (Copley Scientific, UK). Additionally, the capsule actuation time was calculated to achieve an inhalation volume of 4 L per actuation, as recommended by the Ph. Eur <2.9.18> and USP <601> [100, 101].

The fast-screening impactor employs 2 stages, in the first large non-inhalable particles are captured in a liquid and a fine-cut impaction stage of 5 μm , while the fine particle dose is retained by a filter in an external filter holder. The liquid trap was filled with 15 mL of 80:20 w/w of isopropyl alcohol and water mixture and the final stage was lined with a 76 mm diameter glass fiber filter (Pall Corporation, USA). For each run, the capsule, device, and filter were weighed before and after actuation, to determine the ED and FPF. The ED was calculated as the SDI emitted from the capsule after actuation minus the leftover SDI in the device (Eq 3.1) and the FPF as the ratio between the SDI mass retained in the final stage and the ED (Eq 3.2)

$$ED (\%) = \frac{Mass_{capsule} - Mass_{retained\ in\ device}}{Capsule\ load} \times 100 \quad (3.1)$$

$$FPF (\%) = \frac{Mass_{final\ stage}}{ED} \times 100 \quad (3.2)$$

3.2.4 Modelling Strategy

Data correlating the identified pCPPs and pCQAs was collected following a structured design of experiments as detailed in Section 3.2.1. A MLR strategy was followed, enabling the modeling of an output variable (Y) as a function of various input variables ($x=x_1, x_2, \dots, x_n$) and their corresponding regression coefficients ($b=b_1, b_2, \dots, b_n$), along with a constant term (c) (Eq 3.3). [102–104]

$$Y = b_1x_1 + b_2x_2 + \dots + b_nx_n + c \quad (3.3)$$

Therefore, MLR based on the collected data was used to describe the mathematical relationships between input and output variables of the SD process toward the production of composite particles for pulmonary delivery. The considered input variables are the process parameters and output variables are the quality attributes of the SDIs with the greatest impact on the aerosol performance. JMP software (trial version) was utilized to develop models by least squares linear regression coupled with a stepwise variable selection. The stopping criterion for the variable selection was based on minimizing the Akaike Information Criterion (AIC) (Eq3.4), where n is the number of observations, SSE the square sum of residuals and p the number of predictor variables. [95]

$$AIC = n \ln \left(\frac{SSE}{n} \right) + 2p \quad (3.4)$$

An initial EDA was applied to identify relationships between process parameters, assess linearity between them and detect any patterns or trends in the data. Outlier identification was performed by Hotelling's T2 distribution analysis. The models were evaluated with a hypothesis test, whereby the null hypothesis, there is no relationship between input and output variables (all regressions coefficients are equal to zero), must be rejected.

For a given significance level α ($\alpha = 5\%$ in this work) the null hypothesis is rejected if $F_0 > F_{(\alpha, k, n-k-1)}$, where F_0 is the F-statistic, $F_{(\alpha, k, n-k-1)}$ is the F-critical value, k is the number of predictor variables in the model and n the number of observations.

The p-value of the F-statistic was also considered as it corresponds to the evidence against the null hypothesis, which is rejected when $|t_0| > t_{(\alpha/2, n-k-1)}$, where t_0 is the observed statistic of the hypothesis tests and $t_{(\alpha/2)}$ the upper $\alpha/2$ percentage point of the t-Student distribution with $n - k - 1$ degrees of freedom. The model's statistical significance is higher as the p-value is lower. [95, 102]

When the null hypothesis cannot be rejected, when applied to any of the regression coefficients of a single variable, the contribution of said variable has no statistical significance given the other variables in the model [89, 95].

Additionally, to ensure the modeling suitability some assumptions must be met: i) errors are expected to be normally distributed, ii) have a mean equal to zero, iii) have constant variance, and iv) be uncorrelated [95, 102]. To evaluate if the models are statistically significant in explaining the observed output variable variance an ANOVA

analysis was performed, where the sum of squares due to regression, SSR, due to residuals, SSE, and total, SST, are calculated.

Model fitness to the data was evaluated by the coefficient of determination R^2 (Eq 3.5), which explains the fraction of output variable variance that can be mathematically explained by the model. [89, 95]

$$R^2 = \frac{SSR}{SST} \quad (3.5)$$

The adjusted coefficient of determination, R_{adj}^2 (Eq 3.6) was also employed. This coefficient considers the improvement in the predictive power of the model with the addition of predictor variables, valuing simpler models and preventing the selection of overfitted models. [89, 95]

$$R_{Adj}^2 = 1 - \frac{SSE/(n - k - 1)}{SST/(n - 1)} \quad (3.6)$$

Variable selection can cause the addition of correlated input variables, therefore multicollinearity must be checked, since the addition of a second variable to the model, which explains the same amount of the total variance as another predictor, does not add any statistical significance. Multicollinearity was evaluated by the variation inflation factor (VIF) of every variable included in the models and considering a VIF threshold <5 [89].

Lastly, to validate the models, their predictions were compared with a new set of independent experimental data, thus, assessing the predictive capacity of the models (trial #19 to #21 in Section 3.2.1).

RESULTS AND DISCUSSION

4.1 Formulation screening

The selection of a formulation suitable for encapsulation that simultaneously optimizes the SDI's aerodynamic performance is a critical task as different excipients will behave differently during droplet drying. Thus, an initial screening was performed using different AAs with different hydrophobicity characteristics. Depending on the AA's chemical structure, they will arrange differently at the gas-liquid interface influencing shell formation. Three AAs were screened, for a formulation comprising 50% API and 50% AA, Leu as an example of a hydrophobic AA, Lys representing a hydrophilic AA and Gly as a rather neutral AA in terms of hydrophobicity [105]. Additionally, the influence of drying temperature was evaluated between trial #5 and #9 and a formulation comprising Tre and Leu (80:20 %w/w) was also studied, as it is a proposed standard formulation for inhalable spray-dried powders [34, 37, 65, 106–108]. Results are summarized in Table 4.1.

Spectral scans of the API and studied AAs are in Appendix A. Since the AAs do not absorb in the same region of ITZ, API quantification using UV-Vis spectroscopy is viable. The assay was determined at 260 nm according to the method described in section 3.2.2. The theoretical drug load is 50% w/w and the results of the assay are presented in Table 4.1.

Table 4.1: Results of the formulation screening regarding API load, a summary of surface composition by XPS, particle size (n=3), and aerosol performance (n=3). Results expressed as mean $\pm$ standard deviation

Trial	T _{out} (°C)	Shell former	API Load (% w/w)	Cl presence at the surface (XPS)	Particle Size (μ m)			Aerosol performance		
					D_{v10}	D_{v50}	D_{v90}	Span	ED (%)	FPF(%)
#1 ITZ	35	-	-	-	0.8 $\pm$ 0.0	1.9 $\pm$ 0.0	4.6 $\pm$ 0.1	2.0	-	-
#2 Leu	80	-	-	-	-	-	-	-	94.3 $\pm$ 4.6	77.5 $\pm$ 4.1
#3 Lys	80	-	-	-	-	-	-	-	-	-
#4 Gly	80	-	-	-	-	-	-	-	-	-
#5	62	Leu	51.5 $\pm$ 1.9	Barely	0.7 $\pm$ 0.0	2.3 $\pm$ 0.0	5.3 $\pm$ 0.1	2.0	92.6 $\pm$ 3.8	62.6 $\pm$ 6.3
#6	62	Leu:Tre	49.6 $\pm$ 1.3	Yes	0.6 $\pm$ 0.0	3.0 $\pm$ 0.2	7.6 $\pm$ 0.4	2.3	95.4 $\pm$ 6.6	34.1 $\pm$ 5.6
#7	62	Lys	55.4 $\pm$ 1.4	No	3.1 $\pm$ 0.1	7.7 $\pm$ 0.1	74.4 $\pm$ 9.5	9.2	94.7 $\pm$ 2.0	14.3 $\pm$ 4.0
#8	62	Gly	49.6 $\pm$ 3.4	Yes	3.3 $\pm$ 0.1	5.9 $\pm$ 0.1	42.8 $\pm$ 5.8	6.8	80.0 $\pm$ 7.7	31.6 $\pm$ 6.8
#9	40	Leu	58.4 $\pm$ 1.5	No	0.8 $\pm$ 0.0	2.6 $\pm$ 0.1	7.5 $\pm$ 0.2	2.6	97.4 $\pm$ 2.0	46.8 $\pm$ 0.8

During Trial #7 and #9, F_{feed} of the solutions changed over time, as a consequence the aqueous solution was not completely fed to the spray dryer, resulting in SDIs with higher ITZ concentrations. When comparing the results from the trials in which the F_{feed} of both feed solutions was kept constant throughout the SD trial, the results demonstrate that when spray drying with a 3FN, homogenous composite SDIs with the expected theoretical composition can be obtained.

The XPS spectra of individual components and the developed formulations can be found in Appendix B. Unlike the investigated AAs, ITZ has chlorine atoms, thus it can be detected on an XPS spectrum solely by the presence of its characteristic Cl peaks (270 eV and 200 eV). The purpose of this analysis is to ascertain the presence of an AA shell covering the API and not necessarily quantify the surface composition, thus a summary of Cl presence in the XPS spectra is presented in Table 4.1.

For trial #5, a residual Cl peak is observed, indicating near-complete coverage of the API by Leu. It is worth noting that since samples are in tablet form, the possibility of broken particles, due to the high pressure applied, cannot be ruled out, potentially accounting for the residual presence of API on the surface. Additionally, trial #9, consisting of API and Leu displays no Cl peaks, further evidencing that Leu forms an outer shell, completely covering the API. Regarding trial #6, where Tre was added to the formulation with Leu, subsequently reducing the AA content (theoretically 10%), a distinct Cl peak is evident. This suggests insufficient Leu content to fully encapsulate the API. The presence of Lys on the formulation also led to API encapsulation, with no identifiable Cl peaks in its spectrum. Conversely, Gly does not form a shell, as Cl presence is evident on the surface.

The 3FN is proposed to yield multilayered droplets, as the solvent sprayed via the outer channel may coat the solvent of the inner channel during a brief atomization process [38, 39, 41], therefore it is expected that the components of the feed in the outer channel are prone to be on the droplet's surface. This was verified for Leu and Lys, which enriched at the surface forming a hydrophobic and hydrophilic shell, respectively, however, Gly did not behave in the same manner. Gly molecules (MW=75.1 g/mol) are smaller than Leu (MW=131.2 g/mol) or Lys (MW=146.2 g/mol), therefore it is expected that Gly molecules have a faster diffusion coefficient, diffusing more easily towards the core of the droplet whilst drying, ultimately leading to a uniform distribution of Gly on the particle instead of its enrichment at the surface.

Regarding the PSD, it was verified that under the same SD conditions, the investigated formulations lead to different particle sizes, evidencing the influence of the different AA in the formulation. The results reveal that only the SDIs containing Leu (trial #5, 6 and 9) are suitable for inhalation, exhibiting a $D_{v50} < 5 \mu\text{m}$. The minimum D_{v50} was achieved with the drying conditions of trial #5. Both the addition of Tre (trial #6) and drying at a lower temperature and feed rate (trial #9) resulted in slightly larger particles and wider distributions. While all Leu formulations had monomodal size distributions, at the selected pressure it was not possible to de-agglomerate trial #7 and #8, which exhibit a bimodal distribution. Bimodal distributions are unlikely to be a result of the

nozzle, therefore the observed lack of de-agglomeration is indicative of highly cohesive particles. According to the XPS results (Table 4.1), the material of trial #8 shows that API is at the surface, this can lead to increased cohesiveness [12, 40], as was observed in the PSD analysis. Regarding trial #7, the hydrophilic shell might lead to increased agglomeration and adhesion forces due to high capillarity [109]. Furthermore, it becomes evident that the D_{v50} follows the same trend as the AA hydrophobicity, with the most hydrophobic AA yielding the finer particles and the most hydrophilic yielding the largest. These observations align consistently with the nature of the formed shell, wherein the hydrophobic shell leads to highly dispersible particles, due to reduced surface energy and weakened interparticle forces [40, 51, 109], whilst the hydrophilic shell, characterized by increased capillarity forces [109], exhibits poor dispersibility, corroborated by the bimodal distribution, leading to larger particles sizes.

The aerosol performance results are aligned with the obtained PSD for each trial, and as expected, the FPF is higher for materials with a lower PSD, since the aerodynamic diameter is a function of D_{v50} , particle density, and a shape factor [12, 25]. It is noticeable that formulations containing Leu, having a hydrophobic shell, exhibit the most promising aerosol performance, while the formulation containing Lys, inducing a hydrophilic shell, exhibits the poorer performance, whilst the formulation containing Gly presents intermediate results. Based on the obtained results for the formulation screening tests it might be concluded that the FPF is likely influenced by the presence and hydrophobicity of the AA shell, as the FPF presents a linear trend with AA hydrophobicity, also confirming that the outer layer of the manufactured materials is composed by the AAs, as expected.

Regarding the SDIs containing a hydrophobic Leu shell, these show superior aerosol performance having emitted doses above 92%. Additionally, the influence of drying temperature and feed flow rate is noticeable on the aerodynamic behavior of the particles since there are differences in the aerodynamic performance between the materials from trial #5 and #9. The addition of Tre in trial #6 as glass former and bulking agent [25, 40], results in poorer aerosolization likely due to incomplete API encapsulation. Additionally, the presence of Tre is expected to contribute to denser particles, due to its higher molecular weight, negatively affecting aerosolization. Nonetheless, without a glass stabilizer ITZ can recrystallize over time, altering the SDI aerodynamic performance. Trial #6 outperforms trial #7 and #8, demonstrating that, even an incomplete Leu shell (as demonstrated by the XPS results shown in Table 4.1) enhances SDI aerodynamic properties. Although trial #8 has the lowest ED, it resulted in a higher FPF than the trial containing Lys and only 3% less than the formulation containing Leu and Tre. The lower ED is in accordance with the PSD data, since for this trial a larger D_{v50} was observed. Furthermore, during the PSD analysis, it was not possible to fully deagglomerate the particles, which can be correlated to higher cohesiveness of the material. Therefore, when the particles are properly dispersed, they have better aerodynamic properties than the SDI containing Lys, suggesting that the hydrophilic shell has no benefits in terms of aerosolization enhancement.

2D fluorescence was employed to support XPS data as neither of these techniques is

fully established for the evaluation and confirmation of shell formation. In Figure 4.1 the data extracted from the EEMs is plotted according to the two first principal components (PCs).

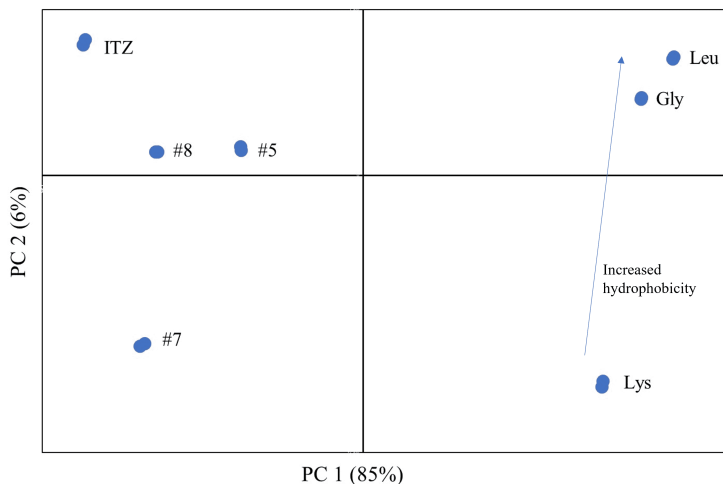


Figure 4.1: Data projection in the principal component space according to the first two PCs

The first and second PCs account for more than 90% of data variability, 85% and 6% respectively, therefore their representation might be used to represent most of the data variability. The PCA effectively segregates the data: single AAs are situated on the right-hand side, while the API and formulations occupy the left-hand side. The unique EEMs of the AAs, exhibiting low fluorescence, starkly differ from the EEMs of the API and formulation, which display a distinctive signal (see Appendix C). This disparity contributes to their distinct separation. Moreover, there is a clear pattern related to AA hydrophobicity, with Lys-containing formulations being on the bottom part of the plot and the AAs being arranged according to their hydrophobicity. The API is individualized in the left top corner, with trial #5 (Leu) positioned the farthest along the PC1 axis. Even though this data is merely qualitative it demonstrates that trial #8 is the closest to the API, with trials with Leu and Lys being more distant, supporting XPS data, where the Gly formulation exhibited API at the surface while the AAs yielded a shell. The data interpretation of EEMs and their deconvolution are not sufficient to confirm the presence of a shell. However, using this technique, it is possible to observe a tendency that corroborates the XPS results, supporting the evidence of a shell. Thus, coupled with XPS, this approach can be applied to the study of shell formation.

To further corroborate the XPS observation regarding shell formation on Leu-containing formulations (trial#5 and #9), trial #5 was analyzed by means of SEM-FIB and SEM-EDS, as depicted in Figure 4.2. Both figures reveal a distinct outer shell and an API core, characterized by the presence of Cl (green dots). Thus, the utilization of the 3FN enables the preparation of encapsulated particles with a ITZ : Leu (1:1) formulation. Simultaneously, it was verified that the shell was melting during analysis (due to the local temperature

increase on the particle surface), thus the use of this technique for shell characterization will need to be further improved.

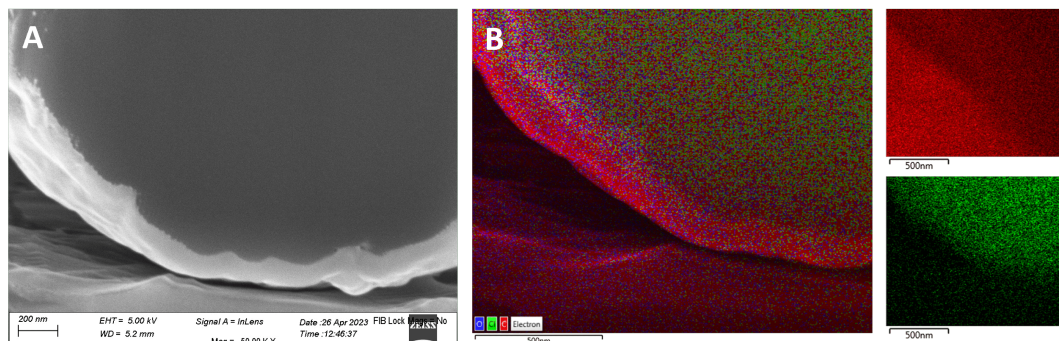


Figure 4.2: SEM analysis of trial #5: A - EDS (green and red colors correspond to Cl and C elements, respectively) and B - SEM micrograph of FIB cross-section of a spherical particle

Upon a comprehensive analysis of all the results, it becomes evident that Leu is the optimal AA for the purpose of encapsulated composite inhalable particles. It was the only AA with suitable D_{v50} , alongside confirmed shell formation supported by different techniques. In this case, the hydrophobic nature of the shell most likely reduces cohesive forces [51], improving dispersibility and yielding an enhanced ED and FPF. Leu's hydrophobicity, surface activity, and apolar character favor its organization and enrichment at the gas-liquid interface [28, 29]. This preferred surface arrangement coupled to Leu limited water solubility results in early precipitation and consequent shell formation at the droplet's surface [28, 51, 52, 65]. Moreover, the hydrophobic shell reduces surface energy and inter-particulate forces, due to surface rugosity/ asperities and reduced capillary forces [28, 40, 54, 110].

4.2 Three-fluid nozzle: Comprehensive study

4.2.1 Exploratory data analysis

To study the impact of the 3FN and SD operating parameters on the particle properties, a DoE was performed, where T_{out} , F_{atom} , and M_{ratio} were considered as the pCPPs. An initial exploratory data analysis (EDA) of the results was conducted to identify and determine the nature of the relationships between process parameters and powder quality attributes. The SDIs were characterized in terms of the PSD, aerosol performance, residual water and solvent content, and surface morphology. Table 4.2 summarizes the results.

Yield and API Load

The process yield under the tested conditions was mostly above 60% w/w. A gradual increase in process yield is expected as the particles adhere to the glass chamber, however, this was not verified. The observed decrease in the process yield was likely due to SDI accumulation in the cyclone, leading to reduced separation efficiency. Regarding the API

4.2. THREE-FLUID NOZZLE: COMPREHENSIVE STUDY

Table 4.2: API load (n=4), particle size (n=3) and aerosolization performance (n=4) results according to the investigated DoE levels. Results are expressed as mean $\pm$ standard deviation.

Trial	T_out (°C)	F_atom (kg/h)	M_ratio (-)	API Load (%w/w)	Particle Size (μm)			Aerosol performance		
					D_{v10}	D_{v50}	D_{v90}	Span	ED(%)	FPF(%)
#10	65	3.0	3.8	50.9 $\pm$ 2.0	0.9 $\pm$ 0.0	2.9 $\pm$ 0.0	7.2 $\pm$ 0.1	2.2	97.1 $\pm$ 4.7	50.2 $\pm$ 3.0
#11	50	3.0	3.8	48.2 $\pm$ 2.5	1.0 $\pm$ 0.0	3.6 $\pm$ 0.0	9.3 $\pm$ 0.1	2.3	95.3 $\pm$ 3.6	50.6 $\pm$ 2.3
#12	65	2.1	3.8	50.0 $\pm$ 2.9	1.0 $\pm$ 0.1	3.5 $\pm$ 0.1	8.5 $\pm$ 0.1	2.1	98.5 $\pm$ 2.5	42.3 $\pm$ 2.9
#13	50	2.1	3.8	52.3 $\pm$ 1.1	1.1 $\pm$ 0.1	3.9 $\pm$ 0.1	10.3 $\pm$ 0.1	2.4	98.3 $\pm$ 1.9	37.7 $\pm$ 5.1
#14	65	3.0	2.8	45.7 $\pm$ 1.1	0.9 $\pm$ 0.0	3.0 $\pm$ 0.0	7.5 $\pm$ 0.1	2.2	98.9 $\pm$ 1.3	53.4 $\pm$ 5.4
#15	50	3.0	2.8	50.4 $\pm$ 2.4	0.9 $\pm$ 0.0	3.0 $\pm$ 0.0	7.9 $\pm$ 0.0	2.3	97.2 $\pm$ 3.6	53.2 $\pm$ 1.2
#16	65	2.1	2.8	51.6 $\pm$ 1.5	1.0 $\pm$ 0.0	3.4 $\pm$ 0.0	8.4 $\pm$ 0.0	2.2	97.0 $\pm$ 2.5	40.7 $\pm$ 5.5
#17	50	2.1	2.8	50.8 $\pm$ 2.1	1.1 $\pm$ 0.0	4.2 $\pm$ 0.1	11.5 $\pm$ 0.2	2.5	95.9 $\pm$ 1.9	47.2 $\pm$ 4.3
#18	57.5	2.5	3.3	53.7 $\pm$ 0.8	0.9 $\pm$ 0.0	3.4 $\pm$ 0.0	10.5 $\pm$ 0.2	2.8	96.0 $\pm$ 4.5	50.0 $\pm$ 3.4

load, it is within a 10% error from the theoretical load for all trials, further ensuring that when the correct pumping ratios are employed the 3FN can produce homogenous SDIs.

Residual water and solvent content

The residual water content was below 0.4% in all trials, a relatively low value. Additionally, the variations in water content among different trials were not significantly different (p-value > 0.05), indicating that the investigated factor levels did not affect the moisture content. Similar behavior was verified for the DCM content, which was below the limit of quantification (30 ppm) of the used method. Therefore, practically no DCM remained in the SDIs, confirming compliance with ICH's limit (600 ppm [111]) and further demonstrating the suitability of the 3FN for preparing dry powders. This notably low residual solvent and water content suggest high solvent evaporation rates and, consequently very rapid drying kinetics. Although a low residual water content is desirable both for aerodynamic performance and stability, such low content can also difficult downstream processing such as capsule or device filling processes. Nonetheless, this can still be optimized by means of process parameter adjustment during both spray drying and downstream processes.

Particle size

The D_{v10} , D_{v50} , D_{v90} and span of the DoE points are reported in Table 4.2. For all the samples the particles are within the inhalable range [9, 24, 29], have monomodal distributions and similar narrow spans. These findings indicate that the 3FN, within the studied design space, is appropriate to prepare composite inhalable particles displaying homogenous size distribution. Given that particle size is a CQA of dry powders, it was analyzed as a response of the DoE. The impact of the different combinations of levels (section 3.2.1), on the D_{v50} is summarized in Figure 4.3.

From Figure 4.3, the two levels of M_ratio seem to have a limited influence on the D_{v50} , except for the trial with T_out on the low level and correspondent F_atom on the high level. Regardless of the outlet temperature, reduced F_atom leads to larger particles, due

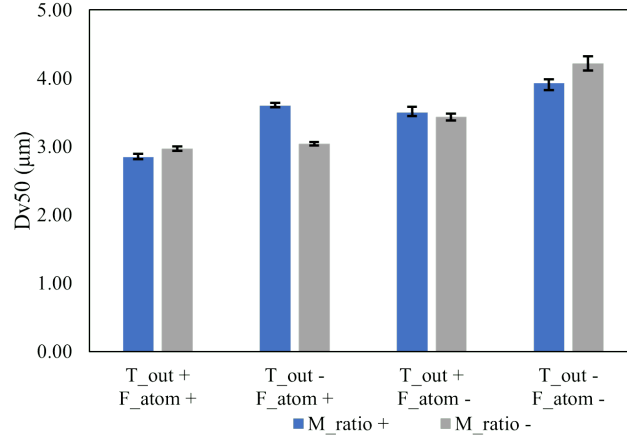


Figure 4.3: D_{v50} across DoE combinations, the high level of the factors is denoted as + and the low level as -.

to the increased droplet size. Similarly, at the same atomization flow rate a lower T_{out} results in bigger particles. Such observation is unexpected as the outlet temperature does not influence particle size directly, however, it can influence the solvent vapor pressure inside the droplet and increase the RS_{out} , having the potential to alter drying kinetics. Furthermore, when the M_{ratio} is on the low level and F_{atom} is on the high level the outlet temperature seems to not affect the D_{v50} parameter, demonstrating that in cases where feed rate is reduced, suitable atomization flow rate can still yield particles with the desired size, without the influence of drying kinetics (T_{out}). It is also noticeable, that the experiments with T_{out} on the low level are more sensitive to the changes in the feed rate, underlining the potential influence of RS_{out} . The most favorable factor combination for achieving a minimal D_{v50} involves having both the outlet temperature and atomization on the positive level.

Particle size is recognized to be influenced by the feed rate and atomization flow rate, as these dictate the amount of liquid entering the chamber and the available energy to disperse the liquid into droplets [12, 29]. The D_{v50} was plotted as a function of F_{atom} and M_{ratio} (as manipulating this variable resulted in different feed rates, described in Section 3.2.1) in Figures 4.4A and 4.4B, respectively, to identify if there are relationships between input and output variables. Additionally, a simple linear regression was fitted to the data.

There is an apparent linear relationship between D_{v50} and F_{atom} , which is reinforced by the central point's placement at an equal distance between the two levels. This pattern is consistent, as increased atomization leads to finer particles, due to the increased energy available to break up the liquid. Regarding the M_{ratio} , the linear relationship is not as clear and there is high variability between the investigated levels. Only F_{atom} exhibits statistically significant differences between the studied levels ($p\text{-value} < 0.05$), further corroborating the observed tendencies and the essential role of the atomization feed on the definition of the particle size.

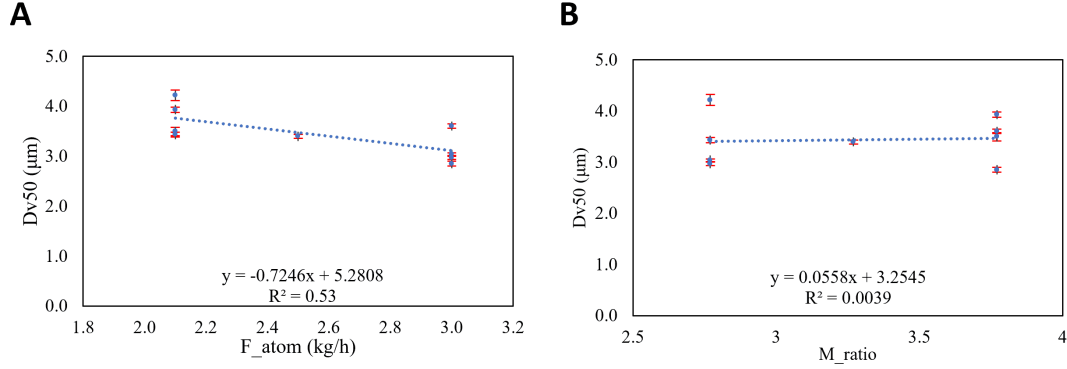


Figure 4.4: Influence of the DoE factors on the D_{v50} response, A - F_{atom} , B - M_{ratio} .

In vitro aerosolization

The aerosol behavior of the SDIs is another quality attribute of paramount importance, as it determines lung deposition, thus the ED and FPF were also analyzed as DoE responses and pCQAs. Beyond atomization flow rate and feed rate (F_{atom} and F_{feed}), these quality attributes are also affected by the RS_{out} , as it influences heat and mass transfer, altering particle shape and density [42]. The obtained FPF and ED from the DoE levels are summarized in Table 4.2. The ED is consistently above 95% across all trials, almost reaching 100%. This high value indicates that most of the SDI effectively exits the capsule and the device, highlighting the enhanced dispersibility and low cohesion forces of this formulation, which are pivotal for a successful dry powder formulation. In terms of the FPF the highest value is approximately 53% and the lowest 38%. These are reasonable values; however, they could be further optimized to yield maximum lung deposition. A summary of the DoE results for the ED is displayed in Figure 4.5

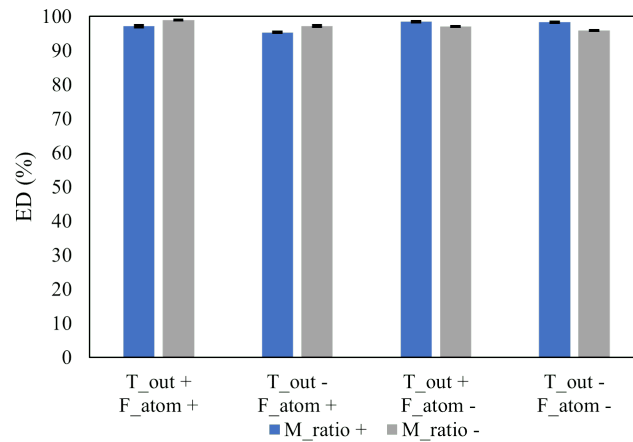


Figure 4.5: ED across DoE combinations, the high level of the factors is denoted as + and the low level as -.

Analyzing the chart reveals that the ED remains unaffected by the SD conditions, with neither of the factors exhibiting statistical significance ($p\text{-value} > 0.05$). Regardless, it was plotted against F_{atom} , M_{ratio} , and RS_{out} , in Figures 4.6A,B and C, respectively.

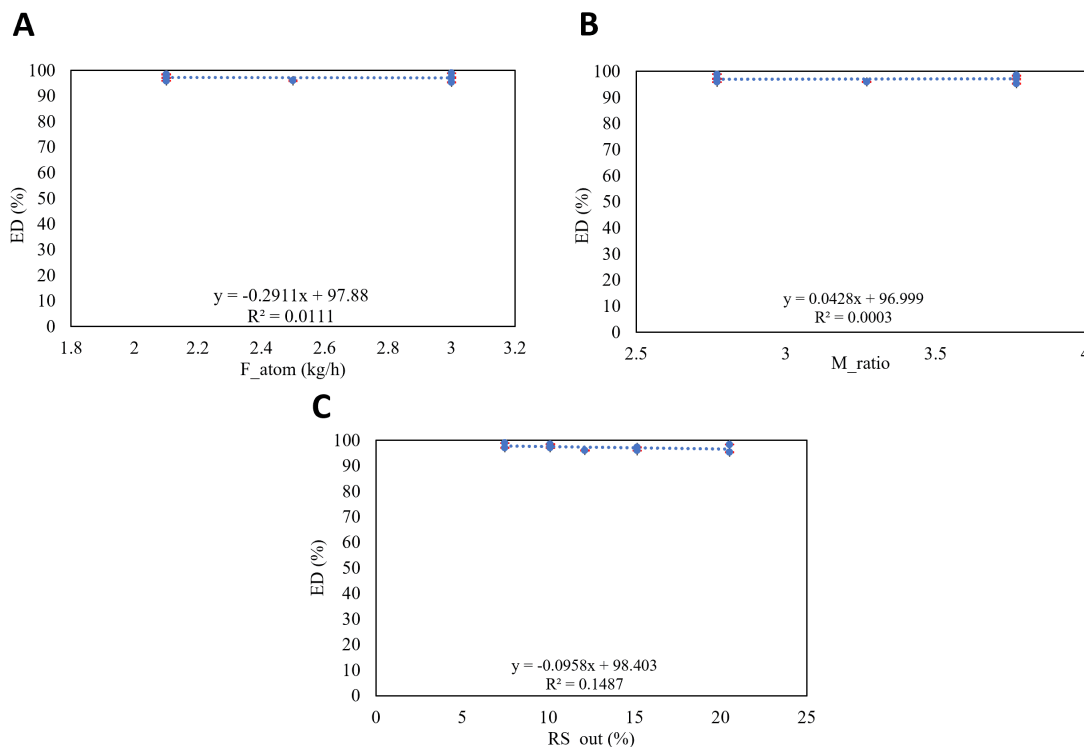


Figure 4.6: Influence of the DoE factors on the ED response, A - F_{atom} , B - M_{ratio} , C - RS_{out} .

In the plots, the ED resembles a straight line across the factor levels, further confirming that it is minimally influenced by the operating parameters. Consequently, these results suggest that the ED is mostly influenced by the formulation chosen for this work, whereby the high mass fraction of Leu, increases significantly SDI dispersibility, potentially masking any possible effects linked to the SD conditions. The increased dispersibility is attributed to the formation of the hydrophobic shell and its reduced surface energy, as discussed previously in section 4.1.

Regarding the FPF, the DoE results are summarized in Figure 4.7.

Similarly to the observations made for D_{v50} , limited differences were detected among the tested levels of M_{ratio} ($p\text{-value} > 0.05$). The only exception is the worst-case scenario, characterized by both the outlet temperature and atomization flow rate being on the low level. Under these conditions, an increased feed adversely affected the FPF. This adverse effect stems from the increased liquid volume, that led to the formation of larger droplets, which were simultaneously subject to a slower drying process due to the elevated solvent content in drying gas ($RS_{\text{out}} = 20.5\%$). Furthermore, when the atomization flow rate was set to the higher level, the impact of variations in T_{out} and M_{ratio} on the FPF is

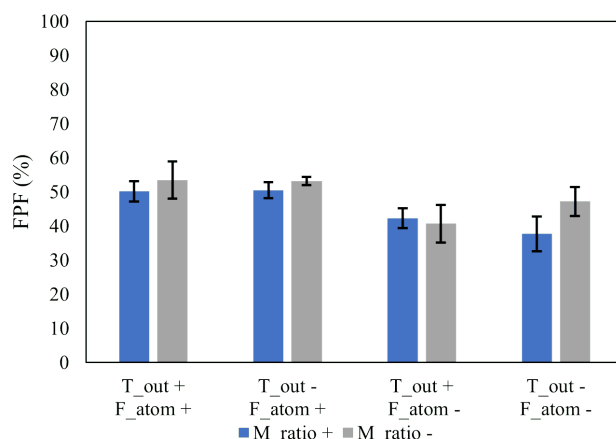


Figure 4.7: FPF across DoE combinations, the high level of the factors is denoted as + and the low level as -.

minimal, with the SDIs exhibiting a FPF in the range of 50% to 53%. Conversely, when the atomization flow rate is reduced, the influence of T_{out} on the FPF starts to become perceptible. These observations indicate that maintaining the F_{atom} at an optimal level, thereby achieving a specific droplet size distribution, is sufficient to effectively disperse even the higher liquid feed rates. Moreover, it appears that the acquired droplet size distribution exerts a more significant influence on the FPF than the drying kinetics (T_{out}). In parallel with observations regarding the D_{v50} , F_{atom} is the sole factor displaying statistical significance (p -value < 0.05), emphasizing the pivotal role of the atomization flow rate. The combination of T_{out} and F_{atom} on the high level with M_{ratio} on the low level yields the maximum FPF, appearing to be the most suitable conditions for SDI preparation. The FPF was plotted as a function of F_{atom} , M_{ratio} and RS_{out} , Figure 4.8A,B and C.

As anticipated, the FPF shows a discernable linear relationship with F_{atom} , further supported by the placement of the central point. Additionally, as expected, the FPF follows the same trend as the D_{v50} , with increased atomization resulting in finer particles and consequently higher FPF values. Regarding the M_{ratio} , the linear pattern is not so visible, but it is present. Lastly, RS_{out} does not seem to influence aerosolization in a clear manner, as it accounts for both the effect of temperature and feeding rate, which were not significant.

Surface Morphology

SEM micrographs of ITZ, Leu and four of the DoE tests are displayed in Figure 4.9. Overall, the particles from the DoE resulted in very similar morphologies thus only material from trial #10, 13, 14 and 17 are pictured as they are the best (F_{atom} and T_{out} on the high level) and worst (F_{atom} and T_{out} on the low level) cases for each M_{ratio} tested. SEM micrographs of the remaining trials can be found in Appendix D.

The spray-dried API leads to smooth spherical particles. Conversely, Leu is observed to be in a flake-like form, suggesting that under the SD conditions applied the particles burst.

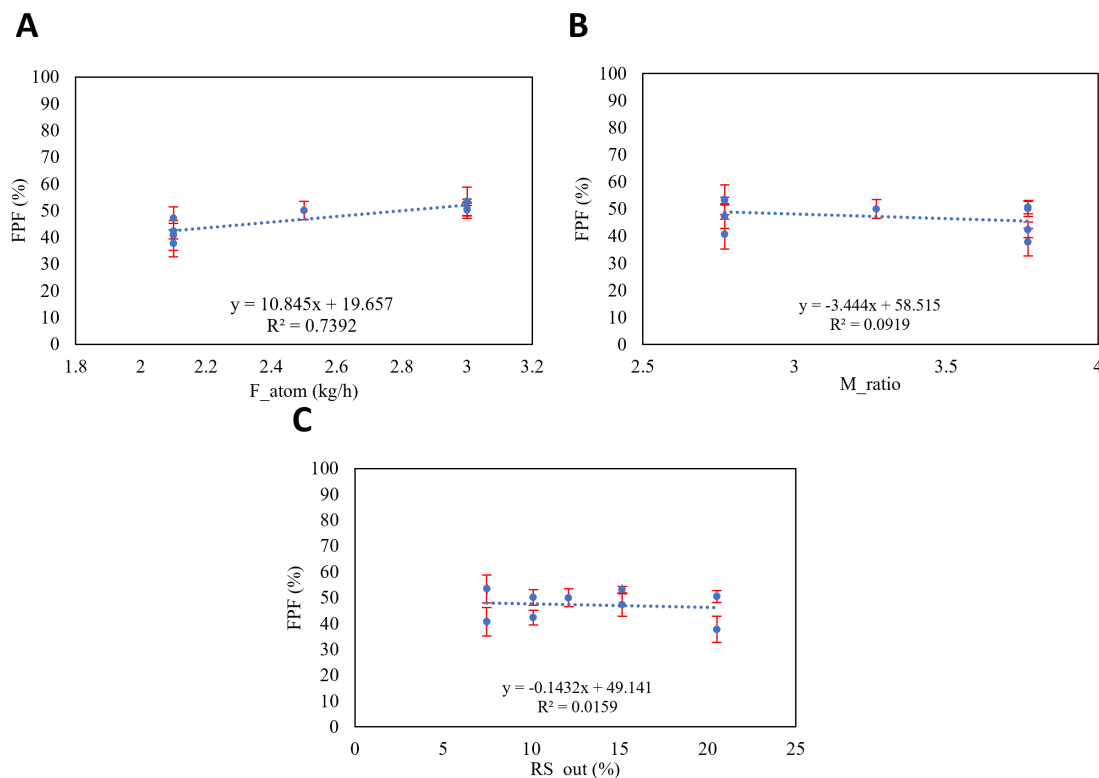


Figure 4.8: Influence of the DoE factors on the FPF response, A - F_atom, B - M_ratio, C - RS_out.

Burst particles are a result of drying kinetics, which can be influenced by temperature and the solvent system. Regarding the DoE trials (Figure 4.9 C to F), composed of 50% ITZ and 50% Leu, they are very similar, exhibiting fragments of broken particles, Leu flakes, larger particles with raisin-like surfaces, and smaller, smooth spheres. SEM micrographs 4.9C to 4.9F suggest that the SD conditions coupled with Leu's fast shell formation led to burst particles. Leu, characterized by its poor water solubility and surface activity, prompts the early development of a thin shell on the droplet's surface. However, the combination of high drying temperatures, and the use of a solvent system comprising both water and DCM, which only mix under high shear conditions and possess highly distinct vapor pressures, can result in an increased solvent evaporation rate due to DCM's high volatility. This causes a pressure gradient inside and outside the droplet, changing its morphology and ultimately resulting in burst particles. Therefore, rapid shell formation can entrap solvent vapors inside the droplet, and if the shell is too thin and cannot withstand the increasing solvent pressure, the particles will rupture. The presence of burst particles further supports the observed minimal solvent/water content, as barely any residual solvents remained entrapped in the particles. Regardless, considering the differences observed in the spheres' surface morphology, between trial #1 (API alone) and trial #10,13,14 and 17 (API with Leu) the surface rugosity is indicative of Leu shell

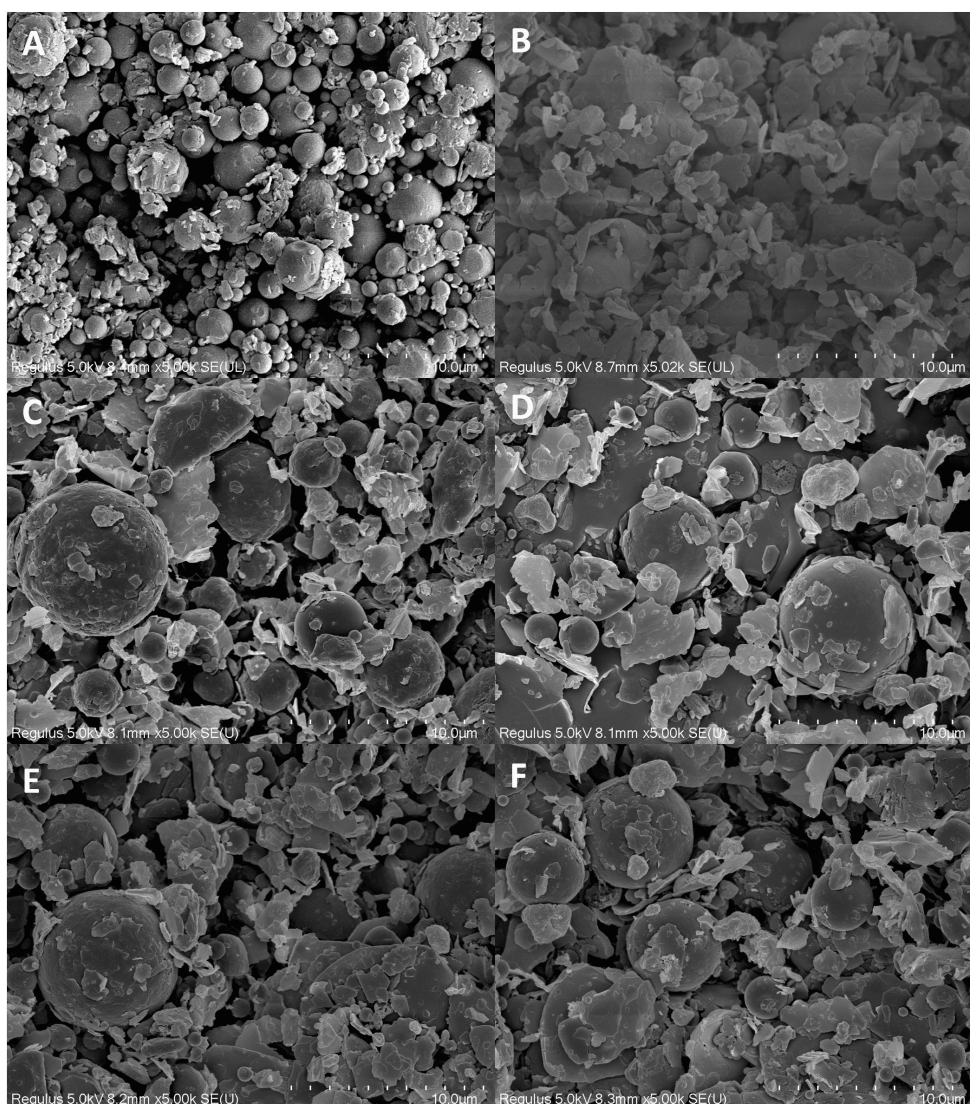


Figure 4.9: SEM micrographs of the prepared SDI at a x5000 magnification, A - trial #1 ITZ, B - trial #2 Leu, C - trial #10, D - trial #13, E - trial #14 and F - trial #17

formation. The relatively smaller and smooth spheres are proposed to be solely API that was not encapsulated. Overall, an examination of particle morphologies and sizes across the different DoE levels indicates that the different combinations of factors do not significantly affect the surface morphology, yielding similar SDIs.

To address the formation of broken particles, formulation optimization strategies can be implemented. For instance, the addition of an excipient with the potential to increase particle mechanical resistance, potentially calcium chloride, could reduce the likelihood of particle rupture. Alternatively, further optimization of SD conditions, namely increasing the RS_{out} to induce slower drying kinetics, could be explored by means of increasing the feed flow rate or transitioning into a closed-loop system.

4.2.2 Empirical modeling

Insights from the EDA in section 4.2.1 enabled the identification of the nature of the relationships between pCQAs such as D_{v50} and FPF, with pCPPs, enabling the identification of the adequate mathematical relationships that help explain those effects and variability [89]. In this case, it appears through the EDA that linear dependencies govern the observed variability of the pCQAs with the pCPPs. Another primary finding of the EDA was that the ED appears to be independent of SD conditions, attributed solely to the formulation properties. Empirical models based on the DoE were developed for these responses as a tool to optimize SD coupled with a 3FN and obtain process knowledge on how SD factors influence the output variables.

Before developing the models, the data was checked for outliers, applying a multi-variate control chart. This method assesses outliers by plotting the Hotelling's T2 statistic distribution for an α level of 5%, for the determined upper control limit (UCL), shown in Appendix E. This approach did not identify any outliers, thus all data points were included in the model.

The data set comprises 8 observations, a small set but with a suitable range for the studied variables, based on the performed DoE. Additionally, it is essential to acknowledge that the validity of models derived from this data set is constrained by the specific ranges of variables included, as these ranges delineate the design space within which the models hold applicability.

4.2.2.1 Modeling strategy

The development of the regression models considered DoE factors as predictors, as these are the process pCPPs. Additionally, second-order interactions between input variables were also included, so that intrinsic relationships between variables and their influence on the output could be inferred. Thus, ensuring the model thoroughly explains the response variables.

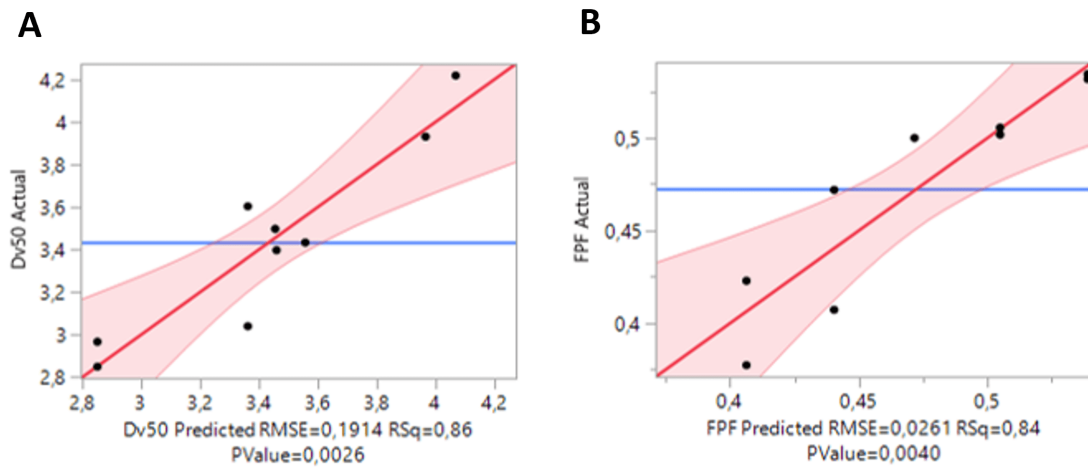
The models' mathematical functions were established by least squares regression, with stepwise variable selection by minimization of the AIC as a stop criterion. Even though the ED was not significantly affected by SD conditions, model development was still attempted to confirm the EDA observations. In this case, the AIC criterion could not be minimized with the SD factors tested range, further supporting the assumption that the ED is governed by the formulation. The resulting model terms, quality of fit, and VIFs are displayed in Table 4.3. The models were developed with F_atom at a different unit (mm), thus a conversion factor ¹ must be applied to determine F_atom in kg/h.

Figure 4.10 illustrates the observed outputs as a function of the predicted values, for both models. D_{v50} can be modeled as a function of atomization flow rate and outlet temperature, with atomization having the most significance (p-value < 0.05), aligning with the observation from the EDA. Both T_out and F_atom have a positive effect in minimizing

¹F_atom (kg/h) = 0.0789 F_atom (mm) – 1.791

Table 4.3: Empirical modeling results: Model expression, fit and parameters' VIFs

Output variable	Model	AIC	RMSE	Fit		Multicollinearity	
				R^2	R^2_{adj}	Term	VIF
D_{v50}	$9.294 - 0.034 T_{out} - 0.071 F_{atom}$	10.1	0.19	0.86	0.82	T_{out}	1.0
						F_{atom}	1.0
						F_{atom}	1.0
FPF	$0.042 + 0.010 F_{atom} - 0.034 M_{ratio}$	-25.7	0.03	0.84	0.79	F_{atom}	1.0
						M_{ratio}	1.0

Figure 4.10: Actual vs Predicted plots for the developed models, A - D_{v50} and B - FPF.

particle size. Additionally, the R^2 and R^2_{adj} of approximately 0.80 reflect a suitable model fitness to the data. This can also be visualized in Figure 4.10A, with most points being near the perfect fit, suggested by the diagonal line. Furthermore, both predictors present a VIF of 1 indicating a total absence of collinearity [112]. The inclusion of T_{out} in the model was not expected as temperature is not estimated to influence droplet size directly. However, it is hypothesized that T_{out} might have a potential impact on D_{v50} by influencing the solvent vapor pressure inside the droplet and/or shell which in extreme cases might lead to shredded particles as observed in Figure 4.9C-F, thus reducing the measured D_{v50} . As seen in Table 4.3, the M_{ratio} was not considered for the model explaining D_{v50} variability, which is unexpected, as it is well known that feed flow rate influences particle size, as greater amounts of liquid will lead to larger droplets if atomization flow rate remains constant. Such observation was not verified in the EDA nor in the model. This can be attributed to the narrow variation range for M_{ratio} . Additionally, Leu's surface activity decreases the surface tension of the liquid sheet, thus reducing the resistance to droplet formation and narrowing the possibility to enlarge the tested range for droplet formation at this scale. Possibly, also the used C_{feed} , as typically for SD process for manufacturing of inhalable products might have prevented testing a significant variability on surface tension.

The FPF model encompassed F_{atom} and M_{ratio} as predictor variables, consistent with the EDA observations. F_{atom} has a positive influence on the FPF, as it is associated with a decrease in particle size, and thus a lower D_{ae} . On the other hand, M_{ratio} has a negative influence, i.e., the higher the amount of solution in the drying chamber the slower will be the drying kinetic for the same drying gas flow rate and temperature profile, as the drying gas gets more saturated with solvent, reducing the driving force for heat and mass transfer. Such effects can influence particle density and morphology, consequently altering aerosolization. The model resulted in an R^2 and R^2_{adj} of 0.84 and 0.79, respectively, both indicative of a robust fit of the model to the experimental data, which can also be visually assessed by Figure 4.10B. Furthermore, there is no collinearity between the selected predictors ($VIF = 1$). For this model T_{out} was not included, emphasizing that it does not describe any additional information in terms of the FPF variability.

The predictor variables were not subject to normalization, rendering a direct comparison between regression coefficients unfeasible. Nonetheless, considering the nature of the studied values, wherein T_{out} spans from 50 to 65 °C and F_{atom} ranges from 50 to 60mm (equivalent 2.1 to 3kg/h), both of which are within a similar order of magnitude, it becomes evident that in the D_{v50} model, that F_{atom} exerts the most impact on altering the particle size, as indicated by its regression coefficient being higher than T_{out} 's. Regarding the FPF, it's important to note that the variables display different orders of magnitude, with M_{ratio} ranging from 2.8 to 3.8. Therefore, despite the lower numerical value of the regression coefficient associated with F_{atom} compared to that of M_{ratio} , F_{atom} 's influence on the FPF is more pronounced.

Additionally, it's important to highlight a limitation of these models, specifically concerning their prediction when the predictor variables are set to zero. In such cases, the predicted D_{v50} and FPF values are 9.3 μm and 4.2%, respectively. Evidently, these values lack physical meaning and are a consequence of the model's adjustment to the empirical data. In both models, there was an absence of second-order interactions, suggesting that the optimization of the SD process does not demand the simultaneous manipulation of two or more variables, simplifying the optimization process.

To assess the statistical significance of the developed models they were subject to an ANOVA, the results are summarized in Table 4.4.

Table 4.4: ANOVA results of the developed models.

Model	Sum of Squares			Degrees of Freedom			Mean Squares		$p - value$	F0	
	Model	Error	Total	Model	Error	Total	Model	Error		F-ratio	F_{crit}
D_{v50}	1.39	0.22	1.61	2	6	8	0.694	0.037	0.003	18.9	5.1
FPF	0.02	0.00	0.03	2	6	8	0.011	0.001	0.004	15.9	5.1

Considering a significance level of 5%, the ANOVA reveals that both models are statistically significant in explaining the observed variation on the output variables. This is evident from the F-ratio being higher than the F_{crit} and the p -value < 0.05 [89]. To ensure that the least squares regression approach is suitable for the data set, it is crucial

to analyze the model residuals. In Appendix E, the residuals of both models were plotted against the predicted values and the order of observation. These plots show no distinct patterns, with the points being randomly distributed with zero mean. These observations suggest that residuals have a zero average with constant variance [89, 102]. Additionally, the random distribution of residuals is evidence that they are independent of the order in which the experiments were performed. The distribution of residuals also resembles a normal distribution. Therefore, this approach is suitable for the models as the assumptions for the least regression were met based on the residual analysis.

4.2.2.2 Model validation

The predictive capacity and accuracy of the models can be evaluated by performing an external validation, whereby a new independent data set is compared to the model's predictions. This validation data set comprised two points (trial #19 and #20), that were randomly defined and executed within the established design space. An additional point was tested with T_{out} and M_{ratio} above their upper levels of the DoE (trial #21). In this trial, the F_{atom} remained within the tested limits since decreasing would result in a wet drying chamber and increasing would not be possible due to the limitations of the equipment ($F_{atom} \leq 3.0$ kg/h). The results from the validation data set, regarding particle size and aerosol performance are presented in table 4.5.

Table 4.5: Particle size distribution (n=3) and aerosol performance (n=3) for the validation data set. Results are expressed as mean $\pm$ standard deviation.

Trial	T_{out} (°C)	F_{atom} (kg/h)	M_{ratio} (-)	Particle Size (μm)			Aerosol performance		
				D_{v10}	D_{v50}	D_{v90}	Span	ED (%)	FPF(%)
#19	54	3.0	3.0	0.8 ± 0.0	2.9 ± 0.1	8.4 ± 0.1	2.6	100.0 ± 0.0	50.2 ± 0.9
#20	62	2.5	3.5	0.9 ± 0.0	3.7 ± 0.0	10.1 ± 0.1	2.5	98.2 ± 2.0	51.4 ± 1.4
#21	75	3.0	4.5	0.9 ± 0.0	3.4 ± 0.1	8.8 ± 0.1	2.3	99.6 ± 0.4	49.7 ± 5.4

These trials displayed yields between 65-80%, with API load near the theoretical value and a moisture content of 0.3%, being similar to the remaining tests. To visualize how these results fit within the observed tendencies in the EDA, they were plotted against the F_{atom} and M_{ratio} , Figure 4.11.

As it can be observed, for both D_{v50} and FPF, the external validation points lie within the previously observed range. For the M_{ratio} , the point outside the design is in accordance with the trend line, plotted for the DoE points.

Additionally, parity plots including the validation data for each model are in Figure 4.12. As can be observed in Figure 4.12, the external validation data set fits well within the predictions of both models, with the relative error between prediction and actual values being below 10% for the D_{v50} and FPF (Appendix E). Testing input variables outside of a model's defined range is not advised. However, the FPF model was demonstrated to accurately predict the point outside of the defined limits, maintaining an error below 10%.

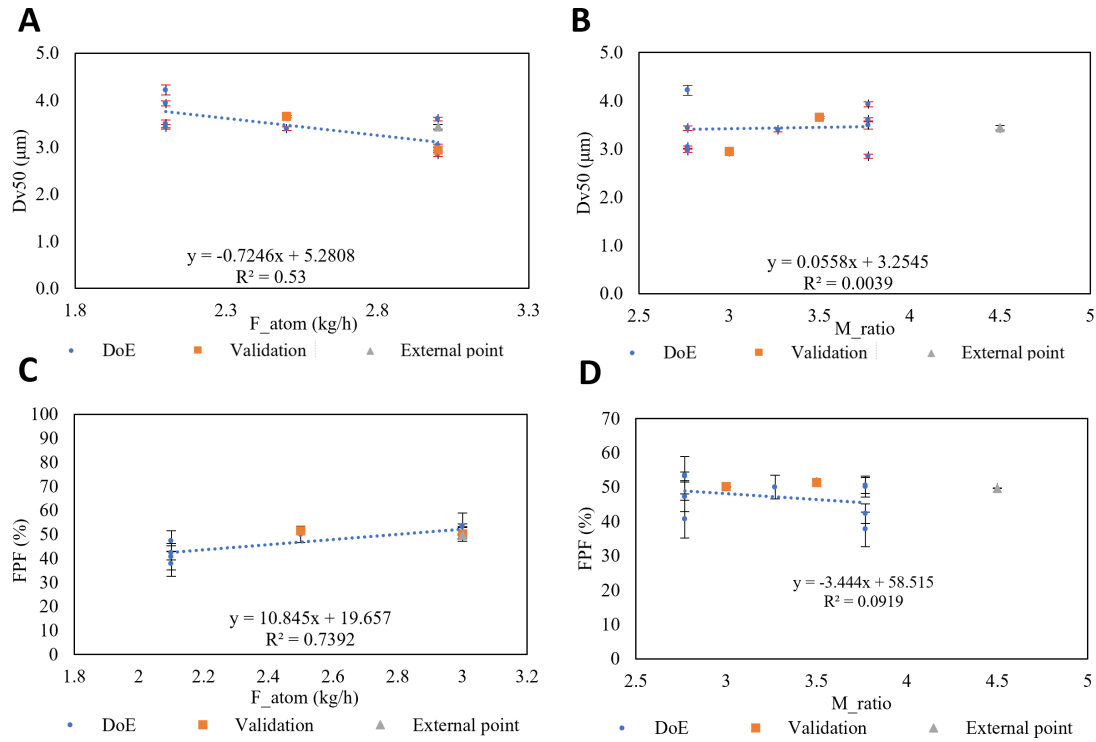


Figure 4.11: Observed tendencies for the validation dataset, A - D_{v50} as a function of F_{atom} , B- D_{v50} as a function of M_{ratio} , C - FPF as a function of F_{atom} and D - FPF as a function of M_{ratio} .

Therefore, the FPF models' prediction capacity seems to be robust, nevertheless, any use of the model outside of the validated range is not advised. Conversely, the D_{v50} model did not accurately predict the external point with conditions outside of the model range, as it is greatly influenced by the temperature. This is due to the incongruence between the model's expected size reduction with temperature increase, swaying the model to predict a smaller particle size, when in fact the contribution of temperature for D_{v50} is expected to be lower than that predicted by the model. Considering the external validation results and the tested conditions outside of the model ranges, the built empirical models explaining D_{v50} and FPF can be considered reliable, showing good predictive power within the studied design space.

The residuals analysis of the validation tests (Appendix E) confirmed they are randomly distributed with zero mean, indicating they have constant variance. These results confirm model's suitability for the prediction of the results from the external validation dataset.

4.2.2.3 Sensitivity analysis

Sensitivity analysis aims at understanding how a reference point can be disturbed by variations in the operating conditions, thus identifying input variables with the most effect on the output as per the developed models. The central point of the DoE was

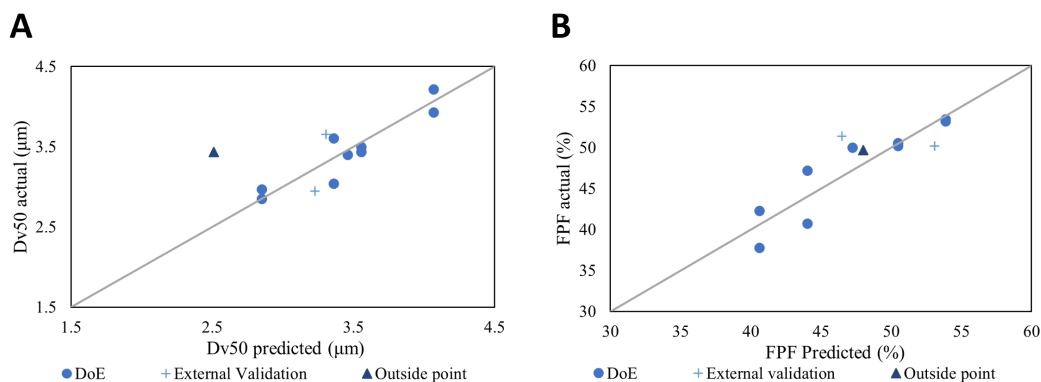


Figure 4.12: Actual vs Predicted plots for the developed models including the validation data set, A - D_{v50} and B - FPF.

selected as the reference state, and operating conditions were varied within the limits of the design space. The output variables were normalized by dividing the predicted value by the maximum predicted value so that the influence of the factors was comparable. In Figure 4.13 are summarized the sensitivity plots of each model as a function of the investigated operating conditions. Of the three predictor variables, F_{atom} has the highest impact on the output variables, influencing both to a similar extent. This is expected as atomization plays a pivotal role in the definition of the D_{v50} , which in turn directly influences the D_{ae} and consequently the FPF. The M_{ratio} has a discernible effect on the FPF, notwithstanding to a lesser extent than F_{atom} , aligning with the trends observed in the EDA. Lastly, T_{out} impacts the D_{v50} in a similar manner to F_{atom} , however, this impact is not expected, as has been discussed.

Overall, the models provide insight into the optimization of the SD process to finely tailor powder properties, particularly concerning API encapsulation with a 3FN. They accurately translated the tendencies that were identified in the EDA and enabled the identification of the actual CPPs for the investigated CQAs. Furthermore, the developed models reinforce that the optimal process conditions, that yield the lowest D_{v50} and highest FPF, require having F_{atom} and T_{out} on the high level (3.0 kg/h and 65°C, respectively) and M_{ratio} on the low level (2.8), corresponding to trial #14. F_{atom} was found to be a key predictor variable in explaining the variation of D_{v50} and FPF. No second-order interactions were included in the stepwise selection, demonstrating that the pivotal role of atomization coupled with the single influences of the other factors are enough to explain data variability. Validation further demonstrated model validity, especially for the FPF model, which is more reliable than the D_{v50} model. To further improve these models it is proposed to expand the DoE, by developing a central composite design with broader factor range. Additionally, the study of the ratio between the atomizing gas flow rate and liquid flow rate (R_{atom}) as a pCPP could be interesting as this factor reflects, in a scale-independent way, the droplet formation process. Such efforts would add useful

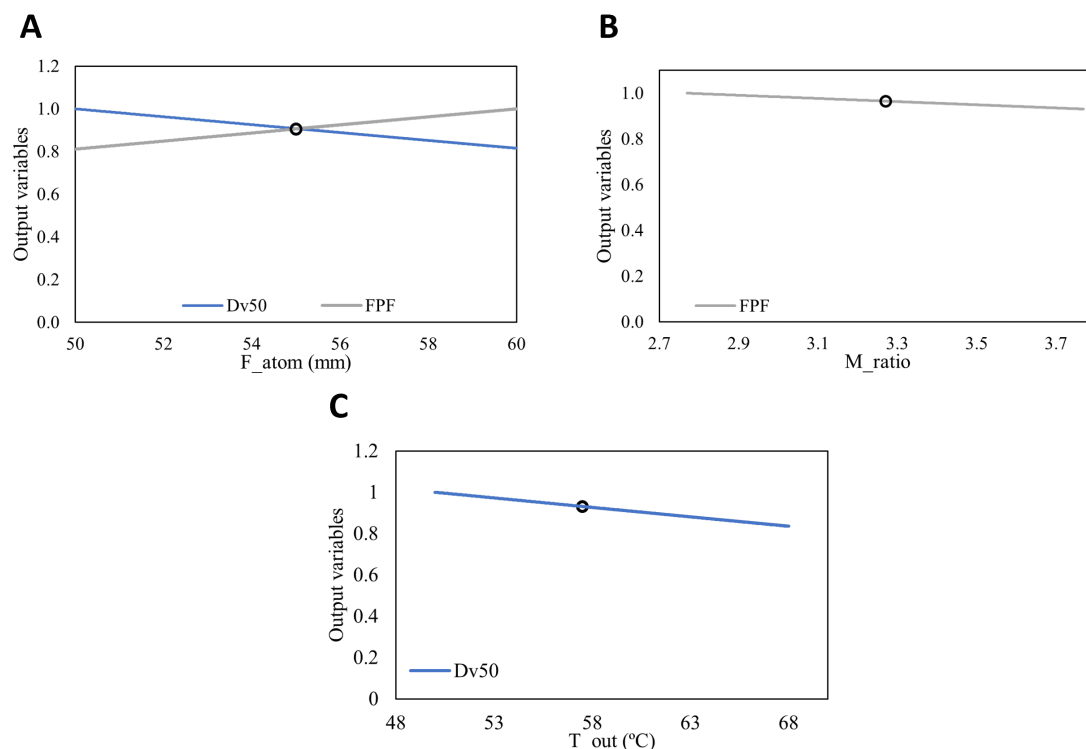


Figure 4.13: Sensitivity analysis plots of the developed models for the operating conditions A - F_{atom} , B - M_{ratio} , C - T_{out} .

information to the model, enabling the investigation of potential second-order interactions or curvatures in the output data, rendering a more robust and broader applicable model.

4.2.3 Proof of concept: poorly water-soluble API

To assess the adaptability of this methodology for encapsulating other poorly soluble APIs, the same formulation was prepared, using RIF as model API (trial #25), under the optimal DoE conditions (trial #14). RIF is an antibiotic used to treat tuberculosis [113], with water solubility of 2.5mg/ml at 25°C [114], significantly more soluble than the previously studied ITZ (1 ng/ml at pH 7 [69, 97]). The particle characterization results of the RIF formulation are reported in Table 4.6. SEM micrographs of the particles are displayed in Figure 4.14

This trial resulted in a yield of approximately 80% and the target API load. In terms of characterization, the particles with RIF exhibit a D_{v50} within the inhalable range, which was significantly lower than the results obtained with ITZ (approximately 40% decrease), despite being prepared using similar spray drying conditions. Even the obtained D_{v90} is $< 5 \mu\text{m}$, being suitable to be deposited in the lungs, which was not observed for the ITZ trials. This difference can be attributed to the alteration in the organic solvent composition. For RIF particles, the organic phase consisted of 80% DCM and 20% Methanol (instead

Table 4.6: API load (n = 4), particle size (n =3) and aerosol performance (n =3) of the formulation with RIF as the API and comparison with ITZ-based formulation. Results are expressed as mean $\pm$ standard deviation.

Trial	API Load (%w/w)	Particle Size (μm)				Aerosol performance	
		D_{v10}	D_{v50}	D_{v90}	Span	ED (%)	FPF(%)
#14	45.7 $\pm$ 1.1	0.9 $\pm$ 0.0	3.0 $\pm$ 0.0	7.5 $\pm$ 0.1	2.2	98.9 $\pm$ 1.3	53.4 $\pm$ 5.4
#25	45.3 $\pm$ 1.1	0.8 $\pm$ 0.0	1.8 $\pm$ 0.0	4.4 $\pm$ 0.0	2.0	98.5 $\pm$ 1.4	78.1 $\pm$ 2.2

of 100% DCM), thus altering droplet size, which depends on solvent properties. In this particular situation, methanol's surface tension (22.7 mN/m [115, 116]) and density (0.79 g/cm³ [115]) are lower than DCM's (26.5 mN/m [116] and 1.3 g/cm³ [117]), leading to the formation of smaller droplets [118] and consequently smaller particles. This observation reveals the critical role of solvent selection, which in this case had a significant impact on particle size.

A direct consequence of the reduced particle size is the enhanced aerodynamic behavior, with the material of this trial displaying an FPF of 78%, approximately a 46% increase from the ITZ formulation. Furthermore, the high ED indicates that the SDI remained highly dispersible despite the low PSD.

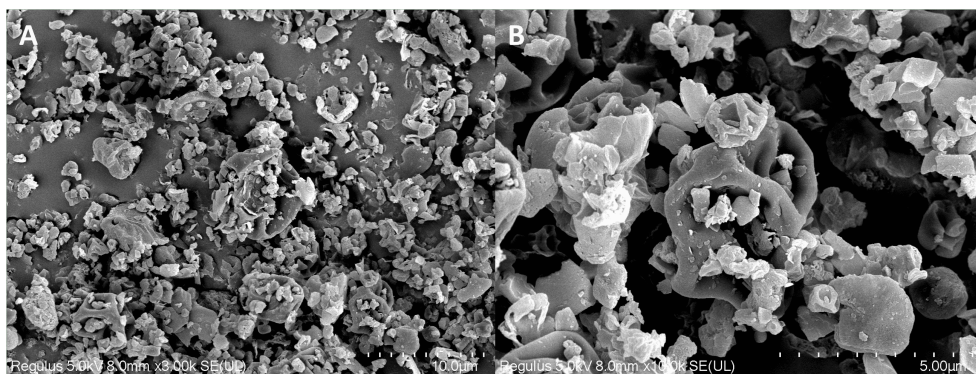


Figure 4.14: Particle surface morphology of RIF composite particles with Leu as SEM micrographs, A - 3000x magnification and B - 10000x magnification

SEM images reveal distinct particle types, burst particles, raisin-like particles, and the previously observed Leu flakes. Notably, the observed morphology differs from that observed in the ITZ trials (illustrated in Figure 4.9). This morphology variation can be attributed to the different model API and/or the utilized solvent system. Considering the molecular weight of RIF (822.9 g/mol [113]), which is roughly the same as that of ITZ (705.6g/mol [119]), it can be inferred that both molecules possess comparable diffusion coefficients. Consequently, it is expected similar and comparable behavior during droplet drying. On the other hand, the introduction of methanol, characterized by its reduced volatility compared to DCM (vapor pressure of 12.9 kPa at 20°C [115] vs. 47.4 kPa at 20°C for DCM [117]) that was used for ITZ trials, likely leads to a lower evaporation rate.

Hence, more time is available for shell formation, ultimately yielding particles with highly corrugated surfaces.

Given the absence of distinctive elements in RIF's molecular compositions that would facilitate its differentiation from the AAs, XPS analysis was not performed. To definitively confirm shell formation a more thorough and quantitative XPS analysis would be required. Additionally, SEM-FIB could potentially serve as an alternative method, as the observed shades of grey change according to material density, possibly enabling the distinction between materials. Nevertheless, efforts should be directed toward the establishment of more robust methods for confirming shell formation in this type of materials.

4.2.4 Proof of concept: hydrophobic amino acid shell

In the screening tests, section 4.1, it was verified that a hydrophobic Leu shell (trial #5 and #9) yields highly dispersible SDIs with enhanced aerosol performance, whereas a hydrophilic Lys shell (trial #7) does not contribute positively to the FPF. Thus, it becomes crucial to ascertain if the favorable aerodynamic properties (ED and FPF) are primarily attributed to the formation of a hydrophobic shell, expected to decrease surface energy, cohesive forces and moisture sorption [24, 54, 110, 120], or if they are inherently linked to Leu's properties and its drying kinetics during droplet drying.

To address this hypothesis, an experiment was conducted with Val (trial#24), an example of another hydrophobic AA, in place of Leu. In this trial, Valine was used to encapsulate the API under the exact same processing conditions utilized on the optimal DoE point (trial#14). Val is slightly less hydrophobic than Leu [105], exhibiting a water solubility of 88.5 g/L at 25°C [121]. This difference in solubility is anticipated to delay shell formation during droplet drying, as it will take longer for Val to reach supersaturation and precipitate. The formulation was kept with 50% of AA since Val's molar weight is similar to Leu's (117.5 g/mol [121] vs. 131.2 g/mol [98] for Leu), thus maintaining a similar molar ratio to the API. The resultant SDI properties are summarized in Table 4.7, while SEM micrographs and SEM-EDS are in Figure 4.15.

Table 4.7: API load (n = 4), particle size (n =3) and aerosol performance (n =3) of the formulation with Val as a shell former and comparison with Leu-based formulation. Results are expressed as mean $\pm$ standard deviation.

Trial	AA	API Load (%w/w)	Particle Size (μm)				Aerosol Performance	
			D_{v10}	D_{v50}	D_{v90}	Span	ED (%)	FPF(%)
#14	Leu	45.7 $\pm$ 1.1	0.9 $\pm$ 0.0	3.0 $\pm$ 0.0	7.5 $\pm$ 0.1	2.2	98.9 $\pm$ 1.3	53.4 $\pm$ 5.4
#24	Val	53.7 $\pm$ 1.6	0.8 $\pm$ 0.0	3.1 $\pm$ 0.0	7.3 $\pm$ 0.1	2.1	93.4 $\pm$ 1.8	30.4 $\pm$ 2.8

From the SEM analysis, two types of particles were observed: spherical and burst particles. To confirm the composition of the particles, elemental analysis was also performed (EDS). From this analysis, shown in Figure 4.15C, it can be seen that the Cl element is well distributed in the spherical particles which are attributed to the presence of API, however

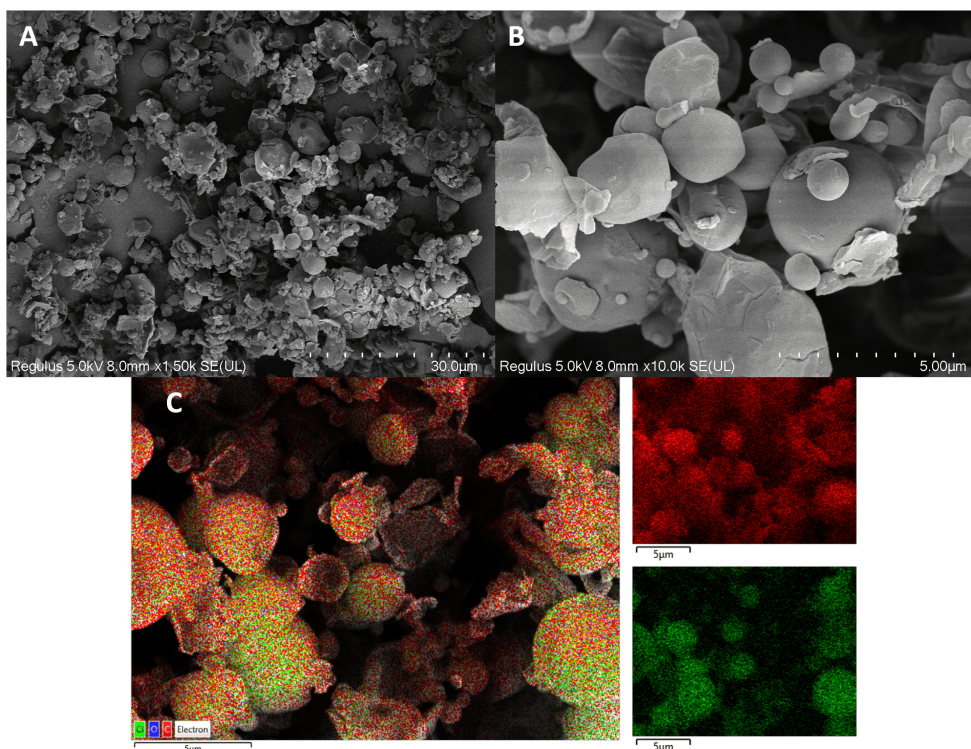


Figure 4.15: SEM micrographs of the SDIs containing Val as a shell former, A - 1500x magnification, B - 10000x magnification and C - SEM-EDS.

for burst particles, Cl was not detected which can be indicative of the presence of Val-rich particles. These results suggest that Val's drying kinetics are slower than Leu's, resulting in a delay of shell formation of the AA and eventually leading to a lack of shell formation.

Particles prepared with Val maintained the same PSD as the ones prepared with Leu, as they were prepared under identical conditions, with the same solvent system and solid concentration, thus droplet size on the 3FN was kept unchanged. Although having equivalent PSD results, particles prepared with Val and Leu exhibit slightly distinct FPF, with Val composite particles displaying a 43% decrease in FPF.

Aerosolized material density, namely bulk density and true density are material quality attributes that are indicated to influence D_{ae} [25] and consequently FPF, apart from others, such as D_{v50} as discussed previously. The surface morphology, as indicated by the SEM images, differs from prior observations. In this case, the spheres are mostly smooth, resembling API particles. Smooth API particles exhibit increased contact area, resulting in heightened adhesion forces between individual particles, consequently hindering aerosolization [28], similar to what was observed with Gly in section 4.1. Therefore, the lack of API encapsulation, supported by the SEM-EDS, further corroborates the poorer aerodynamic behavior.

Shell formation and particle morphology are greatly influenced by drying kinetics [40, 52]. Leu tends to recrystallize during SD, which coupled with its limited water solubility is associated with early precipitation during droplet drying, culminating in the

formation of a solid crust, that encapsulates the material inside [40, 51, 52, 65]. Moreover, Leu's concentration in the trials approaches its solubility limit, thereby facilitating its precipitation and shell formation around the drying droplet. However, the phenomenon of early precipitation is not as rapid for Val, due to its higher water solubility, which compromises API encapsulation, as evidenced in the results. Despite being hydrophobic and apolar [105], Val's arrangement within the droplet might differ from that of Leu's, owing to Val's three carbon side chain (in contrast to Leu's four carbons), influencing hydrophobicity and surface activity of this AA and consequently affecting shell formation.

Therefore, for a straightforward encapsulation, Leu proves to be the better choice. Nevertheless, a system employing Val could possibly be optimized to induce early precipitation of the AA and consequently early shell formation.

The properties of the SDIs prepared with RIF and Val were predicted by the models and plotted in parity plots, as displayed in Figure 4.16.

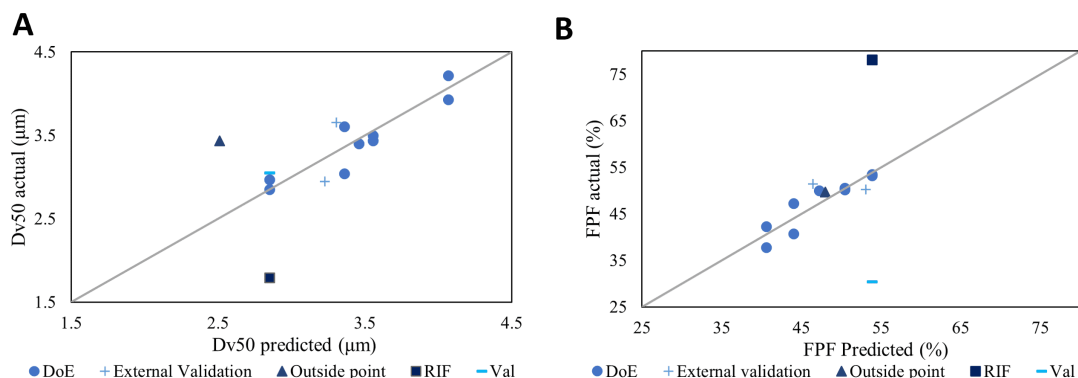


Figure 4.16: Actual vs Predicted plots including trial #24 and #25, A - D_{v50} and B - FPF

Both trials yielded results that differed from those obtained with the ITZ:Leu formulation, as evidenced by the data points displaying a significant deviation from the perfect fit. The only exception is D_{v50} from the material of trial #24, which closely resembles the D_{v50} of the Leu formulation, as it employs the exact same SD conditions and solvent system. The model could not predict the D_{v50} from the SDI containing RIF (trial #25) as the utilization of methanol alters the liquid's surface tension, a phenomenon for which the model was not calibrated.

The FPF is notably more difficult to compare to the model results, as expected given the essential role of API encapsulation for improved aerosol performance. The lack of fit in trial #24 and #25 may be attributed to the use of a different AA and API, respectively, for which the model was not standardized. Alternatively, these deviations may result from the absence of API encapsulation, as it was concluded that the SDI with Val was not encapsulated, thus falling outside the model's predictive capacity. These observations highlight the important role of theoretically evaluating formulation components before establishing process parameters and characterization techniques.

Furthermore, the observed deviations from the models' predictions are expected, as the validity of empirical models is limited to the studied conditions, in this case, the specific ITZ:Leu formulation, the selected solvent systems and the SD parameters. Thus, these models should only be applied within the context of the specific formulation from which they were developed.

The development of models with the capacity to distinguish between various formulations would require a larger dataset, incorporating in the DoE formulation factors such as AA content, different AAs and diverse APIs. Additionally, the inclusion of solvent properties, such as viscosity and density, in the model could enable more accurate predictions regarding the particle size, as it was concluded that under the same SD conditions the solvent influences the PSD. Therefore including formulation and solvent parameters would result in the creation of more complex and comprehensive models.

4.2.5 Proof of concept: Template-assisted SD methodology

It is well established that particles with a $D_{ae} < 5 \mu\text{m}$ will deposit in the deep lung, however, the actual physical diameter of the particle can be above those dimensions if particle density is reduced, due to high porosity [6, 11, 122]. Such particle morphology is named large porous particles, which also have the added benefit of escaping alveolar macrophage uptake, due to their increased size, potentially increasing drug bioavailability.[6, 15, 122]. These particles can be prepared via template-assisted SD, whereby a pore-forming agent is added to the formulation, originating the pores during droplet drying. There are various pore-forming agents, but the most used are volatile salts, that sublime during particle formation, leaving behind the pores [12, 123].

This methodology was attempted with the 3FN as it enables the possibility of having the pore former either on a suspension in the organic feed or in solution in the aqueous feed. Sodium bicarbonate (NaHCO_3) was used as a pore-forming agent, as it decomposes into sodium carbonate, water, and CO_2 at temperatures above 80°C [124]. Consequently, the SD outlet temperature had to be adjusted to ensure salt sublimation, the atomization was tested on both levels of the DoE, to assess if it influenced the pore-forming mechanism and M_{ratio} was kept at 2.8, according to the optimal DoE level. Furthermore, the formulation contained 1% w/w of NaHCO_3 , a value within the tested range in the literature [125]. The results and comparison with the formulation without the pore former (trial #14 and #16) are summarized in Table 4.8.

Aqueous solution of NaHCO_3

The theoretical API load was not attained in this experience, with the SDIs exhibiting a prevalence of Leu, also noticeable on the SEM micrographs, in Figure 4.17, where there are predominantly Leu flakes and shattered particles.

Regarding the PSD, the trial with $F_{\text{atom}} = 3.0 \text{ kg/h}$ (trial #22A) displayed a larger D_{v90} since it presents a bimodal distribution, under the studied pressure (3.5 bar). However, this

Table 4.8: Particle characterization results of the trials containing the pore-forming agent and comparison with the formulations without a pore-forming agent. Results are expressed as mean $\pm$ standard deviation (n=3).

	ITZ:Leu		NaHCO ₃ solution		NaHCO ₃ suspension	
Trial	#14	#16	#22A	#22B	#23A	#23B
M_ratio (-)			2.77			
T_out (°C)	65				85	
RS_out (%)	7.5		3.2		3.2	
F_atom (kg/h)	3.0	2.1	3.0	2.1	3.0	2.1
D_{v10} (μm)	0.9 ± 0.0	1.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	1.4 ± 0.0	2.5 ± 0.1
D_{v50} (μm)	3.0 ± 0.0	3.4 ± 0.0	3.1 ± 0.2	2.8 ± 0.0	4.9 ± 0.1	7.9 ± 0.4
D_{v90} (μm)	7.5 ± 0.1	8.4 ± 0.0	19.6 ± 2.6	8.7 ± 0.2	11.5 ± 0.2	17.8 ± 0.6
ED (%)	98.9 ± 1.3	97.0 ± 2.5	98.4 ± 2.0	99.4 ± 0.4	98.7 ± 0.4	95.8 ± 4.7
FPF (%)	53.4 ± 5.4	40.7 ± 5.5	62.8 ± 5.0	61.7 ± 1.1	51.9 ± 3.7	39.1 ± 5.1
Density (g/cm ³)	1.1 ± 0.0	-	1.0 ± 0.0	-	1.0 ± 0.0	-
BET Area (m ² /g)	7.9 ± 0.5	-	6.2 ± 0.4	-	8.4 ± 0.5	-
Pore Volume (cm ³ /g)	0.03	-	0.02	-	0.03	-
Pore size (nm)	16.0	-	12.0	-	13.7	-
API Load (%)	45.7 ± 1.1	51.6 ± 1.5	39.1 ± 0.8	38.2 ± 1.6	44.3 ± 1.5	45.6 ± 0.9

bimodal distribution was not consistent across different observations at varying pressures, suggesting a heterogenous SDI, as also observed in SEM micrographs. Although the D_{v50} was similar to the formulation without the pore former (trial #14), these SDIs have a higher FPF. This discrepancy is not attributed to the preparation of porous particles with enhanced aerosolization but rather to the presence of particles containing solely Leu, which have increased dispersibility. Additionally, the ED remains near 100% due to the presence of Leu-rich particles.

Analyzing the SEM micrographs, even though Leu flakes are prevalent in both trials, the two levels of atomization led to distinct morphologies. With atomization at 2.1 kg/h mm, Figure 4.17C and D, particles resemble the morphology of the material obtained from the DoE trials (Figure 4.9) displaying surface asperities. Furthermore, SEM-EDS results in Figure 4.17 confirm that the irregular shapes/flakes are likely Leu-rich particles, while the larger spherical particles show Cl, indicating that API encapsulation was not complete.

NaHCO₃ is more soluble in water (96 g/L [126]), than Leu, thus during droplet drying it might start to precipitate after the Leu shell is formed. Additionally, it will not diffuse towards the DCM layer as it is insoluble in this solvent. It is the diffusion of CO₂, resulting from the thermal degradation, towards the gas-liquid interface that originates the pores [125]. However, if NaHCO₃ precipitates within the shell and only then degrades, the production of CO₂ and water will further increase the pressure inside the droplet. This pressure increase can interrupt the shell formation, leaving behind burst pieces of Leu, which is in accordance with the SEM images. Unfortunately, the observation of pores by SEM would not be effective. Therefore, trial#22A underwent analysis to determine

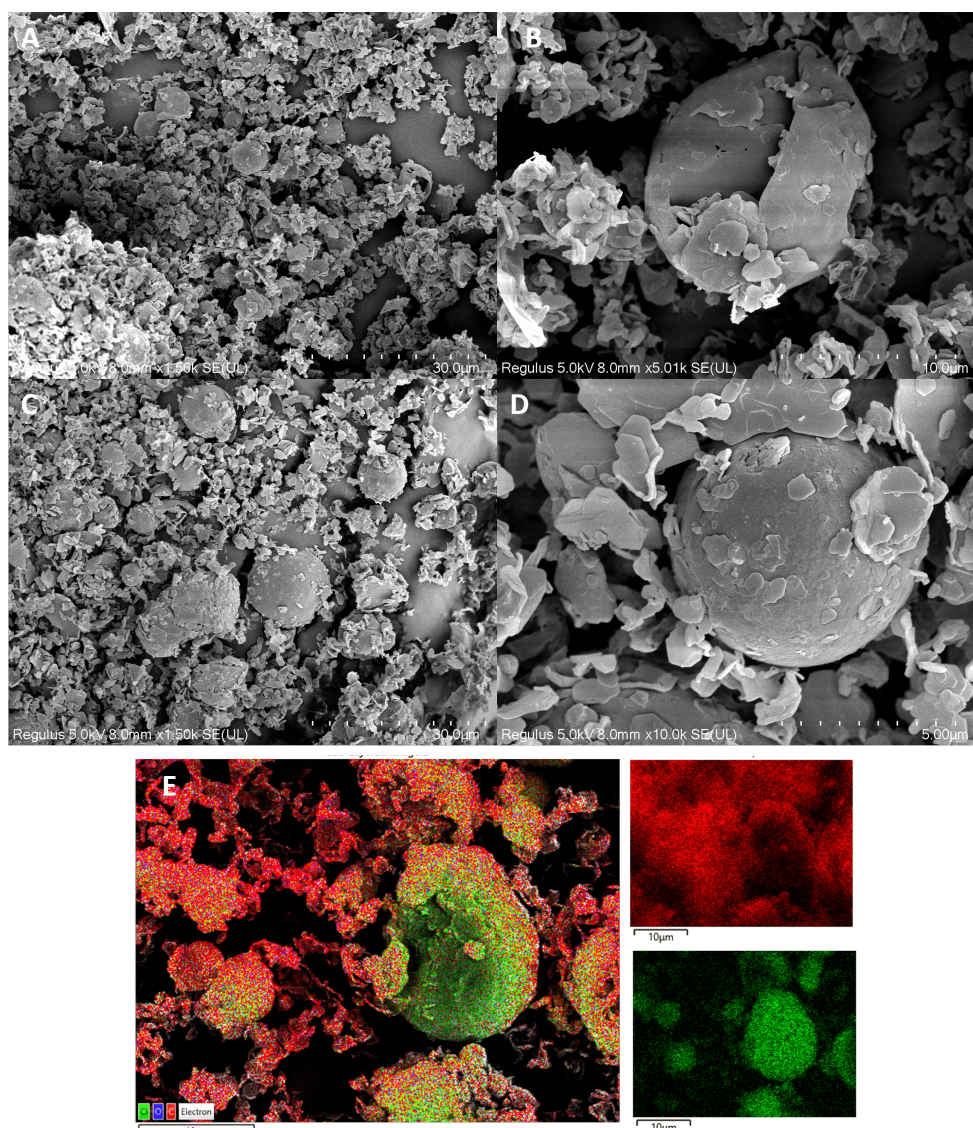


Figure 4.17: SEM micrographs of SDIs obtained with NaHCO_3 in solution, A - trial #22A 1500x magnification, B - trial #22A 10000x magnification, c - trial #22B 1500x magnification, D - trial #22B 5000x magnification and E - SEM-EDS

surface area, pore properties and true density. The surface area was slightly lower than the material of the formulation without the pore-former, whilst the material's true density, pore size, and volume remained similar. It is anticipated that the materials should exhibit identical true densities, as this parameter is solely dependent on the material constituting the particles and not on the density of each individual particle. The remaining results are inconclusive, possibly attributed to the high prevalence of shattered particles and SDI heterogeneity.

Suspension of NaHCO_3

In these experiments (#23A and B) the API load was in accordance with the theoretical value, which in combination with the monomodal PSD results confirms the presence of a homogeneous material. The SDIs prepared with NaHCO_3 in suspension resulted

in larger particles when compared to the formulation without the pore former. Despite their increased size, these SDIs (#23A and 23B) display FPF values similar to the reference formulation (trial #14 and #16, respectively). Particularly in the case of trial #23B, where a D_{v50} two times larger (3.4 vs 7.9 μm) displayed a 1.6 % lower FPF. This observation confirms and highlights the low density of the prepared particles, which are presumed to maintain a $D_{ae} < 5 \mu\text{m}$, regardless of the larger geometric size.

Moreover, noticeable differences in density were visually detected for trial #23A, which exhibited a volume three times larger than trial #22A and #14. However, further analysis failed to corroborate this observation, as trial #23A displayed comparable values with trial #22A and #14, concerning true density and pore volume, with only a slightly higher surface area. These analytical results did not confirm the observed differences in density, probably due to the scale of the test which was not adequate for this material. Potentially, the measurement of bulk and tapped density would validate the distinct observed densities, unfortunately, such analysis could not be performed due to insufficient quantities of manufactured SDI.

Regarding particle morphology, it is noticeably distinct from trial #22A and #22B. In the SEM micrographs 4.18, Leu flakes were less prominent, indicating that the material kept its integrity. Such observation can be explained by the mechanism of NaHCO_3 degradation when suspended. In this context, depending on the rate of solvent evaporation, the salt will lixiviate from the DCM towards the water. Once in the water, it can dissolve and subsequently precipitate or remain already in its solid form and start to thermally degrade. This process is expected to be slower, compared to when NaHCO_3 is already dissolved in water. Thus, it enables the formation of a more robust Leu shell that can withstand the pressure increase inside the droplet without rupturing so promptly.

Increasing the atomization flow rate resulted in the generation of more shredded and burst particles, as evidenced when comparing Figures A and B (trial #23A) with Figures C and D (trial #23B). Nevertheless, in the same figures, highly porous particle surfaces are observed. Furthermore, from the SEM micrographs, some particles appear to be hollow, a characteristic of rapid droplet drying and consequent early shell formation [19, 52], which justifies the observed reduced density. SEM-EDS analysis was performed on two ruptured particles to examine the inner and outer surfaces of the particles. The upper particle, in Figure 4.18E displayed Cl on its interior whilst below, the shell of the ruptured particle exhibits reduced Cl content. This image suggested that, in this case, the API was encapsulated, however, the particles are ruptured. The rupture phenomenon may be attributed to various causes, including the particles' inability to withstand the solvent pressure inside, particle interactions, high shear on the cyclone, and sample handling, all of which could contribute to these observations.

This proof-of-concept experiment showcased the feasibility of producing large porous particles with enhanced aerosolization, utilizing the 3FN. This method is particularly suitable for the preparation of such particle morphology when the pore-forming agent is in suspension. Additionally, the potential of enlarged surface area is beneficial in terms of

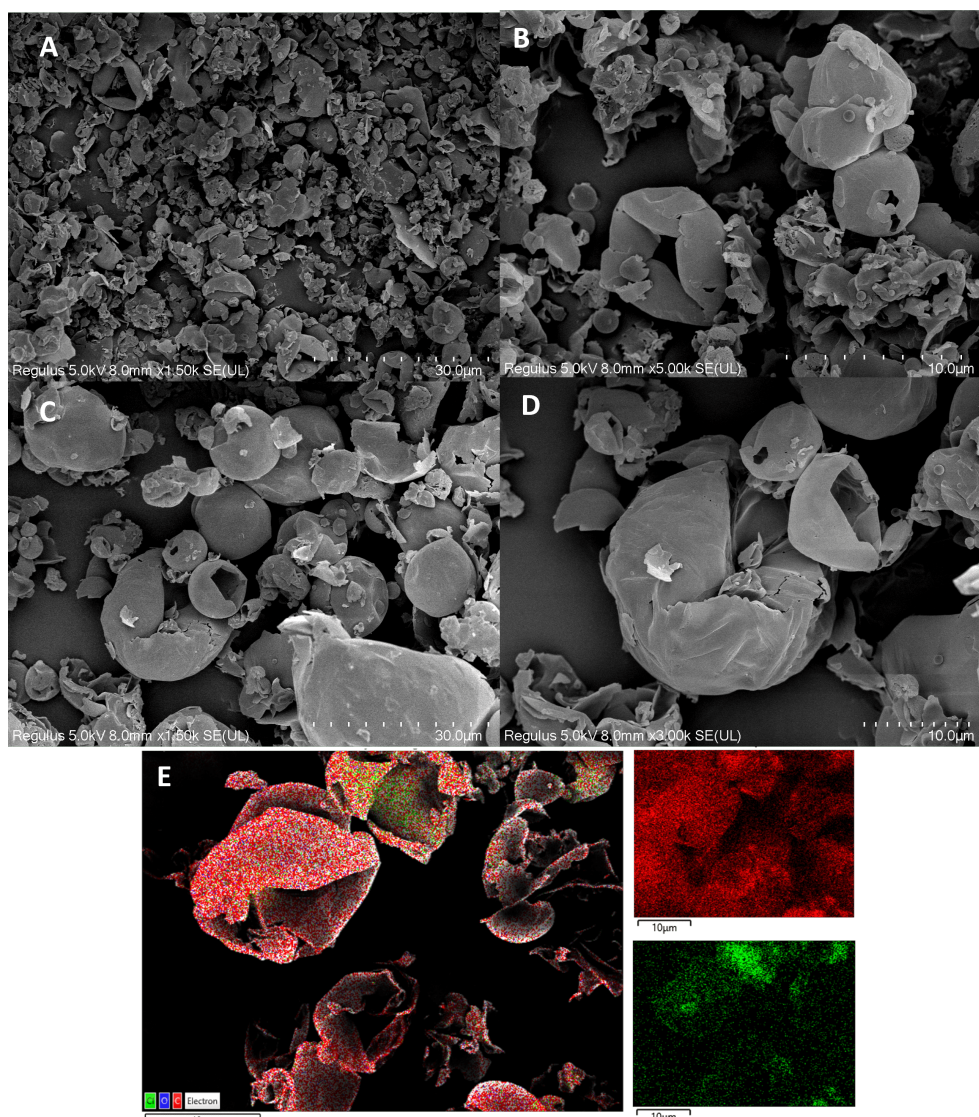


Figure 4.18: SEM micrographs of SDIs obtained with NaHCO_3 in suspension, A - trial #23A 1500x magnification, B - trial #23A 3000x magnification, c - trial #23B 1500x magnification, D - trial #23B 5000x magnification and E - SEM-EDS

facilitated mass transfer, which can promote increased bioavailability. Nonetheless, both process and formulation must be optimized to yield the best results. The mass fraction of the pore-forming agent needs to be optimized according to the formulation under study. Moreover, it should degrade at lower temperatures, enabling smoother SD conditions. Alternatively, pore formers with other pore-forming mechanisms may be explored [125]. Only the optimization of these two parameters can prevent particle rupture and more consistent template-assisted methodologies.

CONCLUSIONS AND FUTURE PRESPECTIVES

Throughout the course of this work, inhalable composite encapsulated particles were successfully produced applying a 3FN as an atomization strategy within the SD process. This study confirmed the 3FN's potential to encapsulate APIs while simultaneously overcoming solubility limitations that arise from the API's highly lipophilic nature and hydrophilic nature of the shell-forming excipients. The implementation of the 3FN strategy enabled the encapsulation of the poorly water-soluble ITZ using Leu as shell-forming excipient. This formulation resulted in SDIs with the desired CQAs, exhibiting a D_{v50} within the inhalable range ($D_{v50} < 5 \mu\text{m}$), ED above 95%, and FPF above 38%. Additionally, the residual solvent levels are below ICH limits. In this specific case, the 3FN enabled the use of a solvent system comprising two immiscible solvents, water and DCM, enabling the independent introduction of two solutions. Thus, the 3FN potentiates the use of less complex solvent systems, leading to improved process throughput and enhanced economic feasibility. In fact, the major identified challenge when using 3FN is ensuring the correct feed ratios to achieve the desired API load in the final product.

To comprehensively understand the process dynamics with the 3FN, and how process parameters influence the powder CQAs, a structured experimental design was performed. This systematic approach not only facilitated a thorough analysis of how process parameters impacted SDI's properties but also enabled the identification of the CPPs and the definition of optimal conditions, within the defined parameter range. Despite the attainment of the desired properties, SEM micrographs revealed the presence of broken particles. This observation is a consequence attributed to the rapid drying kinetics of Leu and its early shell formation within the studied solvent system. The use of DCM and water represents an example of worst-case conditions due to the substantial difference in vapor pressure between these solvents and the required high shear needed during droplet formation to prevent phase separation. Proposed solutions to prevent particle rupture and obtaining SDI with improved integrity include the incorporation of excipients with enhanced mechanical resistance and optimizing the SD conditions to increase the RS_{out}, thus delaying drying kinetics.

Concerning the CQAs of the manufactured SDIs the obtained results indicate that, for

the studied formulation, the ED appears to be independent of process conditions, solely depending on the formulation. On the other hand, the results indicate that both the FPF and D_{v50} , are dependent on SD parameters, exhibiting a clear linear relationship with F_{atom} , the sole factor found to have statistical significance in explaining FPF and D_{v50} variability. Conversely, the influence of factors M_{ratio} and T_{out} is not as noticeable. Moreover, the FPF results were in accordance with the D_{v50} , whereby increased atomization flow rate led to finer particles and consequently higher FPF values, confirming the correlation between volumetric and aerodynamic diameter.

Additionally, this study successfully established empirical models as QbD tools for the development of processes employing the 3FN as atomization strategy for the manufacture of inhalable encapsulated composite particles. The models accurately predicted the D_{v50} and FPF of the prepared SDIs, exhibiting good predictive capacity within the studied design space, as evidenced by the R^2 and R^2_{adj} values of approximately 0.8. Notably, the models further highlighted the great impact of the atomization flow rate on both studied CQAs, enhancing process comprehension.

The observations from the EDA and the models reinforce the pivotal role of atomization flow rate, which was found to be a crucial predictor, greatly influencing particle size distribution and aerosolization, due to its role in determining droplet size. However, according to the models, relying solely on this parameter is insufficient to predict powder properties, with M_{ratio} and T_{out} enhancing predictive accuracy to the FPF and D_{v50} models, respectively. The developed models allowed the identification of the actual critical process parameters that influence the defined CQAs. Regarding improvements, a lower range of temperatures should be assessed in the DoE, to ensure the formation of intact and more homogeneous particles, in the specific case of the employed solvents. Furthermore, at lower T_{out} , the influence of M_{ratio} might become more prominent, as these two factors jointly influence the RS_{out} , altering the drying kinetics and potentially particle properties such as particle density and shape.

The feasibility of encapsulating another API with Leu using the 3FN was evaluated. The resulting powders displayed the required properties, further confirming the suitability of the developed methodology across other poorly water-soluble APIs. Additionally, a comparison of Leu's role as a shell former with another hydrophobic AA, Val, revealed that Leu formulations grant a more straightforward encapsulation process. Further optimization by tailoring formulation parameters such as AA content and process parameters, namely the selected solvent, and the solid concentration, can fine-tune Leu's supersaturation at the droplet's surface leading to faster shell formation. Lastly, the feasibility of utilizing the 3FN in template-assisted SD for the preparation of large porous particles with enhanced aerosolization was explored. In this context, $NaHCO_3$ worked as a pore former, being added either in solution or suspension. These proof-of-concept experiments suggested that when in suspension, $NaHCO_3$ can yield geometrically larger particles with reasonable FPFs. However, it became evident that achieving optimal results in this context requires a comprehensive optimization of both process parameters and formulation.

This methodology, encompassing an experimental design, parameter analysis and empirical modeling, offers a comprehensive and robust strategy for process development, fitting into a QbD framework. The ability to identify CPPs, optimize conditions, and predict outcomes using empirical models grants a structured framework for process development and optimization. With additional efforts, such as a more complex DoE with a broader variation range, this methodology can enhance product quality and streamline process development, reducing costs.

A structured pathway for process development contributes to a simple and effective innovation process, facilitating the optimization and scale-up of a SD process coupled with the 3FN. In an industrial setting, this approach serves as a guiding tool, aiding in the transition from laboratory-scale to large-scale production. Furthermore, the availability of predictive tools accelerates the optimization process.

Even though the developed systematic approach was applied to the production of composite particles via SD coupled with a 3FN, its applicability extends beyond this specific scope, as it works as a valuable tool for process development. The methodology can be adapted and extended to the preparation of other types of particles and pharmaceutical processes, by investigating the relevant CQAs of the desired product and linking them to their CPPs. Moreover, it has a versatile application that expands to formulation development. The organized investigation of formulation variables, such as excipient type and ratios, can aid in the design of formulations with target attributes. Therefore, this methodology, hereby designed for process development can be a valuable tool towards innovation across various stages of pharmaceutical development.

BIBLIOGRAPHY

- [1] J. M. Lourenço. *The NOVAthesis L^AT_EX Template User's Manual*. NOVA University Lisbon. 2021. URL: <https://github.com/joaomlourenco/novathesis/raw/main/template.pdf>.
- [2] F. Neves, C. Moura, and E. Costa. *Method of production of inhalable composite particles using a three-fluid nozzle* WO 2017/064481 A1. 2017-04.
- [3] S. Levine et al. "The Global Impact of Respiratory Disease". In: (2021).
- [4] World Health Organization. *The top 10 causes of death*. 2020. URL: <https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death>.
- [5] S. S. Gaikwad et al. "Dry Powder Inhaler with the technical and practical obstacles, and forthcoming platform strategies". In: *Journal of Controlled Release* 355 (2023-03), pp. 292–311. ISSN: 18734995. DOI: 10.1016/j.jconrel.2023.01.083.
- [6] S. He et al. "A roadmap to pulmonary delivery strategies for the treatment of infectious lung diseases". In: *Journal of Nanobiotechnology* 20 (1 2022-12). ISSN: 14773155. DOI: 10.1186/s12951-022-01307-x.
- [7] N. Shetty et al. "Surface Composition and Aerosolization Stability of an Inhalable Combinational Powder Formulation Spray Dried Using a Three-Fluid Nozzle". In: *Pharmaceutical Research* 37 (11 2020-11).
- [8] K. Knap et al. "Inhalable microparticles as drug delivery systems to the lungs in a dry powder formulations". In: *Regenerative Biomaterials* 10 (2023-01). DOI: 10.1093/rb/rbac099.
- [9] A. Baldinger et al. "Quality by design approach in the optimization of the spray-drying process". In: *Pharmaceutical Development and Technology* 17 (4 2012-07), pp. 389–397. ISSN: 10837450. DOI: 10.3109/10837450.2010.550623.
- [10] S. J. Healthcare, M. B. Dolovich, and R. Dhand. "Aerosol drug delivery: developments in device design and clinical use". In: *www.thelancet.com* 377 (2011), pp. 1032–1077. DOI: 10.1016/S0140. URL: www.thelancet.com.

- [11] Y. Ye, Y. Ma, and J. Zhu. "The future of dry powder inhaled therapy: Promising or discouraging for systemic disorders?" In: *International Journal of Pharmaceutics* 614 (2022-02). ISSN: 18733476. DOI: 10.1016/j.ijpharm.2022.121457.
- [12] A. Lechanteur and B. Evrard. "Influence of composition and spray-drying process parameters on carrier-free DPI properties and behaviors in the lung: A review". In: *Pharmaceutics* 12 (1 2020). ISSN: 19994923. DOI: 10.3390/pharmaceutics12010055.
- [13] A. M. Research.
- [14] L. Chen et al. "Amorphous powders for inhalation drug delivery". In: *Advanced Drug Delivery Reviews* 100 (2016-05), pp. 102–115. ISSN: 18728294. DOI: 10.1016/j.addr.2016.01.002.
- [15] Y. Guo et al. "Pharmaceutical strategies to extend pulmonary exposure of inhaled medicines". In: *Acta Pharmaceutica Sinica B* 11 (8 2021-08), pp. 2565–2584. ISSN: 22113843. DOI: 10.1016/j.apsb.2021.05.015.
- [16] M. Hoppentocht et al. "Technological and practical challenges of dry powder inhalers and formulations". In: *Advanced Drug Delivery Reviews* 75 (2014-08), pp. 18–31. ISSN: 18728294. DOI: 10.1016/j.addr.2014.04.004.
- [17] S. Focaroli et al. "The use of a three-fluid atomising nozzle in the production of spray-dried theophylline/salbutamol sulphate powders intended for pulmonary delivery". In: *Pharmaceutics* 12 (11 2020-11), pp. 1–18. ISSN: 19994923. DOI: 10.3390/pharmaceutics12111116.
- [18] B. Chaurasiya and Y. Y. Zhao. "Dry powder for pulmonary delivery: A comprehensive review". In: *Pharmaceutics* 13 (1 2021-01), pp. 1–28. ISSN: 19994923. DOI: 10.3390/pharmaceutics13010031.
- [19] S. Focaroli et al. "A Design of Experiment (DoE) approach to optimise spray drying process conditions for the production of trehalose/leucine formulations with application in pulmonary delivery". In: *International Journal of Pharmaceutics* 562 (2019-05), pp. 228–240. ISSN: 0378-5173. DOI: 10.1016/J.IJPHARM.2019.03.004.
- [20] G. Ledet et al. "A second-generation inhaled insulin for diabetes mellitus". In: *American Journal of Health-System Pharmacy* 72.14 (2015-07), pp. 1181–1187. ISSN: 1079-2082. DOI: 10.2146/ajhp140540. eprint: <https://academic.oup.com/ajhp/article-pdf/72/14/1181/28338892/ajhp1181.pdf>. URL: <https://doi.org/10.2146/ajhp140540>.
- [21] R. A. Hauser et al. "Orally inhaled levodopa (CVT-301) for early morning OFF periods in Parkinson's disease". In: *Parkinsonism related disorders* 64 (2019-07), pp. 175–180. ISSN: 1873-5126. DOI: 10.1016/J.PARKRELDIS.2019.03.026. URL: <https://pubmed.ncbi.nlm.nih.gov/30992235/>.

-
- [22] B. Pacciardi, A. Calcedo, and T. Messer. "Inhaled Loxapine for the Management of Acute Agitation in Bipolar Disorder and Schizophrenia: Expert Review and Commentary in an Era of Change". In: *Drugs in RD* 19 (1 2019-03), pp. 15–25. ISSN: 1179-6901. DOI: 10.1007/S40268-019-0262-3. URL: <https://pubmed.ncbi.nlm.nih.gov/30721526/>.
- [23] R. Scherließ et al. "Particle engineering in dry powders for inhalation". In: *European Journal of Pharmaceutical Sciences* 172 (2022-05). ISSN: 18790720. DOI: 10.1016/j.ejps.2022.106158.
- [24] A. Minne et al. "Optimization of the aerosolization properties of an inhalation dry powder based on selection of excipients". In: *European Journal of Pharmaceutics and Biopharmaceutics* 70 (3 2008-11), pp. 839–844. ISSN: 09396411. DOI: 10.1016/j.ejpb.2008.06.013.
- [25] N. Alhajj, N.J. O'Reilly, and H. Cathcart. "Designing enhanced spray dried particles for inhalation: A review of the impact of excipients and processing parameters on particle properties". In: *Powder Technology* 384 (2021-05), pp. 313–331. ISSN: 1873328X. DOI: 10.1016/j.powtec.2021.02.031.
- [26] Y. J. Son, D. P. Miller, and J. G. Weers. "Optimizing spray-dried porous particles for high dose delivery with a portable dry powder inhaler". In: *Pharmaceutics* 13 (9 2021-09). ISSN: 19994923. DOI: 10.3390/pharmaceutics13091528.
- [27] H. G. Lee, D. W. Kim, and C. W. Park. "Dry powder inhaler for pulmonary drug delivery: human respiratory system, approved products and therapeutic equivalence guideline". In: *Journal of Pharmaceutical Investigation* 48 (6 2018-11), pp. 603–616. ISSN: 20936214. DOI: 10.1007/s40005-017-0359-z.
- [28] W. R. Ke, R. Y. K. Chang, and H. K. Chan. "Engineering the right formulation for enhanced drug delivery". In: *Advanced Drug Delivery Reviews* 191 (2022-12), p. 114561. ISSN: 0169-409X. DOI: 10.1016/J.ADDR.2022.114561.
- [29] A. Ziaee et al. "Spray drying of pharmaceuticals and biopharmaceuticals: Critical parameters and experimental process optimization approaches". In: *European Journal of Pharmaceutical Sciences* 127 (2019-01), pp. 300–318. ISSN: 18790720. DOI: 10.1016/j.ejps.2018.10.026.
- [30] F. Lavorini, M. Pistolesi, and O. S. Usmani. "Recent advances in capsule-based dry powder inhaler technology". In: *Multidisciplinary Respiratory Medicine* 12 (2017-05). DOI: 10.4081/mrm.2017.236.
- [31] H. Douafer et al. "Scope and limitations on aerosol drug delivery for the treatment of infectious respiratory diseases". In: *Journal of Controlled Release* 325 (2020), pp. 276–292. DOI: 10.1016/j.jconrel.2020.07.002. URL: <https://hal.science/hal-03084998>.

- [32] A. M. Healy et al. "Dry powders for oral inhalation free of lactose carrier particles". In: *Advanced Drug Delivery Reviews* 75 (2014-08), pp. 32–52. ISSN: 18728294. DOI: 10.1016/j.addr.2014.04.005.
- [33] C. Moura et al. *Manipulation of Spray-dried Composite Particles Physiochemical Properties and In-vitro Performance*. 2015.
- [34] C. Moura et al. *Screening and Optimization of Formulation and Process Parameters for the Manufacture of Inhalable Composite Particles by Spray-drying*.
- [35] D. Leng et al. "Formulating Inhalable Dry Powders Using Two-Fluid and Three-Fluid Nozzle Spray Drying". In: *Pharmaceutical Research* 35 (12 2018-12). ISSN: 1573904X. DOI: 10.1007/s11095-018-2509-z.
- [36] N. Shetty et al. "Influence of excipients on physical and aerosolization stability of spray dried high-dose powder formulations for inhalation". In: *International Journal of Pharmaceutics* 544 (1 2018-06), pp. 222–234. ISSN: 18733476. DOI: 10.1016/j.ijpharm.2018.04.034.
- [37] C. Moura et al. "Optimization of supercritical CO₂-assisted spray drying technology for the production of inhalable composite particles using quality-by-design principles". In: *Powder Technology* 357 (2019-12), pp. 387–397. ISSN: 1873328X. DOI: 10.1016/j.powtec.2019.08.090.
- [38] K. Kondo, T. Niwa, and K. Danjo. "Preparation of sustained-release coated particles by novel microencapsulation method using three-fluid nozzle spray drying technique". In: *European Journal of Pharmaceutical Sciences* 51 (1 2014), pp. 11–19. ISSN: 09280987. DOI: 10.1016/j.ejps.2013.09.001.
- [39] R. M. Pabari, T. Sunderland, and Z. Ramtoola. "Investigation of a novel 3-fluid nozzle spray drying technology for the engineering of multifunctional layered microparticles". In: *Expert Opinion on Drug Delivery* 9 (12 2012-12), pp. 1463–1474. ISSN: 17425247. DOI: 10.1517/17425247.2012.734295.
- [40] M. Ordoubadi, H. Wang, and R. Vehring. "Mechanistic Formulation Design of Spray-Dried Powders". In: *KONA Powder and Particle Journal* (2022), p. 2023012. ISSN: 0288-4534. DOI: 10.14356/kona.2023012.
- [41] Y.-W. Lin et al. "Powder Production and Particle Engineering for Dry Powder Inhaler Formulations". In: *Current pharmaceutical design* 21 (27 2015-09), pp. 3902–3916. ISSN: 1873-4286. DOI: 10.2174/1381612821666150820111134. URL: <https://pubmed.ncbi.nlm.nih.gov/26290193/>.
- [42] M. Mönckedieck et al. "Spray drying of mannitol carrier particles with defined morphology and flow characteristics for dry powder inhalation". In: *Drying Technology* 35.15 (2017), pp. 1843–1857. DOI: 10.1080/07373937.2017.1281291. URL: <https://doi.org/10.1080/07373937.2017.1281291>.

- [43] D. Lechuga-Ballesteros et al. "Trileucine improves aerosol performance and stability of spray-dried powders for inhalation". In: *Journal of pharmaceutical sciences* 97 (1 2008), pp. 287–302. ISSN: 0022-3549. DOI: 10.1002/JPS.21078. URL: <https://pubmed.ncbi.nlm.nih.gov/17823950/>.
- [44] W. Lu et al. "Inhalable co-amorphous budesonide-arginine dry powders prepared by spray drying". In: *International journal of pharmaceutics* 565 (2019-06), pp. 1–8. ISSN: 1873-3476. DOI: 10.1016/J.IJPHARM.2019.04.036. URL: <https://pubmed.ncbi.nlm.nih.gov/30999050/>.
- [45] L. Willis, D. Hayes, and H. M. Mansour. "Therapeutic liposomal dry powder inhalation aerosols for targeted lung delivery". In: *Lung* 190 (3 2012-06), pp. 251–262. ISSN: 1432-1750. DOI: 10.1007/S00408-011-9360-X. URL: <https://pubmed.ncbi.nlm.nih.gov/22274758/>.
- [46] L. G. Sweeney et al. "Spray-freeze-dried liposomal ciprofloxacin powder for inhaled aerosol drug delivery". In: *International journal of pharmaceutics* 305 (1-2 2005-11), pp. 180–185. ISSN: 0378-5173. DOI: 10.1016/J.IJPHARM.2005.09.010. URL: <https://pubmed.ncbi.nlm.nih.gov/16242277/>.
- [47] M. Chougule, B. Padhi, and A. Misra. "Development of spray dried liposomal dry powder inhaler of Dapsone". In: *AAPS PharmSciTech* 9 (1 2008-03), pp. 47–53. ISSN: 1530-9932. DOI: 10.1208/S12249-007-9024-6. URL: <https://pubmed.ncbi.nlm.nih.gov/18446460/>.
- [48] G. Pilcer, T. Sebt, and K. Amighi. "Formulation and characterization of lipid-coated tobramycin particles for dry powder inhalation". In: *Pharmaceutical research* 23 (5 2006-05), pp. 931–940. ISSN: 0724-8741. DOI: 10.1007/S11095-006-9789-4. URL: <https://pubmed.ncbi.nlm.nih.gov/16715383/>.
- [49] Y. Cui et al. "Moisture-Resistant Co-Spray-Dried Netilmicin with l-Leucine as Dry Powder Inhalation for the Treatment of Respiratory Infections". In: *Pharmaceutics* 10 (4 2018-12). ISSN: 1999-4923. DOI: 10.3390/PHARMACEUTICS10040252. URL: <https://pubmed.ncbi.nlm.nih.gov/30513738/>.
- [50] R. P. Aquino et al. "Dry powder inhalers of gentamicin and leucine: formulation parameters, aerosol performance and in vitro toxicity on CuFi1 cells". In: *International Journal of Pharmaceutics* 426 (1-2 2012-04), pp. 100–107. ISSN: 0378-5173. DOI: 10.1016/J.IJPHARM.2012.01.026.
- [51] M. A. Boraey et al. "Improvement of the dispersibility of spray-dried budesonide powders using leucine in an ethanol-water cosolvent system". In: *Powder Technology* 236 (2013-02), pp. 171–178. ISSN: 00325910. DOI: 10.1016/j.powtec.2012.02.047.
- [52] R. Vehring, W. R. Foss, and D. Lechuga-Ballesteros. "Particle formation in spray drying". In: *Journal of Aerosol Science* 38 (7 2007-07), pp. 728–746. ISSN: 0021-8502. DOI: 10.1016/J.JAEROSCI.2007.04.005.

- [53] T. T. Nguyen et al. "Formulation and evaluation of carrier-free dry powder inhaler containing sildenafil". In: *Drug Delivery and Translational Research* 9 (1 2019-02), pp. 319–333. ISSN: 21903948. DOI: 10.1007/s13346-018-0586-5.
- [54] N. Alhajj, N. J. O'Reilly, and H. Cathcart. "Leucine as an excipient in spray dried powder for inhalation". In: *Drug Discovery Today* 26 (10 2021-10), pp. 2384–2396. ISSN: 1359-6446. DOI: 10.1016/J.DRUDIS.2021.04.009.
- [55] M. A. Garg et al. *Microencapsulation techniques in pharmaceutical formulation*. URL: <https://www.researchgate.net/publication/359391954>.
- [56] S. H. Soh and L. Y. Lee. "Microencapsulation and nanoencapsulation using supercritical fluid (SCF) techniques". In: *Pharmaceutics* 11 (1 2019-01). DOI: 10.3390/pharmaceutics11010021.
- [57] I. Kłodová et al. "Encapsulation: A Strategy to Deliver Therapeutics and Bioactive Compounds?" In: *Pharmaceutics* 16 (3 2023-03). ISSN: 14248247. DOI: 10.3390/ph16030362.
- [58] A. H. Chow et al. "Particle engineering for pulmonary drug delivery". In: *Pharmaceutical Research* 24 (3 2007-03), pp. 411–437. ISSN: 07248741. DOI: 10.1007/s11095-006-9174-3.
- [59] J. Rodríguez et al. "Current encapsulation strategies for bioactive oils: From alimentary to pharmaceutical perspectives". In: *Food Research International* 83 (2016-05), pp. 41–59. ISSN: 09639969. DOI: 10.1016/j.foodres.2016.01.032.
- [60] V. Suganya and V. Anuradha. "Microencapsulation and Nanoencapsulation: A Review". In: *International Journal of Pharmaceutical and Clinical Research* 9 (3 2017-03). DOI: 10.25258/ijpcr.v9i3.8324.
- [61] M. Iqbal et al. "Double emulsion solvent evaporation techniques used for drug encapsulation". In: *International Journal of Pharmaceutics* 496 (2 2015-12), pp. 173–190. ISSN: 18733476. DOI: 10.1016/j.ijpharm.2015.10.057.
- [62] Y. Lu and K. Park. "Microencapsulation: Methods and Pharmaceutical Applications". In: (2012). DOI: 10.3109/9781841848204.000.
- [63] S. Kandasamy and R. Naveen. "A review on the encapsulation of bioactive components using spray-drying and freeze-drying techniques". In: *Journal of Food Process Engineering* 45 (8 2022-08). ISSN: 17454530. DOI: 10.1111/jfpe.14059.
- [64] T. Sunderland, J. G. Kelly, and Z. Ramtoola. "Application of a novel 3-fluid nozzle spray drying process for the microencapsulation of therapeutic agents using incompatible drug-polymer solutions". In: *Archives of Pharmacal Research* 38 (4 2015-04), pp. 566–573. ISSN: 19763786. DOI: 10.1007/s12272-013-0261-9.

- [65] A. Feng et al. "Mechanistic models facilitate efficient development of leucine containing microparticles for pulmonary drug delivery". In: *International Journal of Pharmaceutics* 409.1 (2011), pp. 156–163. ISSN: 0378-5173. DOI: <https://doi.org/10.1016/j.ijpharm.2011.02.049>. URL: <https://www.sciencedirect.com/science/article/pii/S0378517311001840>.
- [66] A. Sosnik and K. P. Seremeta. "Advantages and challenges of the spray-drying technology for the production of pure drug particles and drug-loaded polymeric carriers". In: *Advances in Colloid and Interface Science* 223 (2015-08), pp. 40–54. ISSN: 00018686. DOI: [10.1016/j.cis.2015.05.003](https://doi.org/10.1016/j.cis.2015.05.003).
- [67] T. Parumasivam et al. "Dry powder inhalable formulations for anti-tubercular therapy". In: *Advanced Drug Delivery Reviews* 102 (2016-07), pp. 83–101. ISSN: 18728294. DOI: [10.1016/j.addr.2016.05.011](https://doi.org/10.1016/j.addr.2016.05.011).
- [68] S. Poozesh and E. Bilgili. "Scale-up of pharmaceutical spray drying using scale-up rules: A review". In: *International Journal of Pharmaceutics* 562 (2019-05), pp. 271–292. ISSN: 18733476. DOI: [10.1016/j.ijpharm.2019.03.047](https://doi.org/10.1016/j.ijpharm.2019.03.047).
- [69] Q. T. Zhou et al. "Inhaled formulations and pulmonary drug delivery systems for respiratory infections". In: *Advanced Drug Delivery Reviews* 85 (2015-05), pp. 83–99. ISSN: 18728294. DOI: [10.1016/j.addr.2014.10.022](https://doi.org/10.1016/j.addr.2014.10.022).
- [70] Research and Markets. *Pharmaceutical Spray Drying Market (2nd Edition)*, 2018-2028. URL: <https://www.researchandmarkets.com/reports/4542473/pharmaceutical-spray-drying-market-2nd>.
- [71] Mordor Intelligence. *Pharmaceutical Spray Drying Market Size Share Analysis - Industry Research Report - Growth Trends*. URL: <https://www.mordorintelligence.com/industry-reports/global-pharmaceutical-spray-drying-market>.
- [72] R. Siekmeier and G. Scheuch. "Inhaled insulin—does it become reality?" In: *Journal of Physiology and Pharmacology* 59 Suppl 6 (2008-12), pp. 81–113. (Visited on 2023-08-03).
- [73] J. Vicente et al. "Fundamental analysis of particle formation in spray drying". In: *Powder Technology* 247 (2013), pp. 1–7. ISSN: 0032-5910. DOI: <https://doi.org/10.1016/j.powtec.2013.06.038>. URL: <https://www.sciencedirect.com/science/article/pii/S0032591013004476>.
- [74] A. Kauppinen et al. "Efficient production of solid dispersions by spray drying solutions of high solid content using a 3-fluid nozzle". In: *European Journal of Pharmaceutics and Biopharmaceutics* 123 (2018-02), pp. 50–58. ISSN: 18733441. DOI: [10.1016/j.ejpb.2017.11.009](https://doi.org/10.1016/j.ejpb.2017.11.009).

- [75] N. Marasini et al. "Development of excipients free inhalable co-spray-dried tobramycin and diclofenac formulations for cystic fibrosis using two and three fluid nozzles". In: *International Journal of Pharmaceutics* 624 (2022-08). ISSN: 18733476. DOI: 10.1016/j.ijpharm.2022.121989.
- [76] F. Wan et al. "One-Step Production of Protein-Loaded PLGA Microparticles via Spray Drying Using 3-Fluid Nozzle". In: *Pharmaceutical Research* 31 (8 2014), pp. 1967–1977. ISSN: 1573904X. DOI: 10.1007/s11095-014-1299-1.
- [77] C. Corell et al. "Application of a three-fluid nozzle for the preparation of amorphous solid dispersions". In: *Drying Technology* 0.0 (2023), pp. 1–14. DOI: 10.1080/07373937.2023.2177308. URL: <https://doi.org/10.1080/07373937.2023.2177308>.
- [78] G. Kasten et al. "Process Optimization and Upscaling of Spray-Dried Drug-Amino acid Co-Amorphous Formulations". In: *Pharmaceutics* 11 (2019). ISSN: 1999-4923. DOI: 10.3390/pharmaceutics11010024. URL: <https://www.mdpi.com/1999-4923/11/1/24>.
- [79] P. T. Ingvarsson et al. "Engineering of an inhalable dda/tdb liposomal adjuvant: A quality-by-design approach towards optimization of the spray drying process". In: *Pharmaceutical Research* 30 (11 2013-11), pp. 2772–2784. ISSN: 07248741. DOI: 10.1007/s11095-013-1096-2.
- [80] F. Buttini et al. "The application of Quality by Design framework in the pharmaceutical development of dry powder inhalers". In: *European Journal of Pharmaceutical Sciences* (2018), pp. 64–76. DOI: 10.1016/j.ejps.2017.10.042.
- [81] P. Holm et al. "Q8(R2)". In: *ICH Quality Guidelines*. John Wiley Sons, Ltd, 2017. Chap. 20, pp. 535–577. ISBN: 9781118971147. DOI: <https://doi.org/10.1002/9781118971147.ch20>. eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/9781118971147.ch20>. URL: <https://onlinelibrary.wiley.com/doi/abs/10.1002/9781118971147.ch20>.
- [82] S. N. Politis et al. "Design of experiments (DoE) in pharmaceutical development". In: *Drug Development and Industrial Pharmacy* 43 (6 2017-06), pp. 889–901. ISSN: 15205762. DOI: 10.1080/03639045.2017.1291672.
- [83] E. Faulhammer et al. "Development of a design space and predictive statistical model for capsule filling of low-fill-weight inhalation products". In: *Drug Development and Industrial Pharmacy* 42 (2 2016-01), pp. 221–230. ISSN: 15205762. DOI: 10.3109/03639045.2015.1040416.
- [84] J. A. Lopes and M. C. Sarraguça. *Data Processing in Multivariate Analysis of Pharmaceutical Processes*. Elsevier, 2018, pp. 35–51. ISBN: 9780128110652. DOI: 10.1016/b978-0-12-811065-2.00002-3.
- [85] L. X. Yu et al. "Understanding pharmaceutical quality by design". In: *AAPS Journal* 16 (4 2014), pp. 771–783. ISSN: 15507416. DOI: 10.1208/s12248-014-9598-3.

- [86] N. Marasini et al. "Statistical modeling, optimization and characterization of spray-dried solid self-microemulsifying drug delivery system using design of experiments". In: *Chemical pharmaceutical bulletin* 61 (2 2013-02), pp. 184–193. ISSN: 1347-5223. DOI: 10.1248/CPB.C12-00823. URL: <https://pubmed.ncbi.nlm.nih.gov/23370195/>.
- [87] *An introduction to Design of Experiments (DOE) - OPEX Resources*. Accessed on 2023.07.22. URL: https://opexresources.com/introduction-design-experiments-doe_/.
- [88] *Types of Design of Experiments | Introduction to Statistics | JMP*. Accessed on 2023.07.22. URL: https://www.jmp.com/en_in/statistics-knowledge-portal/what-is-design-of-experiments/types-of-design-of-experiments.html.
- [89] R. Churro et al. "Statistical modelling of the amination reaction of cyclohexanol to produce cyclohexylamine over a commercial Ni-based catalyst". In: *Chemical Engineering Research and Design* 170 (2021-06), pp. 189–200. ISSN: 02638762. DOI: 10.1016/j.cherd.2021.03.029.
- [90] Y. Dong et al. "Data-Driven Modeling Methods and Techniques for Pharmaceutical Processes". In: *Processes* 11 (7 2023-07), p. 2096. ISSN: 2227-9717. DOI: 10.3390/pr11072096. URL: <https://www.mdpi.com/2227-9717/11/7/2096>.
- [91] A. Rogers and M. Ierapetritou. "Challenges and opportunities in modeling pharmaceutical manufacturing processes". In: *Computers and Chemical Engineering* 81 (2015-10), pp. 32–39. ISSN: 00981354. DOI: 10.1016/j.compchemeng.2015.03.018.
- [92] J. E. Tabora and N. Domagalski. "Multivariate Analysis and Statistics in Pharmaceutical Process Research and Development". In: *Annu. Rev. Chem. Biomol. Eng* 8 (2017), pp. 403–429. DOI: 10.1146/annurev-chembioeng. URL: <https://doi.org/10.1146/annurev-chembioeng->.
- [93] T. O'Connor et al. *Quality risk management for pharmaceutical manufacturing: The role of process modeling and simulations*. Elsevier Inc., 2017, pp. 15–37. ISBN: 9780081001806. DOI: 10.1016/B978-0-08-100154-7.00002-8.
- [94] G. M. Troup and C. Georgakis. "Process systems engineering tools in the pharmaceutical industry". In: *Computers and Chemical Engineering* 51 (2013-04), pp. 157–171. ISSN: 00981354. DOI: 10.1016/j.compchemeng.2012.06.014.
- [95] D. C. Montgomery. *Design and Analysis of Experiments*. John Wiley & Sons, 2008. ISBN: 9780470128664.
- [96] G. Shi et al. "Pharmaceutical application of multivariate modelling techniques: a review on the manufacturing of tablets". In: *RSC Advances* 11 (14 2021-01), pp. 8323–8345. ISSN: 20462069. DOI: 10.1039/d0ra08030f.

- [97] C. Duret et al. "Solid dispersions of itraconazole for inhalation with enhanced dissolution, solubility and dispersion properties". In: *International Journal of Pharmaceutics* 428.1 (2012), pp. 103–113. ISSN: 0378-5173. DOI: <https://doi.org/10.1016/j.ijpharm.2012.03.002>. URL: <https://www.sciencedirect.com/science/article/pii/S0378517312002013>.
- [98] *Leucine* | C₆H₁₃NO₂ | CID 6106. URL: <https://pubchem.ncbi.nlm.nih.gov/compound/Leucine> (visited on 2023-08-03).
- [99] Copley Scientific. *Driving Results in Inhaler Testing*. 2021.
- [100] European Directorate for the Quality of Medicines and others. *Healthcare (EDQM) Preparations for inhalation: aerodynamic assessment of fine particles*. 2012.
- [101] United States Pharmacopeial Convention. *The United States Pharmacopeia: USP 37: Chapter 601: Physical tests and determinations; Aerosols*. 2014.
- [102] D. C. Montgomery, E. A. Peck, and G. G. Vining. *Introduction to Linear Regression Analysis (4th ed.)* Wiley & Sons, 2006. ISBN: 0471754951.
- [103] J. Fox. *Applied Regression Analysis and Generalized Linear Models*. SAGE Publications, 2008. ISBN: 9780761930426.
- [104] *Multiple Regression - Statistics Solutions*. Accessed on 2023.07.21. URL: <https://www.statisticssolutions.com/free-resources/directory-of-statistical-analyses/multiple-regression/>.
- [105] *Amino acids reference chart*. URL: <https://www.sigmaaldrich.com/PT/en/technical-documents/technical-article/protein-biology/protein-structural-analysis/amino-acid-reference-chart> (visited on 2023-08-03).
- [106] T. Sou et al. "The effect of amino acid excipients on morphology and solid-state properties of multi-component spray-dried formulations for pulmonary delivery of biomacromolecules". In: *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V* 83 (2 2013-02), pp. 234–243. ISSN: 1873-3441. DOI: [10.1016/J.EJPB.2012.10.015](https://doi.org/10.1016/J.EJPB.2012.10.015). URL: <https://pubmed.ncbi.nlm.nih.gov/23183447/>.
- [107] T. Sou et al. "Spray-Dried Influenza Antigen with Trehalose and Leucine Produces an Aerosolizable Powder Vaccine Formulation that Induces Strong Systemic and Mucosal Immunity after Pulmonary Administration". In: *Journal of aerosol medicine and pulmonary drug delivery* 28 (5 2015-10), pp. 361–371. ISSN: 1941-2703. DOI: [10.1089/JAMP.2014.1176](https://doi.org/10.1089/JAMP.2014.1176). URL: <https://pubmed.ncbi.nlm.nih.gov/25714115/>.
- [108] P. T. Mah et al. "The use of hydrophobic amino acids in protecting spray dried trehalose formulations against moisture-induced changes". In: *European Journal of Pharmaceutics and Biopharmaceutics* 144 (2019-11), pp. 139–153. ISSN: 0939-6411. DOI: [10.1016/J.EJPB.2019.09.014](https://doi.org/10.1016/J.EJPB.2019.09.014).

- [109] A. Jüptner and R. Scherließ. "Spray Dried Formulations for Inhalation—Meaningful Characterisation of Powder Properties". In: *Pharmaceutics* 2020, Vol. 12, Page 14 12 (1 2019-12), p. 14. ISSN: 1999-4923. DOI: 10.3390/PHARMACEUTICS12010014. URL: <https://www.mdpi.com/1999-4923/12/1/14/html><https://www.mdpi.com/1999-4923/12/1/14>.
- [110] R. Y. K. Chang and H. K. Chan. "Advancements in Particle Engineering for Inhalation Delivery of Small Molecules and Biotherapeutics". In: *Pharmaceutical Research* 2022 39:12 39 (12 2022-09), pp. 3047–3061. ISSN: 1573-904X. DOI: 10.1007/S11095-022-03363-2. URL: <https://link.springer.com/article/10.1007/s11095-022-03363-2>.
- [111] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. *IMPURITIES: GUIDELINE FOR RESIDUAL SOLVENTS Q3C(R6)*. 2016-10. URL: https://database.ich.org/sites/default/files/Q3C-R6_Guideline_ErrorCorrection_2019_0410_0.pdf.
- [112] *Multicollinearity | Introduction to Statistics | JMP*. URL: https://www.jmp.com/en_is/statistics-knowledge-portal/what-is-multiple-regression/multicollinearity.html.
- [113] *Rifampicin | C43H58N4O12 | CID 135398735 - PubChem*. URL: <https://pubchem.ncbi.nlm.nih.gov/compound/Rifampicin>.
- [114] *Rifampicin - Sigma Aldrich*. URL: <https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/522/389/r3501pis.pdf> (visited on 2023-08-03).
- [115] *Methanol | CH3OH | CID 887 - PubChem*. URL: <https://pubchem.ncbi.nlm.nih.gov/compound/887#section=Density>.
- [116] *Surface tension values of some common test liquids for surface energy analysis*. URL: <http://www.surface-tension.de/>.
- [117] *Methylene Chloride | CH2Cl2 | CID 6344 - PubChem*. URL: <https://pubchem.ncbi.nlm.nih.gov/compound/6344#section=Heat-of-Vaporization>.
- [118] S. Mandato et al. "Liquids' atomization with two different nozzles: Modeling of the effects of some processing and formulation conditions by dimensional analysis". In: *Powder Technology* 224 (2012), pp. 323–330. ISSN: 0032-5910. DOI: <https://doi.org/10.1016/j.powtec.2012.03.014>. URL: <https://www.sciencedirect.com/science/article/pii/S0032591012001775>.
- [119] *Itraconazole | C35H38Cl2N8O4 | CID 55283 - PubChem*. URL: <https://pubchem.ncbi.nlm.nih.gov/compound/55283>.

- [120] M. Ordoubadi et al. "On the Physical Stability of Leucine-Containing Spray-Dried Powders for Respiratory Drug Delivery". In: *Pharmaceutics* 2023, Vol. 15, Page 435 15 (2 2023-01), p. 435. ISSN: 1999-4923. DOI: 10.3390/PHARMACEUTICS15020435. URL: <https://www.mdpi.com/1999-4923/15/2/435/html><https://www.mdpi.com/1999-4923/15/2/435>.
- [121] Valine | C₅H₁₁NO₂ | CID 6287 - PubChem. URL: <https://pubchem.ncbi.nlm.nih.gov/compound/Valine#section=Solubility>.
- [122] D. D. Pham et al. "Formulation of pyrazinamide-loaded large porous particles for the pulmonary route: Avoiding crystal growth using excipients". In: *International Journal of Pharmaceutics* 454 (2 2013-10), pp. 668–677. ISSN: 0378-5173. DOI: 10.1016/J.IJPHARM.2013.04.016.
- [123] L. M. Nolan et al. "Excipient-free nanoporous microparticles of budesonide for pulmonary delivery". In: *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences* 37 (5 2009-07), pp. 593–602. ISSN: 1879-0720. DOI: 10.1016/J.EJPS.2009.05.007. URL: <https://pubmed.ncbi.nlm.nih.gov/19463948/>.
- [124] M. Hartman et al. "Thermal Decomposition of Sodium Hydrogen Carbonate and Textural Features of Its Calcines". In: *Industrial and Engineering Chemistry Research* 52 (31 2013-08), pp. 10619–10626. ISSN: 08885885. DOI: 10.1021/IE400896C. URL: <https://pubs.acs.org/doi/epdf/10.1021/ie400896c>.
- [125] L. Gallo, M. V. Ramírez-Rigo, and V. Bucalá. "Development of porous spray-dried inhalable particles using an organic solvent-free technique". In: *Powder Technology* 342 (2019-01), pp. 642–652. ISSN: 0032-5910. DOI: 10.1016/J.POWTEC.2018.10.041.
- [126] Sodium Bicarbonate | NaHCO₃ | CID 516892 - PubChem. URL: <https://pubchem.ncbi.nlm.nih.gov/compound/Sodium-Bicarbonate> (visited on 2023-08-03).

A

SUPPLEMENTARY UV-VIS RESULTS

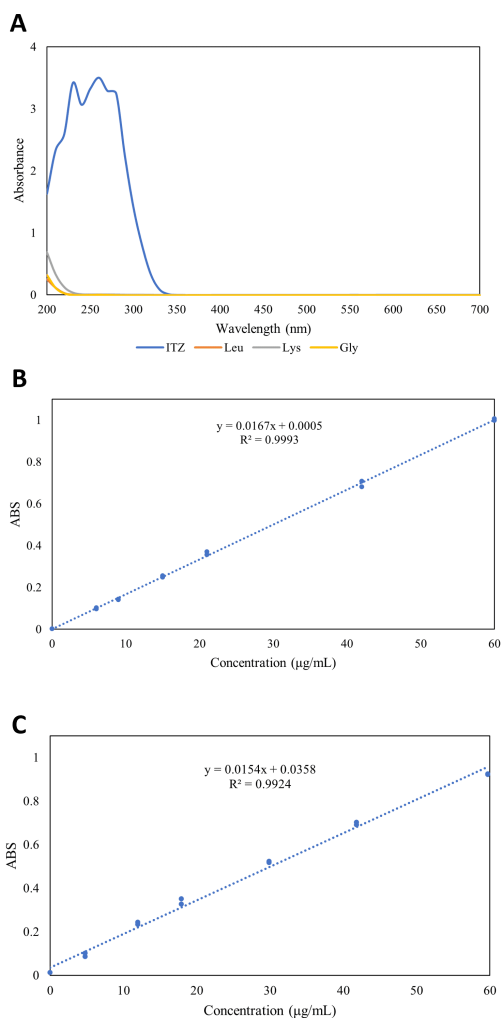


Figure A.1: UV-Vis results, A - API and AA spectral scans, B - ITZ calibration curve at 260 nm, C- RIF calibration curve at 240 nm

B

XPS SPECTRA

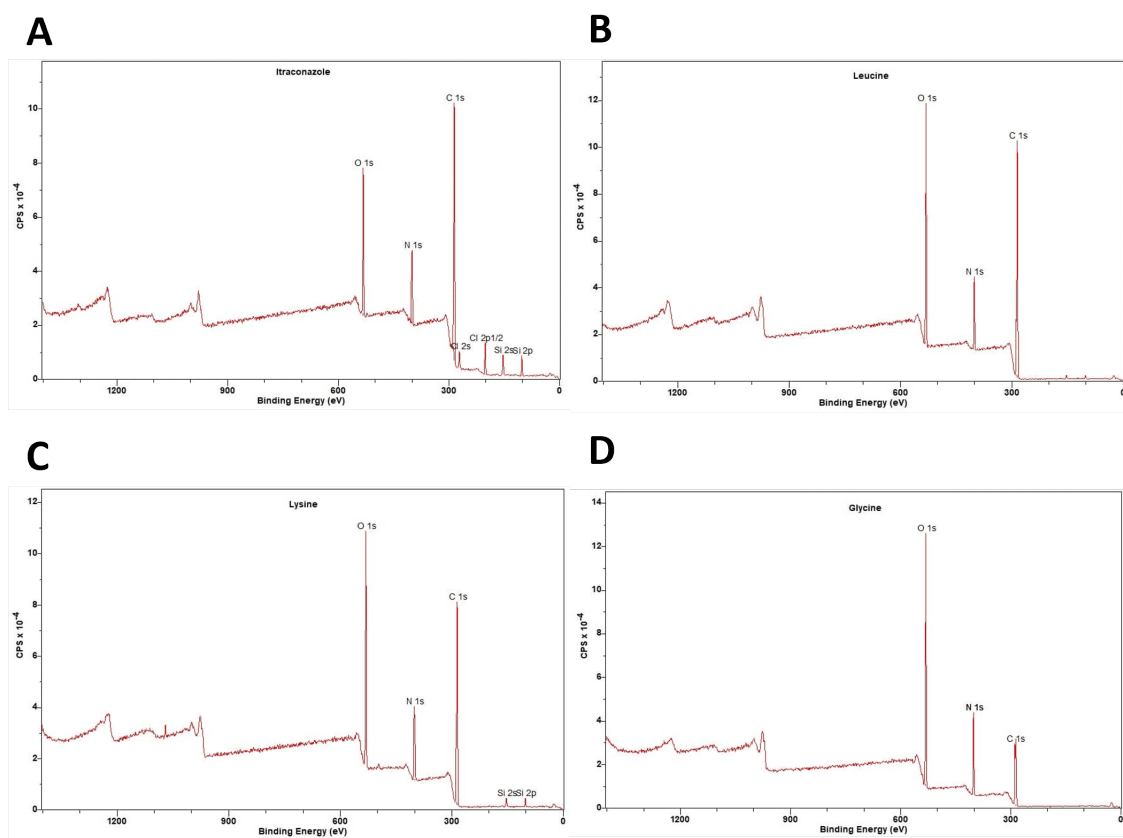


Figure B.1: XPS of single components, A - Itraconazole, B - Leucine, C - Lysine, D - Glycine

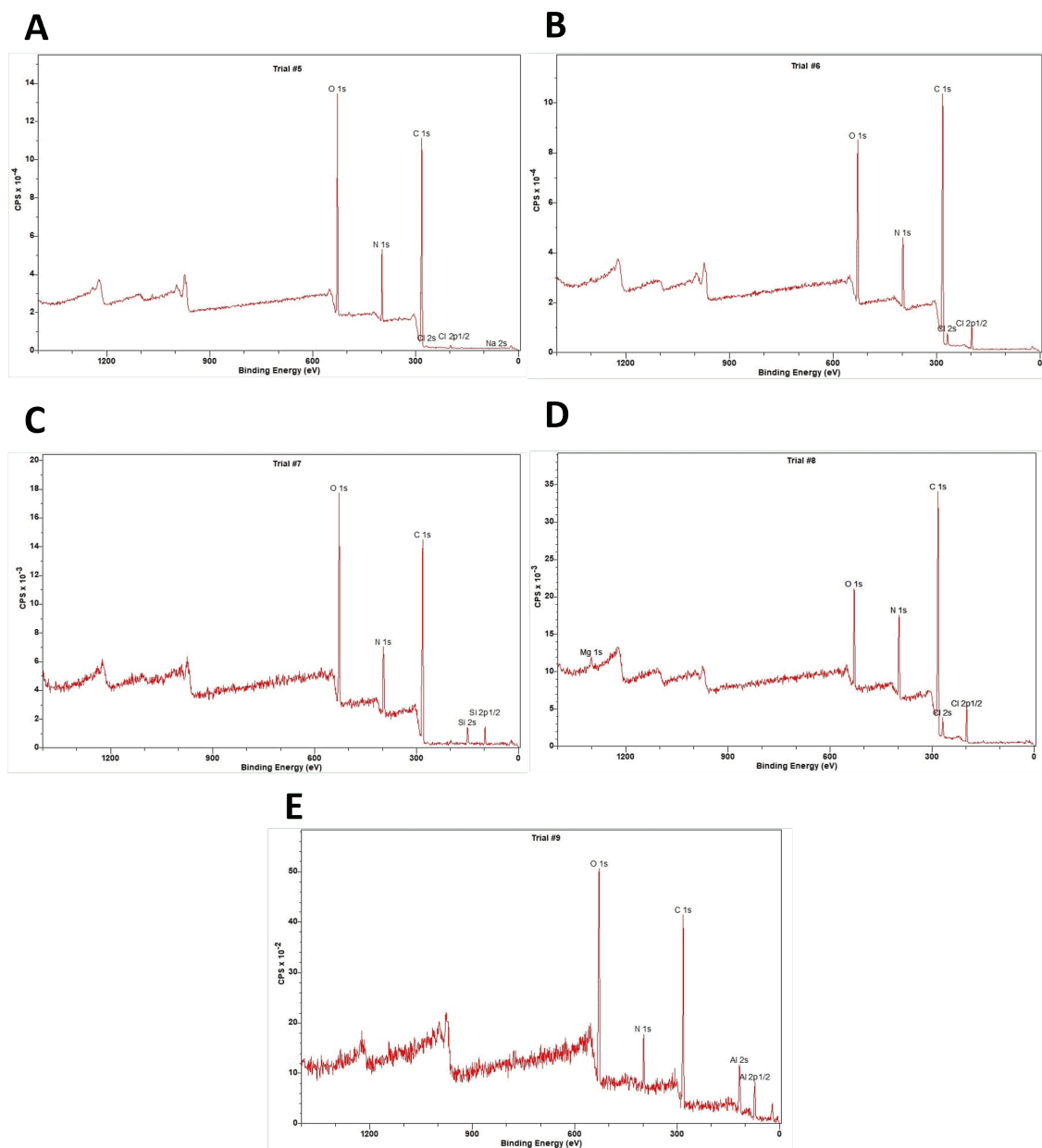


Figure B.2: XPS of formulations, A - trial#5, B - trial#6, C - trial#7, D - trial#8, E- trial#9

EXCITATION-EMISSION MATRIXES

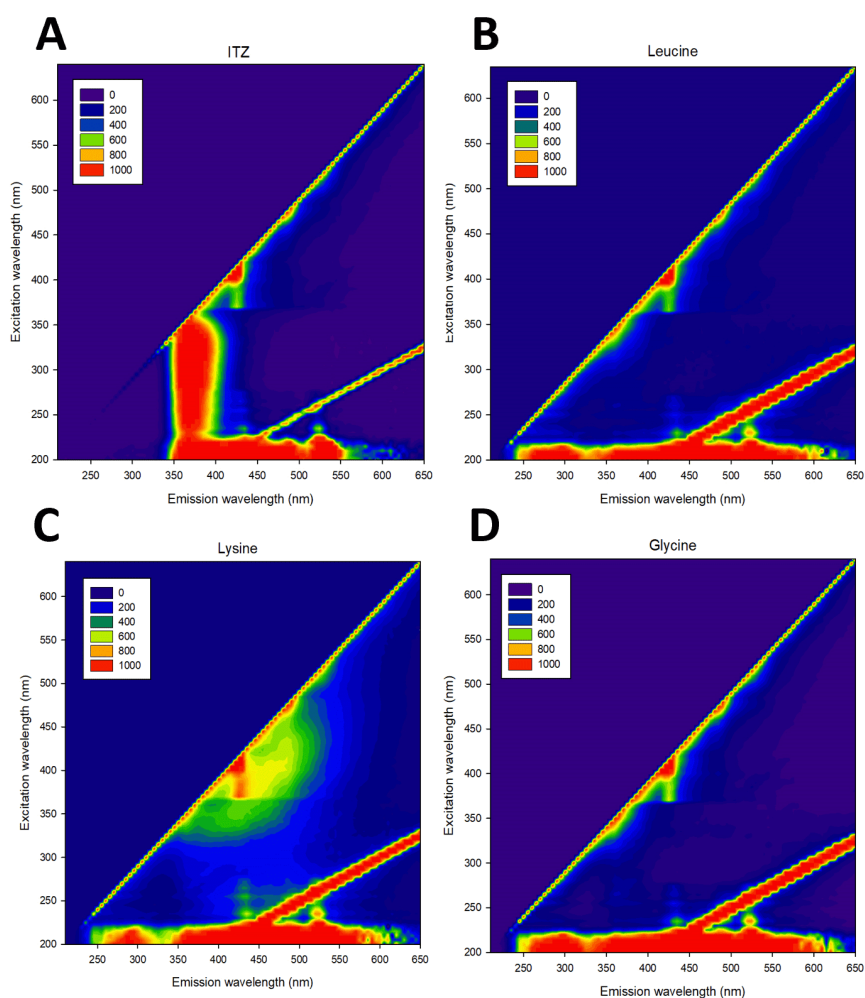


Figure C.1: EEMs of single components, A - Itraconazole, B - Leucine, C- Lysine, D - Glycine

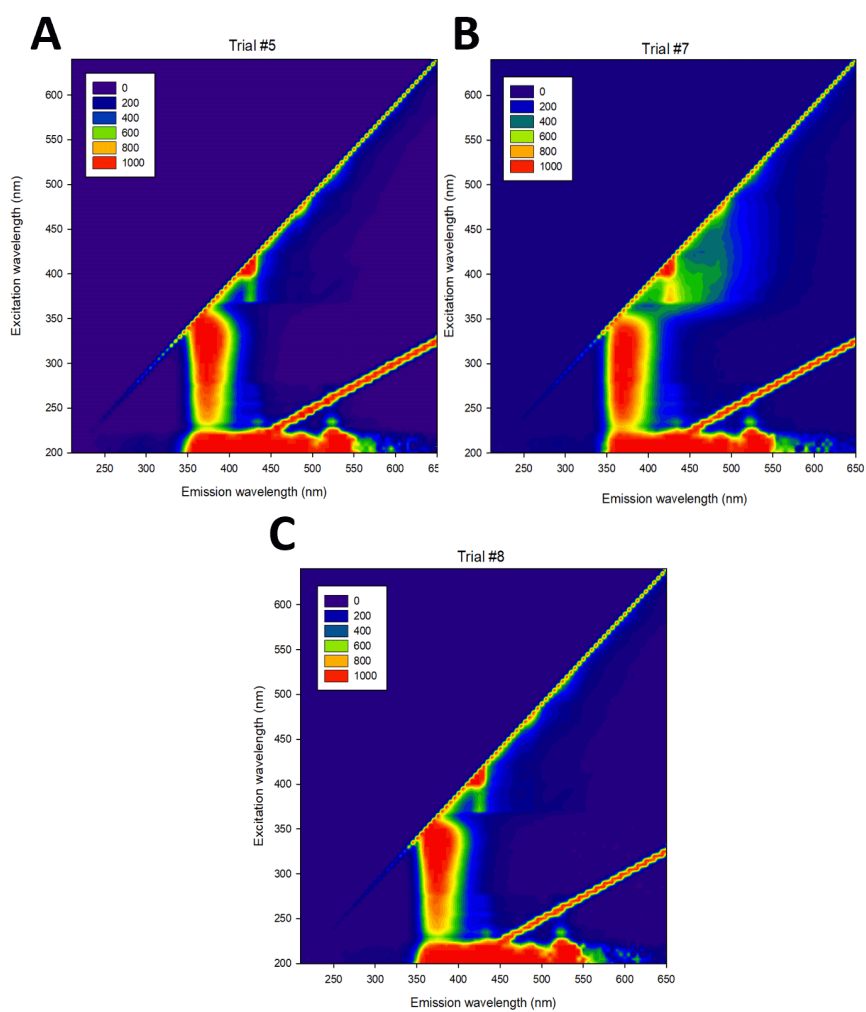


Figure C.2: EEMs of formulations, A - Trial #5, B - Trial #7, C - Trial #8

D

SUPPLEMENTARY SEM MICROGRAPHS

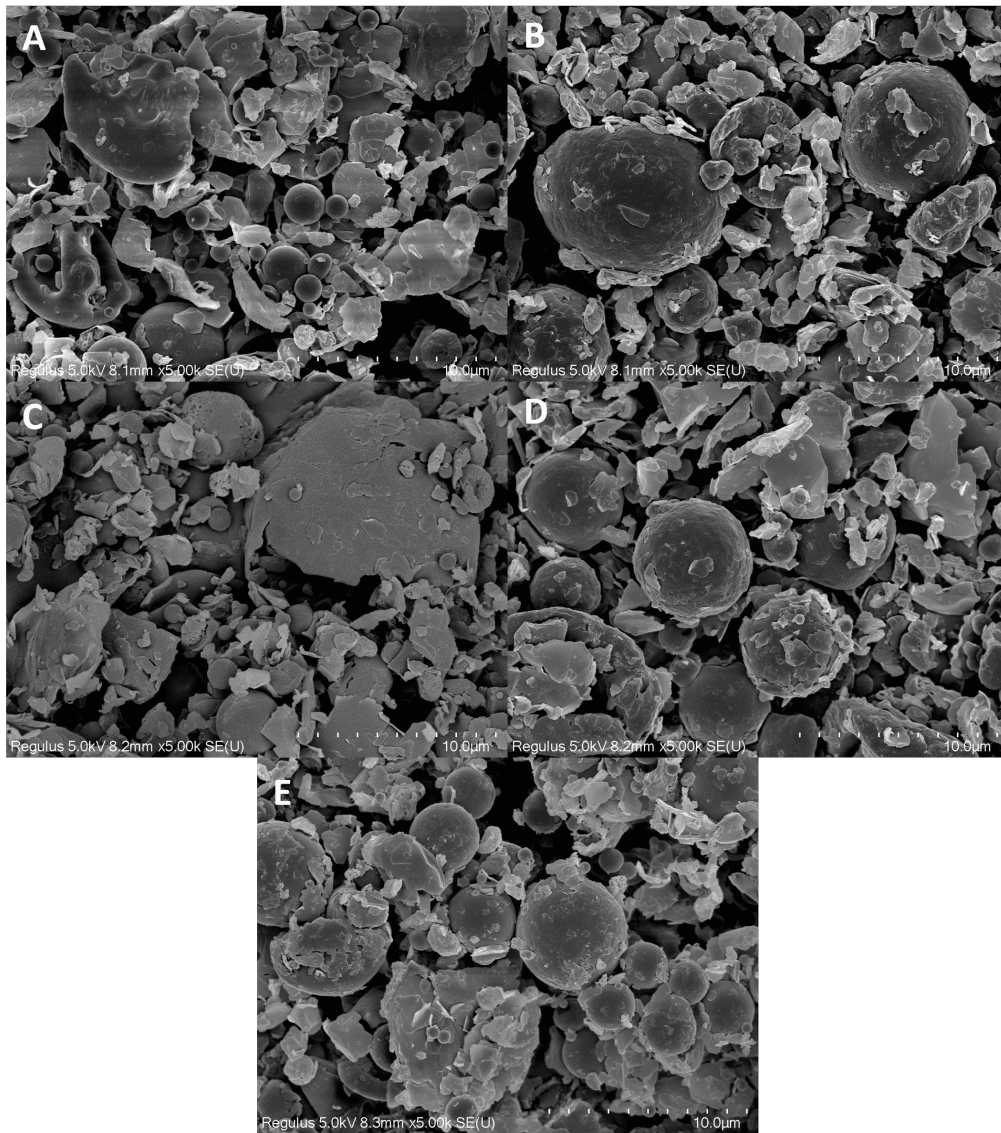


Figure D.1: SEM micrographs at 5000x magnification, A trial#12, B- trial#13, C-trial#15, D-trial#16, E-trial#18

EMPIRICAL MODELING - OUTLIERS AND RESIDUALS ANALYSIS

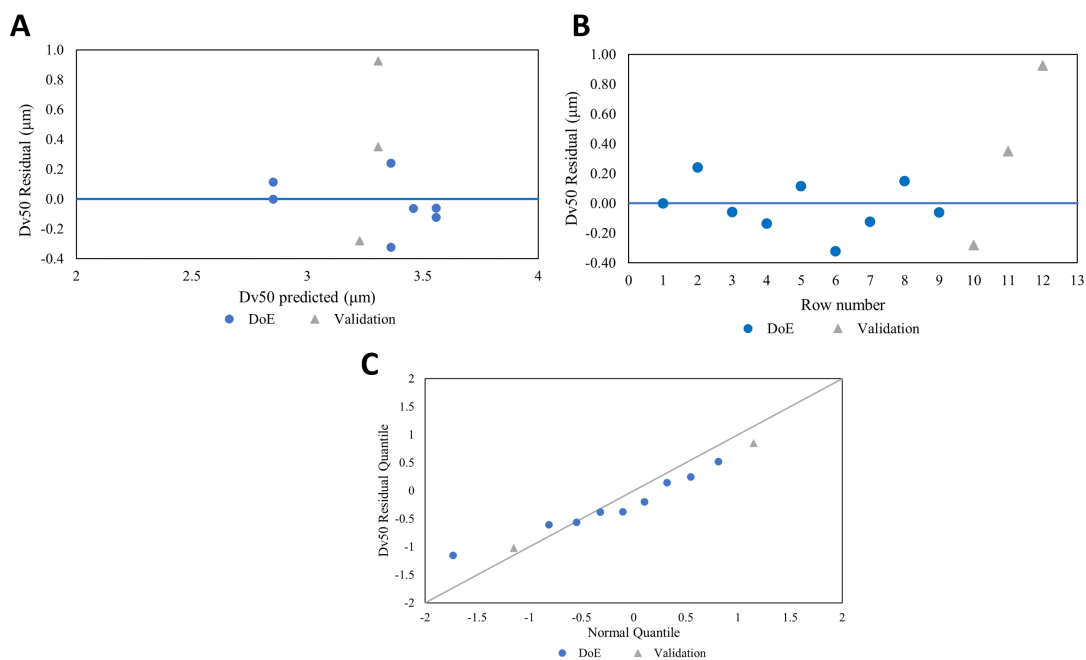


Figure E.1: Residuals analysis for the D_{v50} , A - residuals vs predicted, B- residuals vs row number C - normality plot

Table E.1: Validation dataset actual results, model prediction and relative error

Trial	Predicted D_{v50} (μm)	Actual D_{v50} (μm)	Error (%)	Predicted FPF (%)	Actual FPF (%)	Error (%)
#19	3.2	2.9	9.5	53.1	50.2	5.8
#20	3.3	3.7	9.6	46.5	51.4	9.6
#21	2.5	3.4	26.9	48.0	49.7	3.4

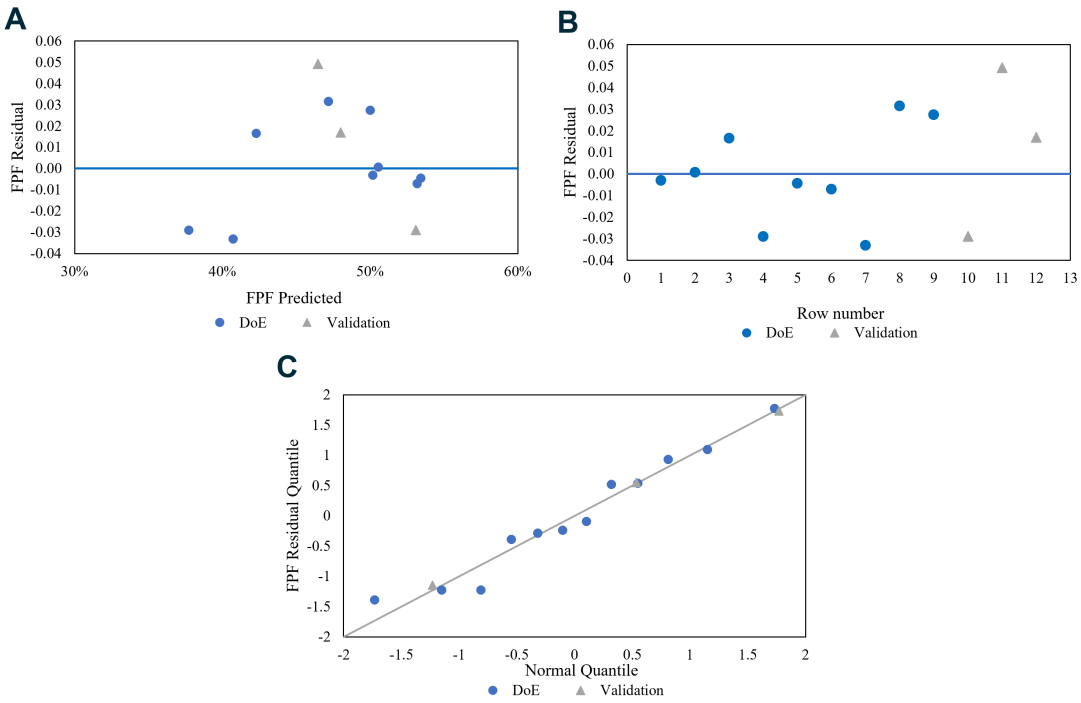


Figure E.2: Residuals analysis for the FPF, A - residuals vs predicted, B- residuals vs row number C - normality plot

