**Sofia da Silva Santos Rebocho Monteiro**
BSC in Chemical and Biochemical Engineering

# Air Stripping Process for Organic Solvent Removal
# Pharmaceutical Process Unit Development

Master dissertation for diploma obtainment in Chemical and Biochemical Engineering

Advisor: Doutor Mike Ugwoke, TEVA
Co-advisor: Doutor Kambiz Farbod , TEVA
Co-advisor: Prof. Doutor Mário Fernando José Eusébio, FCT-UNL

Júri:

President: Prof. Doutor Manuel Luís de Magalhães Nunes da Ponte
Examiner: Prof. Doutora Isabel Maria Rôla Coelhoso
Member: Prof. Doutor Mário Fernando José Eusébio

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

**March 2018**

**Sofia da Silva Santos Rebocho Monteiro**
BSC in Chemical and Biochemical Engineering

# Air Stripping Process for Organic Solvent Removal
# Pharmaceutical Process Unit Development

Master dissertation for diploma obtainment in Chemical and Biochemical Engineering

Advisor: Dr. Mike Ugwoke, TEVA
Co-advisor: Dr. Kambiz Farbod, TEVA
Co-advisor: Prof. Doctor Mário Fernando José Eusébio, UNL-FCT

Júri:

President: Prof. Doctor Manuel Luís de Magalhães Nunes da Ponte
Examiner: Prof. Doctor Isabel Maria Rôla Coelhoso
Member: Prof. Doctor Mário Fernando José Eusébio

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

# Copyright

Air Stripping Process for Organic Solvent Removal - Pharmaceutical Process Unit Development

# Acknowledgments

I would like to dedicate this work to my father, wishing he could have accompanied my academic path.

To Mário Eusébio, my teacher advisor, I would like to highlight and thank the degree of attention and motivation, despite time and distance, throughout the development of the thesis and for always keeping the office door open for every question and doubt I had.

My gratitude and admiration to Mike, who saw the potential in me as an addition to his team, giving me the opportunity and tools to grow as a person and as a professional. To Kambiz, thank you for accompanying every step of the project, all the unwavering guidance and incentive. To my dear office colleagues and friends, thank you for the laughs and the chit chats making work enjoyable and productive. To all TSA group, I will never forget this year and amazing positive vibe. From R&D group, Cheng Cheng and Elena I appreciated all the support and friendship and of course all the heavy lifting help in the pilot plant.

To my partner, João, thank you for these years of support even in all my moody days, everything was made easier thanks to you. To all my friends, Rita, Vânia, Sofia and Justine, thank you for making this life journey agreeable and fun.

Last but not least, to my family I would like to express the utmost gratitude, nothing would have been possible without you. To my mother, Isabel, thank you for always supporting my every step, and siblings, Filipe and Ana, for always giving me so much strength throughout my stay in the Netherlands, not forgetting my nephew, Jonas, which I grew so attached to and brought so much daily joy.

# Resumo

Um produto oncológico farmacêutico complexo está a ser desenvolvido na TEVA, Haarlem bem como toda a envolvente de engenharia de processo e manufactura. Devido à pobre solubilidade da substancia farmacêutica activa, um solvente orgânico é usado durante a fase inicial de constituição e necessita ser posteriormente removido. Devido a longos processos de remoção, certos parâmetros chave do produto são comprometidos, tal como a estabilidade da nano suspensão e precipitação indesejada de cristais do composto activo.

O propósito deste estudo foi a determinação de viabilidade de incorporar uma coluna de enchimento no actual processo de manufactura, cuja implementação bem-sucedida resultaria na aceleração de remoção do solvente por desabsorção. Vários testes à coluna em escala piloto foram realizados, dados experimentais foram recolhidos e analisados, de modo a determinar as condições operacionais e hidrodinâmicas da coluna bem como a sua eficiência e redução do tempo processual com implementação deste equipamento.

Foi observado experimentalmente que, em presença da proteína e de nanopartículas ocorre uma redução da cinética de transferência de massa do solvente, devido ao incremento de resistência da interface e à limitação difusional atribuida ao modelo heterogéneo da nano suspensão. A introdução da coluna resultou numa optimização de processo significativa, reduzindo o tempo de remoção de solvente de 30 para cerca de 5 horas, valor este dependente das condições operacionais e montagem do sistema. A coluna foi adicionalmente dimensionada de modo a satisfazer a eficiência desejada sendo que uma coluna de 1.70 metros de altura consegue teoricamente reduzir o tempo processual para duas horas. Estas duas horas estão limitadas a um caudal máximo de 4L/min devido à formação de espuma, e 30°C para evitar a desnaturação da proteína. A operação unitária não destabilizou a nano suspensão, sendo que o tamanho de partículas e o índice de polidisperção se mantiveram inalterados.

Os resultados favoráveis são indicativo das vantagens em implementar uma coluna de desabsorção na linha de produção do fármaco oncológico e validam o benefício em continuar os estudos e futuro trabalho com esta unidade processual.


---


Keywords: Coluna de adsorção, fármaco oncológico, nano suspensão, cinética de transferência de massa, hidrodinâmica, desenho de experiências.

# Abstract

Oncological protein-drug complex is being developed in TEVA, Haarlem, as well as the encompassing manufacture process engineering buildup. Due to poor solubility of the studied active pharmaceutical ingredient, organic solvent is used during compounding stage and needs to be sub sequentially removed. Its long removal duration impacts key process parameters such as nano-suspension stability and formation of undesired crystals.

The overall purpose of this study was to determine the feasibility of implementing an air stripping process unit in the existing line of production which would be a breakthrough in the current process by accelerating the solvent removal. The column was tested in pilot plant scale in order to collect and analyze experimental data to determine column's operational parameters and hydrodynamic properties as well as its efficiency and overall process time reduction by air stripping unit introduction.

Presence of protein and nano-particles was proved to have delaying effect on solvent mass transfer rate due to increased interface resistance and diffusional limitation due to nano-suspension heterogeneous model. The introduction of the column experimentally showed significant improvement in terms of overall evaporation reduction time; from 30 to about 5 hours, depending on the operating parameters and the set-up. The column was additionally designed to fit the model and could reach a theoretical value of 2 hours of stripping for a 500L solution at a maximum of 4 L/min and 30°C to avoid foam and protein denaturation respectively. The air stripping process did not de-stabilize the nano-suspension, showing constant particle size and polydispersity index.

The optimistic collected results validate the advantages of air stripping column implementation in the complex drug manufacture and suggest future work and research with column can be sustained.


---


Keywords: Air stripping column, API-protein bound drug complex, nano-suspension, mass transfer rate, hydrodynamics, design of experiments.

# Table of Contents

# List of Figures

# List of Tables

# Acronyms:

API – Active Pharmaceutical Ingredient
BMR – Batch Manufacturing Record
BSE/TSE– Bovine/Transmissible Spongiform Encephalopathy
CA – Compressed air
CMC – Critical Micelle Concentration
DF – Degrees of Freedom
DLS - Dynamic Light Scattering
DOE – Design of Experiments
EMA – European Medecines Agency
EU – European Union
FDA – Food and Drug Administration
FMEA – Failure Mode Effect Analysis
GC – Gas Chromatography
GF – Gas Flow Rate (L/min)
HEPT - Height Equivalent to Theoretical Plate
HPH – High Pressure Homogenizer
ISO – International Organization for Standardization
LEL – Lower Explosion Limit
LF – Liquid Flow Rate
PDI – Polydispersity Index
PSD – Particle Size Distribution
PW – Purified Water
R&D – Research and Development
SD – Standard Deviation
TPCH – Teva Pharmachemie
TFF – Tangential Flow Filtration
US – United States
VOC – Volatile Organic Compound
WFI – Water for injection

## Nomenclature:

$(A/W)$ – Air to water ratio

$\Delta P$ – Pressure drop (kg/m$^2$)

$\alpha$ – Packing parameter – constant specific to the packing type

$\beta$ – Packing parameter - constant specific to the packing type

$\varepsilon$ – Bed porosity

$\mu$ – Arithmetic mean

$\rho_L$ – Fluid density (kg/m$^3$)

$\rho_G$ – Gas density (kg/m$^3$)

$\sigma$ – Standard Deviation

$\sigma_T$ – Surface Tension (mN/m)

$A_c$ – Column cross sectional area (m$^2$)

$a$ – Specific interfacial area (m$^2$/m$^3$)

$a_p$ – Packing specific surface area (m$^2$/m$^3$)

$C_{Gi}$ – Concentration of VOC in the gaseous phase (kg/m$^3$)

$C_{Li}$– Concentration of volatile in liquid phase (kg/m$^3$)

$C_{Li(in)}$– Concentration of volatile in liquid entering the stripping column (kg/m$^3$)

$C_{Li(out)}$– Concentration of volatile in liquid exiting the stripping column (kg/m$^3$)

$C_{Li}^*$ - Liquid concentration in equilibrium with the gas phase concentration (kg/m$^3$)

$C_s$ – C-factor (based on tower superficial cross sectional area) (m/s)

$CP$ – Capacity factor (cSt/s)

$D_i$ – Diffusion coefficient

$D_{Li}$ – Diffusion coefficient of the compound in liquid ($m^2/s$)

$d_c$ – Internal column diameter (m)

$d_p$ – Effective particle diameter (m)

$F_p$ – Packing Factor (m$^{-1}$)

$G$- Gas mass flow (kg/s)

$G'$ - Gas mass flux (kg/m$^2$.s)

$g_c$ – Acceleration of gravity

$G_v$ – Gas mass flow rate per unit area (kg/s.m$^2$)

$h$ – Height of the stripping column (m)

$H_{ci}$ – Henry's law coefficient for compound i

$H_{ci(atm)}$ - Henry's law coefficient for compound i (atm)

$h_L$ – Liquid holdup

$L$- Liquid mass flow (kg/s)

$L_v$- Liquid volumetric flow (m$^3$/s)

$L'$ - Liquid mass flux (kg/m$^2$.s)

$LF$ – Liquid Flow Rate (L/min)

$n$– Packing parameter - constant specific to the packing type

$P$– Pressure (bar)

$|p|$ – Number of passes through the column

$ppm$ – Parts per million (µg/mL)

$K_L a$ – Transfer rate constant ($s^{-1}$)

$k_i$ – Compound-dependent constant

$R$ – Universal gas constant (J/K.mol)

$Re$– Reynolds number

$R_G$ – Resistance of mass transfer rate in gas phase boundary layer (s/cm)

$R_L$ – Resistance of mass transfer rate in liquid phase boundary layer (s/cm)

$R_G$ – Interfacial resistance (s/cm)

$R_T$ – Total resistance of mass transfer (s)

$S$ – Stripping factor

$T$ – Temperature (˚C)

$U_L$- Liquid viscosity (kg/m.h)

$u_L$- Liquid superficial velocity (m/s)

$V_L$ – Volume of the liquid ($m^3$)

$V_S$ – Volume of the packing ($m^3$)

$v$ – Kinematic viscosity of liquid (cSt)

$v_s$ – Superficial velocity at density averaged between inlet and outlet conditions ($kg/s.m^2$)

$v_G$ – Superficial velocity of gas through the tubing (m/s)

$W$ – Water/liquid flow rate (kg/s)

## 1. Framework and Motivation

Teva Pharmaceutical Industries Ltd. was established in 1901 and today has a world-leading position in the pharmaceutical field, focusing on the production of generic medicines. Headquartered in Israel, Teva is active in 80 countries worldwide. Teva Pharmachemie, Haarlem, the Netherlands site comprises Research & Development, Production and Sales & Marketing of generic medicines as well as specialty pharmaceuticals. Teva Pharmachemie is renowned for its combined expertise in the treatment of cancer and respiratory diseases with parenteral and aerosol drug production.

Presently in the Haarlem site, a generic protein-drug complex is being developed. This drug complex consists of a protein-bound form of API formulated into a suspension intended for intravenous administration after reconstitution, for the treatment of ovarian and breast carcinoma, non-small cell lung cancer and metastatic adenocarcinoma of the pancreas. It affects the normal dynamic of the cell's microtubules growth, promoting the assembly of microtubules from tubulin dimers and stabilizes microtubules by stimulating its polymerization [1]. This disrupts the normal cycle of cellular mitotic division, leading eventually to cell apoptosis. The API must be in a non-crystalline state since it has impact on its release rate to plasma phase.

The API (active pharmaceutical ingredient) hydrophobic nature poses a challenge to administer to humans. Various alternative systems have been developed, such as carrier systems and drug nanocrystals, to render poorly water soluble drugs injectable [2][3]. The carrier systems include delivery vehicles such as micelles, liposomes, nanoparticles of biodegradable polymers or nanohydrogels[4][5].

Teva Pharmachemie's drug product formulation dissolves the API in a volatile organic solvent, and bounds it to an effective soluble drug delivery system; a surface-active polymer biocompatible and has binding affinity with the API and binds to it extensively, making it thus an ideal nanomedicine carrier of the active substance. Due to poor solubility of API in water, formulation as nanosuspension is employed by applying high pressure homogenization to the solution, this way creating the nano-particles. This approach has been developed for pharmaceutical applications for the last decade and proven to solve problems of poor solubility and bioavailability as well as improve drug safety and efficacy [6]. The active ingredient absorbs onto the surface of the protein, providing charge and steric stabilization to the nanoparticles, preventing their aggregation. API aggregation and crystallization can compromise the final product quality and is to be avoided as will be seen ahead.

The used organic solvent used initially to dissolve the API needs to be removed in subsequent steps before obtaining the final product. Since the drug product will be directly administered through intravenous injection, it needs to be free of the hazardous solvents and microorganisms that can be harmful to patient's health.

To reduce the volatile organic solvent (VOC) level down to desired concentrations, currently ~30h of solution treatment through aeration is performed, which is considered very long. Despite the low vapor pressure and high volatility of the solvent, this process is delayed due to the surfactant nature of the protein which increases interfacial resistance and the diffusional limitation in presence of nano-particles. Removal by desorption of the organic solvent would be a significant improvement of the current manufacturing process by increasing solvent mass transfer rate.

Two different approaches were previously evaluated at Teva as to reduce the process duration by increasing the rate of solvent removal. These include the use of a thin film evaporation and the air stripping column. The former however proved not to be successful due to the foam formation when vacuum was applied to the system. Smaller air stripping column studies were also initiated and proven to be promising, however, the small-scale development was not further pursued due to project priorities.

This dissertation's main objective is to experimentally assess, using a large-scale air stripper column, the overall feasibility of implementing an air stripping process unit in the manufacturing process of the drug product and estimation of the overall time reduction of evaporative process by introducing the column. For this purpose, air stripping experiments will be performed at several conditions, aiming to determine the output of the column in terms of solvent removal efficiency, foam formation and nano-suspension stability. This is a challenging subject since the technique is not GMP-proven nor commonly used in pharmaceutical manufacturing. Moreover, the nano-suspension formulation needs to be strictly kept unchanged in terms of components, suspension stability (particle size) and sterility. The tendency of the nano-suspension to create foam due to the surfactant properties of used protein, also poses a challenge when dealing with air stripping columns unit operations.

The influence of operating parameters on solvent removal rate without compromising the efficiency of neither manufacturing process nor the quality of deliverable product will be additionally determined and optimized. Main manipulated parameters will be temperature, liquid and gas flow rate. The collected data will be analyzed through JMP statistical software to determine the critical parameters and significance of those studied factors.

Finally, the column will be designed to fit the requirement of solvent removal efficiency and the hydrodynamic considerations by estimation of column height and diameter respectively.

# 2. Drug Product Manufacturing Process

## 2.1 Narrative Description of Manufacturing Process

The drug product has a complex manufacturing process covering compounding over multiple days with various manufacturing techniques and unit operations.

The manufacturing unit operation can be divided into two main parts for convenience here: upstream (Figure 2.1) and downstream (Figure 2.2).

The upstream part focuses on the compounding of nano-emulsion from which the nano-suspension is obtained, and involves the following steps: crude emulsion compounding followed by creation of homogenized nano-suspension by applying high pressure and shear forces to the emulsion. The suspension will then be stabilized by aqueous dilution and lastly the organic solvent removed by evaporation.

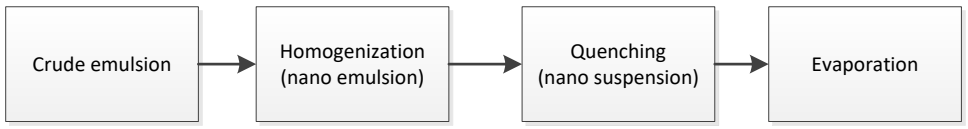


Figure 2.1- Upstream maufacturing of drug product

Before the preparation of the crude emulsion API is dissolved in a small volume of organic solvent and EtOH, necessary due to API's hydrophobic nature. This apolar phase is subsequently added to a protein and water solution and pre-saturated with the organic solvent. The crude emulsion will be formed by submitting the solution to low rotating shear forces. This bulk will then be converted into a nano-emulsion by homogenization using high pressure and shear forces. The equipment used will be a high-pressure homogenizer (HPH). During this manufacturing step, the bulk will recirculate through the HPH and heat exchanger in order to attain the desired temperature. The pressure and temperature used inside the HPH must be high enough to break the particles but must not exceed fixed values to avoid polymerization/oligomerization of the carrier protein.

The nano-emulsion must be subsequently converted into a stabilized nano-suspension by adding the quench solution and removing the solvent. This stability is dependent on the organic solvent content. This can be achieved either by evaporation, dissolving it in an aqueous phase or in this case both. The added aqueous face is a quenching solution of 1% sodium chloride which provides steric stabilization to the suspension by solvation effect. The evaporation is achieved by injection of compressed air in a slightly agitated vessel during approcimately 30h. This time must be reduced as much as possible to avoid API crystallization and nano-particle aggregation. Simplified flowsheet diagram of upstream process can be found in appendix A.

The second essential part, downstream, consists mainly of tangential filtration, which aims at concentrating the solution by removing additional WFI and organic solvent. It also includes sterile filtration and drying of the product by lyophilization. Downstream can be summarized in the following sequential steps; execution 0.2 µm pre-filtration, concentration of the bulk, execution of diafiltration, addition of protein stabilizers, sterile filtration, filling of vials and lyophilization shown in in Figure 2.2.

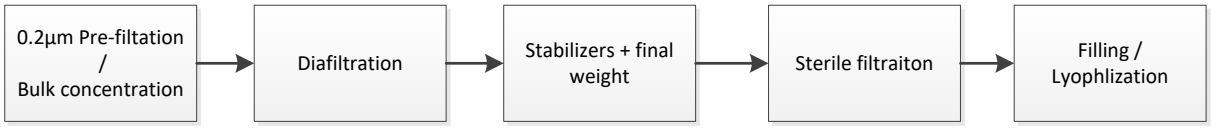


Figure 2.2 - Downstream maufacture of drug product

The remaining organic solvent is removed by tangential flow filtration (TFF). The TFF performs a concentration followed by continuous diafiltration using 0.1% NaCl diafiltration solution. This process also removes water and any API particles that may have crystalized, however, depending on the membrane's molecular weight cutoff the crystalized filter blockage can occur. A pre-filtration of the nano-suspension is for this reason necessary to filter large particles before concentration and diafiltration.

The nano-suspension is filled into glass vials which enter a lyophilization chamber in order to dry the suspension into a white to yellow sterile powder. It is subsequently sleeved and packaged closing the vials with rubber stoppers and aluminum seals. It must be stored in a cold environment and protected from light. Simplified flowsheet diagram of downstream process can be found in appendix B8.B.

## 2.2 Main Controlled Attributes during Manufacturing Process

### Particle Size and Nano-suspension Stabilization

A stable nano-suspension is, by definition, a solution were particle-particle interactions is brought to a level where Van der Waals attractive forces are less that the repulsive steric forces. Stability issues related to a nano-suspension can be categorized into physical and chemical stability. Physical instability common concerns are sedimentation, agglomeration and crystal growth of nano-particles and can be avoided through electrostatic and steric stabilization [7]. Chemical stability however relates to the possibility of chemical reactions and is drug specific. Studies however showed the studied drug nanosuspensions have excellent chemical stability.

One of the main focuses during the development stage was the obtaining of specified average particle size. API tends to aggregate and precipitate, which is why it is important to stabilize the suspension and maintain the attained particle size. The carrier protein adsorbs onto the surface of the API providing the solution charge and steric stabilization by having a high negative zeta potential (surface potential), this is expected since surface-active proteins are known to increase suspension steric stabilization [7]. The NaCl quench solution and the stabilization compounds, contribute to the

steric charge of the solution. The added quenching salt solution as referred will result in repulsion by the droplets mitigating agglomeration and crystallization of the API. Nano-suspension stability can be enhanced if transformed into solid form [7], this is one of the reasons why the suspension is lyophilized and the final product is stored and distributed as a powder for reconstitution.

### Filterability

Filterability is an important factor to be considered and maximize since it can compromise the manufacturing process by clogging the filters during the pre-filtration step. Filterability will decrease during VOC removal by evaporation, due to destabilization of the nanoparticle bulk and particle size increase. The crystallization of API will cause pre-filtration flux decrease by filter blockage. As mentioned above, pre-filtration is performed prior to execution of TFF in order to retain the particulate aggregates and avoid TFF filter blockage. The pre-filters however may also block, therefore, it is important to keep the nano-suspension as stable as possible during upstream processing.

### Sterilized Product

Since the drug product is directly administered via blood circulation it is essential to minimize the presence of microorganisms in the bulk prior to sterile filtration. It is important to keep the sampling line and equipment clean. All tubing and dedicated equipment valves must be rinsed with hot WFI in between productions. The equipment must be cleaned with repeated cycles of 70% EtOH and 80˚C WFI when it is taken to production. Cleaning between each production batch is performed to avoid cross-contamination from or to other products, since not all equipment is dedicated to this drug production only.

### Volatile Organic Solvent Removal Rate

The prolonged evaporation process will result in particle size increase and therefore decrease filterability, which is considered an undesirable attribute during manufacture. It is essential however to keep the nano-suspension in the evaporation phase for sufficient time to reach the desired solvent concentration reduction of 89% of initial concentration. Evaporation manufacturing step must be for this reason developed to attain the removal of desired organic solvent in the least amount of time possible. Decreasing the solvent removal rate would be an improvement to some of the most important controlled parameters during manufacture such as stability, filterability and API recovery. For this reason, effort was put in researching and developing a more fitting and time efficient solutions to accelerate the solvent evaporative phase.

## 2.3 Volatile Organic Solvent Evaporation

The solvent removal process is carried out in a 500L stainless steel vessel after addition of the quenching solution. Currently, compressed air is enters the vessel via a spray ball connected to an inlet on the top of the vessel, allowing the air to come in contact with the VOC, which due to its volatile nature

will be stripped away by the gas from the liquid phase. This process needs optimization since the hold-up time of volume bulk in the vessel is above desired.

To bring the solvent level down to specified concentrations, currently ~30h of contact time between the phases is required. Despite the solvent low vapor pressure and high volatility, this process is delayed both due to the protein surfactant properties of the protein which increases interfacial resistance and presence of nanoparticles. The longer the solution stays in the vessel to reduce VOC concentration, the more API will tend to aggregate and crystalize. This will cause a decrease in solution filterability and can even result in product and material losses due to filter blockage. Furthermore, the entire manufacturing process is delayed which translates in additional production costs and no capacity to promptly answer predicted market demand of the drug product. Since optimizing the evaporation process rate will assure product quality and improve cost of goods, more research on it is valuable.

## 2.4 Legislation and Regulation

National and global pharmaceutical regulatory authorities such as FDA and EMA, from USA and EU respectively, must ensure stringent legislation and regulation on pharmaceutical manufacture, storage, distribution, sale and other related activities, to ensure public health and safety.

The basis of the organized and qualified manufacturing operations for consistent production and control are the Good Manufacture Practices (GMP). Training qualification in GMP guidelines and principles which must be observed during manufacture according to the quality standards is provided by TEVA. Audits and inspections are performed regularly to assure compliance and product quality.

Teva Pharmachemie must ensure the quality of manufactures and/or suppliers of equipment, excipients and active pharmaceutical ingredients by demanding certificates of conformance for each purchased material and performing manufacturer/supplier qualification and/or on-site audits. USP Class VI (United Stated Pharmacopeia), test certificates and BSE/TSE statements are some of the required quality certificates for purchased material and equipment. Depending on the type of material (contact/non-contact material, disposable etc.) and excipients the requirements may vary. Additional sampling and testing is also performed in-house before its release to production. FMEA risk assessment controls are performed to evaluate the impact of newly introduced items.

All controlled waste from the industrial process is monitored by national laws and regulations. The solvent used in the manufacturing process inevitably becomes waste and can be released via waste water or via the air removal system in the production area to the outside environment in a controlled way. Both waste disposal approaches are regulated and strict requirements upon Dutch regulation.

Separate collection of all solvent containing waste will prove logistically very challenging if not impossible. Disposal requirements for solvent concentrations in waste water in Dutch regulations are applicable.

The current industry practice for removal of organic solvents from waste water is the application of techniques that assure 99% reduction of solvent mass coming out of the process via waste water. The solvent removed from the process by evaporation and emitted via air removal system, complies with the Dutch emission guideline for air “Nederlandse Emissie Richtlijn (NeR)” requirements. The worst-case emission of solvent for the emission installation was calculated to be 5 mg/m$^3$.

# 3. Mass Transfer and Air Stripping Unit

## 3.1 Air Stripping/Desorption Units

To remove a volatile component dissolved in a liquid phase, stripping also referred to as gas desorption, unit operations using gas-liquid contacting equipment are implemented. These operations increase contact between the liquid and the gas, so that the VOC can be stripped from the liquid phase and exit the equipment as exhaust gas phase. Depending on the process and toxicity of components to be removed, additional treatment to the gas may be necessary; this follow up treatment usually requires the use of activated carbon or systems to oxidize the organics in the gas stream [8].

These processes are extensively used for wastewater treatment, in chemical and gas/oil industry. Components with reasonable equilibrium vapor pressures at ambient temperature can also be removed from a liquid phase by desorption/stripping [9].

Many techniques have been developed for gas stripping processes, namely air stripping columns, evaporators and aerators. All these unit operations can be used to remove a gas dissolved in a liquid phase, and selection of adequate technology can depend on criteria such as the type of components to be stripped, boiling point of volatile organic compounds, composition of solution and financial limitations.

Air stripper packed columns are extensively used in chemical engineering process units to achieve higher mass transfer rates of the volatile components from the liquid phase to a gas phase.

The fundamental internal components of a counter-current air stripping column include a liquid distributor or aeration nozzle at the top of the column, a gas distributor or gas inlet system and the packed bed or tray allowing the contact between phases and support systems. Optionally it can contain supplementary components, namely liquid redistributors in between packed beds, process control measurement points throughout the column (such as temperature and pressure drop) and demister at top of the column to prevent the escape of water vapor with the exhaust gas.

A column can be designed as either a tray column or a packed column depending on its application. Figure 3.1 provided by Sulzer [8] serves to illustrate an air stripping column with both packing options and internals.

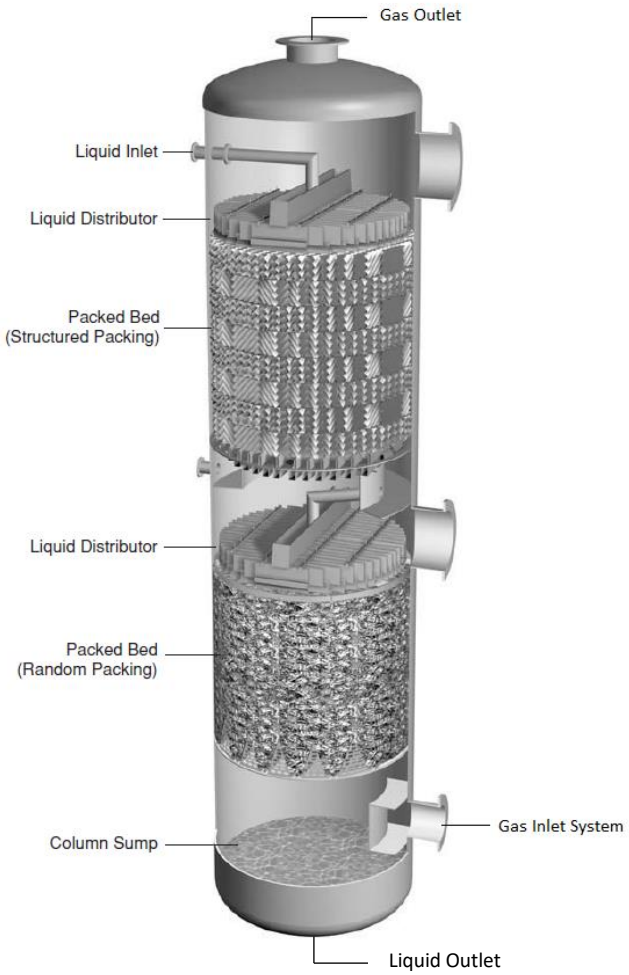


Figure 3.1 - Ilustration of air stripping column [8]

Fouling, which consists of accumulation of solids on the surface of the packing material, must be taken in account when dealing with a packed column. Since the efficiency will decrease and the column quickly loses mass transfer area with fouling, periodic cleaning procedures should be performed on the column. Acidic cleaning solutions can be formulated to include corrosion inhibitors when using metallic equipment [10]. Depending on the implementation of the column (one pass or continuous process), the cleaning process will vary, assuring no interruption of production is required. However, in pharmaceutical industry this cleaning process needs to be thorough since cross-over of material and microbial particles needs to be strictly controlled. A solution would be to use random packing material type with an easy loading and un-loading mechanism to use the packing beds as disposable or a chose a material that is resistant to autoclave temperatures. Cleaning validation also needs to be performed prior to column implementation in GMP environment.

## 3.2 Mass Transfer Principles and Equations

Mass transfer between phases is a kinetic process modeled by mathematical equations that occurs with a relative motion between chemical species driven mainly by concentration gradients. This

process may or may not involve chemical reactions, where species may be produced or consumed. This work will focus on the specific case of mass transfer as a multiphasic liquid-gas mass transfer by evaporation and takes into account evaporative limitation by presence of nano-particles dispersed in the liquid phase – heterogeneous model. For simplicity purposes the compound being diffused shall be referred as i. The theoretical knowledge that follows is essential since the rate of evaporation of the organic solvent will depend on the rate of mass transfer from the liquid to the gas phase.

## Diffusivity

The mass transfer between two phases occurs by diffusion, and if there is a fluid motion (external mechanic driving forces) this can also occur by convection. The overall mass transfer for a volatile compound, considers both convection and diffusion mass transfer [11].

The diffusive flux, governed by Fick's laws of diffusion, will depend on concentration gradient and diffusion coefficient. In parallel, convective flux will depend on solution average velocity. The diffusion coefficient can also be thermal diffusivity if the motion is occurring by heat transport.

The diffusion coefficient can be predicted, and is dependent on other parameters that can be modified during process in order to increase diffusion rate of compound *i*. Assuming binary system, diffusion coefficient, $D_i$, relates on temperature, $T$, and pressure, $P$, according to the eq. 1:

$$D_i \propto \frac{T^{\frac{3}{2}}}{P} \qquad \text{(eq. 1)}$$

This proportionality relationship is relevant to the air stripping process since it gives indication to which parameters should be manipulated to maximize diffusivity and overall mass transfer rate.

## Henry's Law

During evaporative process, compound *i* will be transferred from a liquid phase to a gaseous phase, since its concentration in ambient air is much lower than on the solution. This phenomenon follows the Henry's Law which states [12][13]:

*..."that water takes up, of gas condensed by one, two, or more additional atmospheres, a quantity which, ordinarily compressed, would be equal to twice, thrice, &c. the volume absorbed under the common pressure of the atmosphere."*

This means the concentration of a volatile component in the gas-phase will be in equilibrium with the concentration in the liquid-phase:

$$H_{ci} = \frac{C_{Gi}}{C_{Li}} \qquad \text{(eq. 2)}$$

Where $H_{ci}$ is Henry's law coefficient for compound *i*, $C_{Gi}$ is the concentration of volatile *i* in gas phase and $C_{Li}$ is the concentration of volatile *i* in liquid phase.

The Henry's law coefficient also referred to as Henry's constant measures the overall volatility of the VOC and is one of the equilibrium data necessary to the design of a stripping column. In literature different forms of Henry's law can be found, often partial pressure is used instead of the gas concentration in gas-phase. Therefore, there must be extra attention to Henry's law coefficient units when determining it. In this report most presented equations use Henry's coefficient as the dimensionless value as presented in eq. 2. Henry's law coefficient for a specific compound is hard to determine since it is dependent on liquid-phase mixture composition that alters $H_{ci}$ either by surface activity effects or through solute-solute interaction. Furthermore there is a strongly nonlinear variation of Henry's law constants with temperature [14]. Theoretically the higher the Henry's law coefficient the higher will be the rate of evaporation.

## Overall mass transfer coefficient

It is important to highlight that it is the overall mass transfer coefficient, $K_L a$, that ultimately controls the rate of the removal of the VOC. It is a diffusion rate that relates the mass transfer rate between two phases, mass transfer area, and concentration change as driving force.

The general equation for the rate of two-phase mass transfer across the interface in the air stripper is given by the following equation:

$$\frac{1}{V_L}\frac{dm}{dt} = -K_L a\,(C_{Li}^{*} - C_{Li}) \qquad \text{(eq. 3)}$$

Where $V_L$ is the volume of the liquid, $\frac{dm}{dt}$ is the mass transfer rate, $C_{Li}^{*}$ is the liquid concentration in equilibrium with the gas phase concentration and $C_{Li}$ is the concentration of *i* in liquid phase.

For highly volatile compounds, with high Henry constants, the overall rate of transfer is controlled by the transfer rate at the liquid-phase boundary. In this study case since the organic solvent is relatively insoluble in the liquid bulk, the mass transfer is liquid phase controlled. Any effort aimed at increasing the mas transfer rate should be directed to improving the liquid-phase mass transport coefficient [15].

It is important to notice that the presence of nanoparticles dispersed in the liquid phase will have an impact on the mass transfer rate, since it is in fact a three-phase mass transfer. The overall mass transfer kinetic model will also differ in complexity since it will be dependent on parameters such as particle size and their dispersed volume fraction. The heterogeneous model has therefore rate-limiting effect on the overall mass transfer rate [16].

## 3.3 Governing Parameters of Mass Transfer Rate

The governing parameters of mass transfer are relevant to determine prior performance of design of experiment studies to assess which are applicable and can be used to optimize the process without compromising the drug product quality attributes.

### Temperature and Pressure

Temperature strongly influences multiple variables during the air stripping process namely Henry's law coefficient, mass transfer coefficient, diffusivity of the solute and viscosity of the solution, which will lead to a mass transfer rate increment. However, protein stability was observed at process temperature 15-30˚C up to 48h, and elevated temperatures can lead to denaturation and polymerization of the protein.

Henry's law coefficients depend upon temperature and usually follow an Van't Hoof's relationship [17][18]:

$$\log H_{ci} = -\frac{\Delta H_0}{RT} + k_i \qquad \text{(eq. 4)}$$

Where R is the universal gas constant, T is temperature and $k_i$ a compound-dependent constant. The equation term $\Delta H_0$ is the enthalpy change resulting from the dissolution of the compound in water. This equation allows re-calculation Henry's equation with temperature, and henry's coefficient from literature data[19]. The volatility literature values however are limited to solvent in water at 20°C data only.

Low pressures result in lower saturation concentrations and reduce the boiling point of the solvent, i.e. lowering the pressure allows the column to operate at a lower temperature for the same evaporation efficiency.

### Surface Area

The mass transfer rate of a specific compound or mixture in a vapor-liquid contacting system is given by the product of specific interfacial area with mass transfer coefficient and driving force [18]. The surface area of the packing provides the air-to-water interfacial area, which is why the choice of the packing will have an influence in the column's efficiency.

### Resistance of interface

The organic solvent used, is very volatile and has low boiling point, therefore in air stripping processes the evaporation rate is high. However, for this specific drug manufacture, evaporation process step takes longer than would be predictable. This can be explained by the presence of a surface-active

component in the mixture, the carrier mechanism protein as well as the presence of nano-particles which suppresses evaporation through reducing the accessible interfacial area [20].

Surfactant molecules replace some of the water molecules in the surface and the forces of attraction between surfactant and water molecules are less that those between two water molecules; hence the concentration force is reduced [21][22]. The organic/water interfacial substance tensions are therefore reduced, forming a barrier layer at the interface increasing its resistance to mass transfer during air stripping processes. This interface phenomenon will drastically slow down the solvent removal rate. The surfactant protein acts as the biological carrier of the API also providing charge and steric stabilization to the mixture and with continuous increase of its concentration, mass transfer rate will decrease until reaching critical micelle concentration point (CMC), where it will remain constant due to complete coverage of the interface. Anderson [23] showed the reductions in apparent Henry's constants and how the presence of surfactants substantially reduced the partitioning of volatiles components from liquid interface to the vapor phase. In liquid/liquid systems mass transfer rates were also shown to be reduced with the presence of surfactants due to the change of fluids dynamics and formation of an adsorption layer [17].

The protein concentration used in the bulk solution for the tested drug product is exceedingly above the CMC, decreasing its surface tension down to 10 mN/m when comparing it to the surface tension of pure water.

Presence of surfactants can also be accountable for foam formation. It is important to control and create methodologies to avoid excessive foaming, that results in flooding and pressure drop in stripping column compromising it efficiency [24].

Three well-known theories for gas transfer are used as air stripping modeling; two film, penetration and surface renewal. The simplest model, the two-film model is often used for absorbers and strippers. The theory states the resistance to mass transfer rate consists in the sum of the individual resistances of the two phases [25], and assumes that equilibrium exists at the interface assuming interfacial resistance as negligible, since interfacial resistance of a clean interface is very small [26]. However, the presence of surfactants will have to consider the resistance when estimating mass transfer rate. The total resistance of mass transfer taking into account interfacial resistance is given by:

$$R_T = \frac{1}{K_L a} = R_L + R_G + R_I \qquad \text{(eq. 5)}$$

Where $R_T$ is the total resistance of mass transfer, $K_L a$ the transfer rate constant, $R_L$ the resistance of mass transfer in liquid phase boundary layer, $R_G$ the resistance of mass transfer rate in liquid phase boundary layer and $R_I$ the interfacial resistance

Several studies on partitioning of the VOC into the micellar phase of the surfactant [27], ie. micellar partition coefficients, and its effects on Henry's coefficient have been performed and how it increases the mass transfer and interfacial resistance with increase of surfactant concentration [28] in solution over their critical micelle concentration. A close case study example, consisting in a chlorinated compound removal from micellar solutions, describing the partitioning of the solute between the aqueous solution and the micelles, showed that the presence of surfactant significantly reduced Henry's law coefficient [29].

## Viscosity and Density

Viscosity and density are properties of every solution which influence its fluid dynamics; as so, they dictate the resistance that the fluid has to flowing or sheering out. The viscosity will drop the kinetic energy of the solution molecules have, and therefore, lessen the tendency the particles will have to escape the liquid by evaporation. The viscosity can be reduced with increase of temperature and pressure. For this drug product the protein concentration of amount to an average viscosity of 1.04 centipoise at process operation temperature. This close to water viscosity won't have therefore significant impact on mass transfer rate.

## Air-to-water ratio

The ratio of air-to-water volumetric flow entering the air stripper will control the removal rate of the VOC. The more turbulent the flow inside the column is, the higher the driving force will be contributing to an overall higher mass transfer coefficient. However, if the air flow is too great the column might flood impacting severely the column efficiency. The minimum air-to-water ratio required for stripping depends on the solvent's Henry's law coefficient, and is given for a counter-current process by [30] the following mass balance:

$$\frac{A}{W} = \frac{C_{Li(in)} - C_{Li(out)}}{(H_{ci})C_{Li(in)}} \qquad \text{(eq. 6)}$$

Where, A/W is the air-to-water ratio, $C_{Li(in)}$ the compound *i* concentration entering the column, $C_{Li(out)}$ the compound *i* concentration exiting the column and $H_{ci}$ Henry's coefficient of the compound *i*.

The air-to-water ratio (A/W) can also be estimated relating it to the stripping factor by the following approximation:

$$S = 0.00075 H_{ci(atm)} \left(\frac{A}{W}\right) \qquad \text{(eq. 7)}$$

Where S is the stripping factor, $H_{ci(atm)}$ the henry coefficient of the compound *i* and A/W the air-to-water ratio.

For this eq. 7, $H_{ci(atm)}$ units must come in atm. The stripping factor is the ratio of the slopes of equilibrium and operating lines and is given by the Kemser-Brown equation also used when designing

absorption columns [31]. It determines the theoretical ability of the stripper to remove a specific volatile compound. However, using this equation has a big margin of error associated since as mentioned previously it is very difficult to accurately determine Henry's law coefficient in a solution.

It is possible to operate at lower (A/W) ratios if working at higher temperatures and vice-versa. This relationship is important since it gives information on adequate flow ranges to be applied to the experimental tests and DOE. Furthermore, it minimizes operation costs and design of air stripping column.

In sum, theoretically, the rate of solvent removal is optimized by increase in: temperature, specific interfacial area, turbulent flow and (A/W) ratio, and with decrease in: pressure, resistance of the interface and viscosity. The column should also be designed and operated correctly to achieve its optimal performance. With this background information development and planning of a coherent experimental study by testing the equipment available for evaporation and combining the different possible parameters without compromising the efficiency of the process neither the quality of the final product can be performed.

## 3.4 Hydrodynamic Properties of Packed Column

Knowing upfront column's hydrodynamics is an advantage to know the correct column operation routine and maximize its efficiency. Some limitations on the pilot scale however will only allow a certain range for the tested parameters. The adequate choice of column size and its internals will match desired removal efficiency and reduce processual costs.

Main hydrodynamic properties of packed columns include: pressure drop, liquid holdup, column loading and flooding/flood point. Hydrodynamics of column will determine its specific working flow rates and operational conditions [8]. The higher the gas flow rate the more efficient the evaporative process becomes. However, this parameter is limited to a maximum due to pressure drop that builds up with a high air-to-water ratio and increases the resistance that will be encountered by the down-flowing liquid, which can lead to damage of the packing in the column. If the operating gas flow rate is too high it can furthermore lead to flooding of the column.

The hydrodynamics depend on several operational factors such as the liquid and gas loads, the packing material used and the column's diameter.

As the flow rate of a liquid or gas is increased, the pressure drop increases; the liquid fills up the column and the space for air flow is reduced. The packing should provide for easy liquid drainage and have a low pressure drop for gas flow rate. The pressure drop is most of the times represented as a quotient between pressure and the height of the packing ($\Delta P/h$) since they are correlated terms. The pressure drop of a gas flowing upwards the air stripping column counter-currently to the liquid flow rate is represented graphically in Figure 3.2.

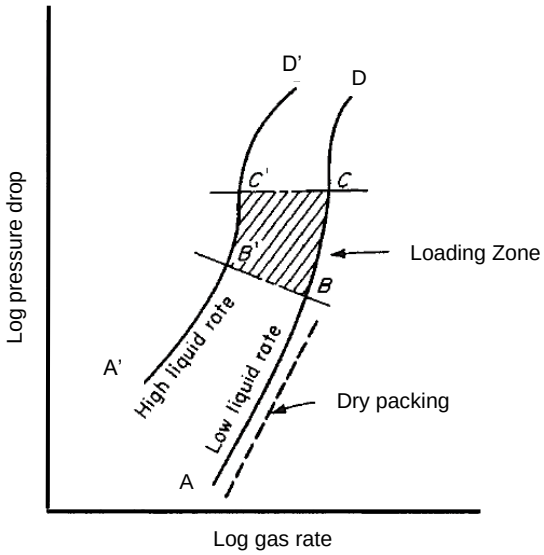


Figure 3.2 - Pressure drop characteristics of packed columns [8]

In Figure 3.2 the crossed area is the loading zone delimited by BB' (loading point) and CC' (flooding point). The area between AA' and BB' is also known as preloading region in which column is being operated at sub-optimal loads (low air-to-water ratios) and the area between CC' and DD' is the flooding region in which the pressure drop sharply increases and the entire column is filled with liquid impacting air stripping process.

When operating in countercurrent and dry packing, the estimation of pressure drop at which liquid and gas flow rate through the column, we rely on a friction factor correlation attributed to Ergun [8]. The pressure drop for dry packing, or at very low liquid flow rates, is indicated in the region AB. It is result of gas flow rate through a series of variable openings in the bed. Pressure drop at these conditions is given by the following equation [32]:

$$\frac{\Delta P}{h}\left[\frac{d_p g_c}{\rho_L v_s}\right]\left(\frac{\varepsilon^3}{1-\varepsilon}\right) = 150\ \frac{1-\varepsilon}{Re} + 1.75 \qquad \text{(eq. 8)}$$

Where, $\Delta P$ is the pressure drop, $h$ is the height of the column, $d_p$ the effective particle diameter, $g_c$is the acceleration of gravity $\rho_L$ is the liquid density, $v_s$the superficial velocity at density averaged between inlet and outlet conditions, $\varepsilon$ is bed porosity and $Re$ is the Reynolds number.

In irrigated columns the pressure drop correlation is given by[32]:

$$\frac{\Delta P}{h} = \alpha(10^{(\beta L'/\rho_L)})\left(\frac{G_v^2}{\rho_G}\right) \qquad \text{(eq. 9)}$$

Where $\alpha$ and $\beta$ are constants specific to the packing type $L'$ and $G_v$ are the liquid and gas mass flow rate per unit area respectively and $\rho_L$and $\rho_G$ the liquid and gas density respectively.

The pressure drop in counter-current packed column is less significant that is the case for tray columns [33]; packed columns need a minimal liquid flow rate so that packing is sufficiently wetted.

The pressure drop by the packing material depends on the F-factor, which is defined as gas superficial velocity ($v_G$) by the gas density ($\rho_G$).

$$FFactor = v_G \times (\rho_G)^{0.5} \quad \text{(eq. 10)}$$

Gas density can be obtained with the ideal gas law, and gas superficial velocity entering the column is given by the coefficient between the gas volumetric flow rate and the tubing cross-sectional area.

There is a limiting condition to the manipulation of flow rates in counter-current operations; when maintaining either the gas or the liquid flow rate constant while increasing the flow rate of the other phase ate a certain point the flooding of the column occurs (CD region of Figure 3.2). The flooding can be identified when liquid appears on top of the bed, and sharp rise of liquid holdup occurs. This results in severe decrease in mass transfer efficiency and is related to operational conditions and column design. A generalized correlation of packed column flood points also called generalized pressure drop correlation, was developed by Sherwood, Shipeley and Holloway [11]:

$$CP = C_s F_p^{0.5} v^{0.05} = v_G \left(\frac{\rho_G}{\rho_L - \rho_G}\right)^{0.5} F_p^{0.5} v^{0.05} \quad \text{(eq. 11)}$$

Where $C_s$ is the C-factor (based on tower superficial cross-sectional area), $F_p$ is the packing factor, $v$ is the kinematic viscosity of liquid and $\mathrm{v_G}$ is the superficial velocity of gas through the column.

The correlation relates the column's capacity factor to the column's diameter, packing material type, liquid viscosity and superficial gas velocity. To achieve maximum air-to-water ratio, it might be a possibility to operate at co-current flow to avoid flooding, co-current columns however have shown lower efficiency than counter-current [34].

The liquid holdup $h_L$, provides the residence time for phase contact and is responsible for the reduction of free cross-sectional area, i.e void fraction. It is given by the volume of the liquid, $V_L$, divided by the volume of the packing, $V_S$:

$$h_L = \frac{V_L}{V_s} \quad \text{(eq. 12)}$$

In other words, liquid holdup is defined as the volume of the liquid per unit volume of the column or the liquid present in the void spaces of the column. This value can be estimated by Engel, Stichlmair and Geipel [8]:

$$h_L = 0.93\left(\frac{u_L^2 a_p}{g_c}\right)^{1/6}\left(\frac{U_L^2 a_p^3}{\rho_L^2 g_c}\right)^{1/10}\left(\frac{\sigma_T a_p^2}{1000\rho_L g_c}\right)^{1/8} \quad \text{(eq. 13)}$$

Where, $u_L$ is the liquid superficial velocity, $a_p$ is the packing specific superficial area, $g_c$ is the acceleration of velocity, $U_L$ is the liquid viscosity and $\sigma_T$ is the surface tension.

Liquid hold-up is necessary to mass transfer of VOC, but it's value should be kept low, since high liquid holdup increases pressure drop across the column [8]. Liquid holdup was found to be 50% lower in random packings than in structured packings [35].

## 4. Design of Experiments Description

The design of experiment (DOE) approach is vastly used as a scientific method for experimental testing involving several different parameters/factors which will vary in between experiments so that the impact on the output response may be observed and measured. This method has been used in several scientific fields, including pharmaceutical research and development, for example to understand the effect of critical formulation variables on spray drying process for crystalline nanosuspensions [36]. The DOE method is applicable to the air stripping column experimental trials to plan, execute and analyze how the changing parameters will influence the measured responses.

An experiment may be performed in one-factor-at-a-time approach, in which a starting point is selected and successively varying each factor over its range while the other factors held constant. This method however fails to consider any possible interaction between the factors, hence a factorial approach is a better alternative, since the factors are varied together instead of one at a time. From a resource point of view, it becomes unfeasible however to perform the full factorial experiment since with the increase of number of factors and respective level there is an exponential increase of number of necessary trials. Due to limitation of number of trials that could to be executed, it was not possible to neither fully replicate the experiment nor perform a full factorial DOE. However, the used data analysis software, JMP, will strategically select and replicate the runs to be performed according to the number of trials to be performed, with conditions that give better output on process variability.

The experiment needs to be correctly planned and conducted so that valid and objective conclusions are obtained through analysis of the resulting data. Prior to start of trials, some aspects need to be taken into account [37], such as the establishment of clear objectives, any relevant background information analysis, prior analysis of possible interaction between factors and assure the execution of random trials. Additionally, it is necessary to select the responses to be measured and the analytical measuring procedures, the controllable factors and their levels as well as the factors to be held constant during the trials.

Depending on the objective of the experiment and the application of the experimental design, different methods are applicable, such as comparative, modeling, screening and optimization models. For these packed column experiments the main goal is the characterization of the process; that is to determine which factors affect the responses – statistically significant effects – how they interact with one another and estimate each magnitude and direction of the factor effects. This method is also referred to as screening model. As will be seen described in the following sections, the used data analysis software, JMP, also allow the determination of the optimal setting of operational parameters – optimization.

## 5. Materials and Methods

Several experimental tests using an air stripping column were performed in TEVA Pharmachemie's pilot plant to assess the column's applicability and optimization efficiency in the oncological drug product manufacturing evaporative process. This project's experimental order is increasingly complex, starting mainly with smaller and easy designs, building up robustness and complexity through previous collected data and knowledge gained from each. Some additional late experiments were performed to bring some clarity on specific findings and linking the overall collected data together.

The used methodology is described in Table 5.1 and illustrated in Figure 5.1 as a chronological chart briefly explaining the objective of each experiment and the reasoning behind each.

In sum a total of ten main experiments were performed. Initially the main experimental focus was on the column's efficiency by testing hydrodynamic limitations, mass transfer rate DOE studies and how the protein solution would respond when being stripped in terms of foaming through empirical observation.

For the recirculating flow experiments with the placebo solution (5, 6, 7, 8 from Table 5.1), two types of experiments were performed, being the first one a screening design DOE with parameters being changed between samples, and the second type of experiments performed at fixed parameters, consisted in a continuous flow of solution being feed to the stripper back to the vessel with periodic samples being collected. This latter was performed a total of three times; first time at lower temperatures (18°C) and higher initial solvent concentration, second time at higher temperatures (25°C) and the third time with compressed air entering the vessel through the spray ball. One additional experiment with placebo solution was performed, experiment 9, using a different setup in which the solution was not re-feed to the same vessel but to an empty one.

The full formulation with API (experiment n° 10 from Table 5.1) included the preparation of the nano-suspension with a smaller-scale high pressure homogenizer prior to evaporation with air stripping column. The parameters during evaporation were fixed and additional samples were taken for particle size evaluation.

For simplicity purposes the additional tests are not included in Table 5.1 but will be presented in the results to support conclusions. All experiments are briefly described in the Table 5.1 with the corresponding parameters.

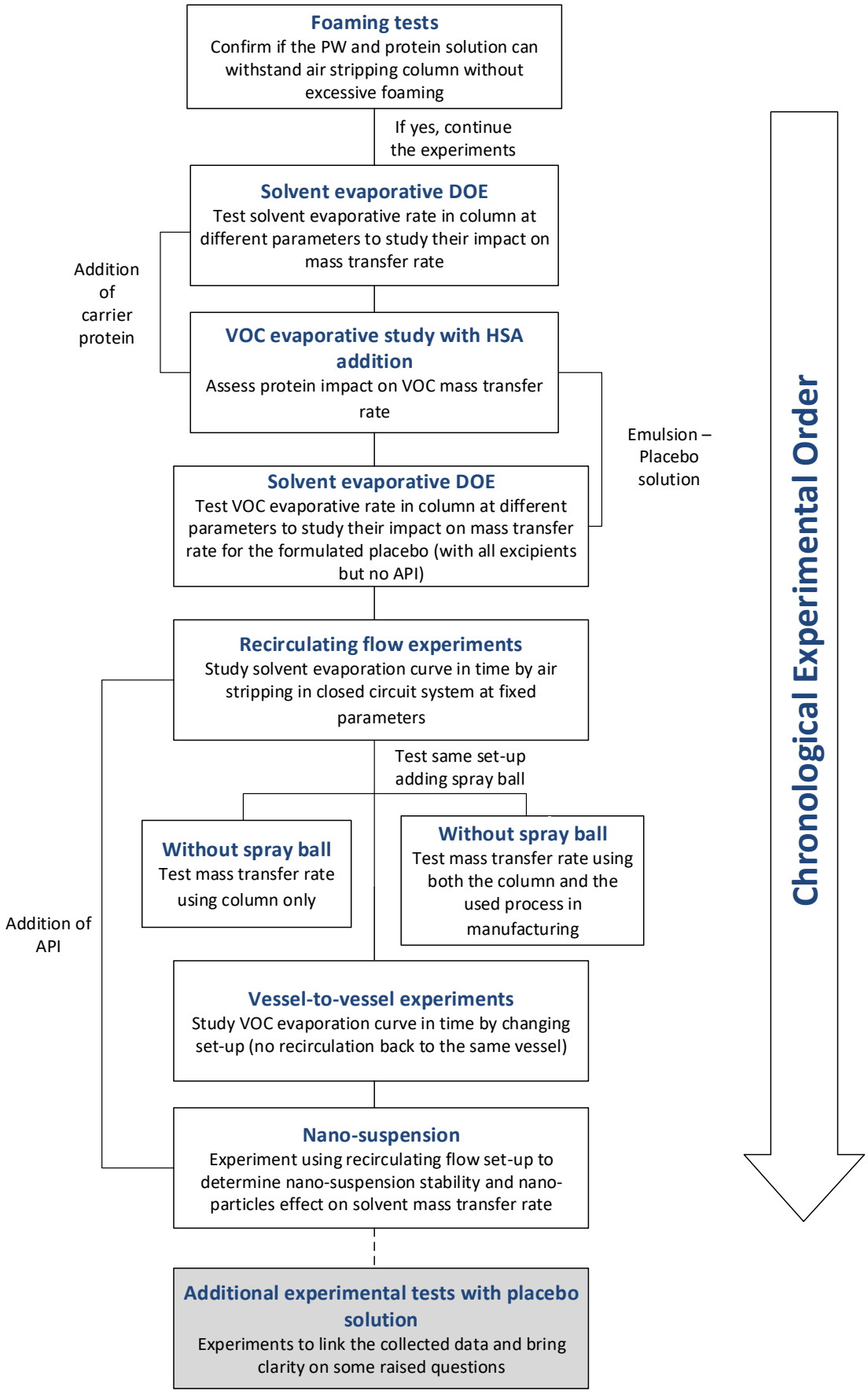


Figure 5.1 - Experimental methodology chart

Table 5.1 - List of performed experiments in TEVA Pharmachemie pilot plant

| Experiments | | Description | Parameters |
|---|---|---|---|
| **Exp n°** | **Initial trials for foaming and DOE evaporation studies** | | |
| **1** | **PW & Protein** | Test foam formation in column with protein at process concentration | T = Room temperature<br>Several LF and GF are tested |
| **2** | **PW & VOC** | VOC evaporative study - Two sets of DOE were performed (first exeperiment's data was rejected) | First experiment:<br>T = Constant room temperature<br>LF = 2,4 and 6 L/min<br>GF = 80, 160, 240 and 320 L/min |
| **3** | | | Second experiment:<br>T = 18 and 30 (°C)<br>LF = 1 to 6 L/min<br>GF = 180, 250 and 320 L/min |
| **4** | **PW, VOC & Protein** | Minor comparison study of evaporative process efficiency when in presence of protein | T = Constant room temperature<br>LF = 2 L/min<br>GF = 200 L/min |
| **Placebo solution (without use of API)** | | | |
| **5** | **Evaporative DOE study** | VOC evaporative study of bulk with DOE statistical study at changing parameters | T = 10, 20 and 30 °C<br>LF = 2, 3, 4 and 6 L/min<br>GF = 180, 260, 320 L/min |
| **6** | **Recirculating flow**<br>No temperature control, no spray ball | Several passes throughout the column in a closed-circuit system at fixed parameters, with periodic sampling for evaporative study | T = 18 °C<br>LF = 2,5 L/min<br>GF = 250 L/min<br>Volume = 125 L |
| **7** | **Recirculating flow**<br>no spray ball | 2nd at higher temperature and lower initial concentration, fixed parameters | T = 25 °C<br>LF = 2,5 L/min<br>GF = 250 L/min<br>Volume = 125L |
| **8** | **Recirculating flow**<br>spray ball | Added compressed air on top of the vessel with spray ball, fixed parameters | T = 25 °C<br>LF = 2,5 L/min<br>GF = 250 L/min<br>Volume = 125L |
| **9** | **Experiment using vessel to vessel set-up** | Setup in which treated product from the bottom of the column is pumped to empty vessel | T=20°C<br>LF= 2 L/min<br>GF = 250 L/min<br>Solution Volume = 50 L |
| **Full formulation with API** | | | |
| **10** | **Nano-suspension Solution** | Preparation of nano-suspension with homogenization followed by stripping process – Evaporation and stability study | T=20°C<br>LF= 2 L/min<br>GF = 250 L/min<br>Volume = 50 L |

## Material and Equipment

The used material and equipment varies for each experiment as will be seen ahead; detailed planning with material quantities and equipment used for each experiment can be found in appendixes E, F, G and H. In addition to the API, excipients used were the surface-active protein, the volatile organic solvent, sodium chloride and ethanol. Purified water, compressed air and glycol system were used as utilities.

In terms of equipment, the air stripping column was the central equipment of each designed set-up and remained unchanged throughout the experimental process. Different sized vessels were used (from 30 to 300 L) and circulation of the solution between the vessel and the packed column was driven by use of peristaltic pumps. Duran glass bottles, caps, weighing scale, spatulas, funnels etc. were used during compounding and weighing. For the preparation of the nano-suspension the GEA Panda Homogenizer and waterbath equipment were additionally used. Other pilot plant routine equipment was used for the setup of the experiments such as butterfly valves, elbows, buffer vessels, suction points, viton tubing, sampling syringes etc. Personal protective equipment was also required throughout the experiment, such as the mask filter during weighing of the solvent as well as a continuous measurement of solvent concentration in working atmosphere by the MiniRae equipment. For handling API additional care was taken with Tyvek suit, filter mask and compounding performed in pharmaceutical isolator equipment.

## Air Stripping Column Hydrodynamic Properties

The tested packed column for the experiments has 1030 mm of height and an external diameter of 270 mm. The structured packing utilized is Mellapak$_{TM}$ 500Y, with 250 mm diameter, which consists in segmented and rotated layers of thin sheet metal, which were loaded into the column stage by stage. Calculations are performed to determine the main hydrodynamic properties as explained in chapter 3.4.

According to Sulzer, column internals manufacturer, the pressure drop per theoretical stage of used packing material of 0.3–1 mbar and the pressure drop at 70-80% flooding is about 2 mbar/m [38]. The pressure drop can also be determined graphically (see Figure 5.2) if the F-factor is known. Replacing the values for gas density and superficial velocity in eq.10, chapter 3.4, F-factor will take values between 0.2 to 1 (m/s)(kg/m3)$^{0.5}$, resulting in a pressure drop of 0.2 to 0.5 mbar/m, according to Sulzer Chemtech provided data on Mellapak 500Y.

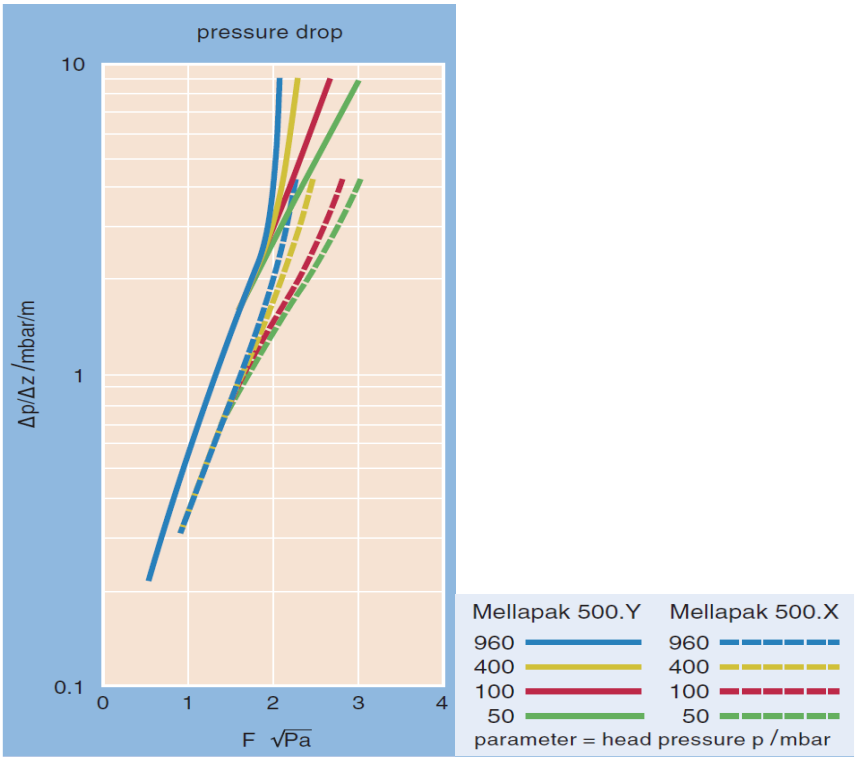


Figure 5.2 - Pressure drop in function of F-value for Mellapak 500 packing

Sulzer's software, Sulcol, was used for the calculation of liquid hold up. The fluid's physical properties, dimensions and type of packing and liquid/gas loads were introduced as input and the calculated liquid hold up was 0.047. Liquid holdup takes values roughly take values between 0.03 and 0.11 but it depends on a multiplicity of variables [39]. The software results can be found in appendix C, and these also include calculated values such as pressure drop and F-factor.

Tests were performed on the air stripping column and, the flooding point was not reached at the range flows that the infrastructure allowed. Meaning the maximum gas flow pressure from utilities is not large enough to reach the flooding point at minimum liquid flow rate across the column.

Before the start of every experiment, the whole system must be first run with purified water to assess its correct functioning. The waterrun is performed to correct any leakage if present any pump that may not perform as expected and to guarantee the system is being properly vented. This good practice avoids any contact and leaks with the bulk containing the organic solvent solvent and cytotoxic material as well as its uncontrolled release into the working environment.

From the first waterrun it was visible that liquid to be not equally distributed in the packing stages with the used single tube liquid distributer, taking preferred flow paths. This phenomenon is called channeling, in which the liquid flow rates down the tower wall rather than through the packing. This is an important factor to be optimized in the current column, since liquid maldistribution may increase the HETP value by a factor as high as 2 or 3 [8].

This is an indicator that either the liquid inlet distributer as droplet discharge system should be replaced to increase efficiency, the column diameter is too large for the liquid charge and/or a liquid collector/redistributor in between packed beds of the column to redistribute the liquid, see appendix D for detailed information of adequate internals for this specific drug product process requirements. The packed bed should be completely irrigated by distribution systems, and equal distribution of liquid over the entire bed-cross section should occur [40].

The head space between the liquid distributer and the packing is too high, compromising the efficacy of the process and resulting in higher foam formation due to splashing and decreased solvent evaporation. By lowering the distributer with stainless steel parts close to the packing level, the splashing was avoided, however column height was lost.

The air-to-water ratio will depend on the VOCs Henry's law coefficient and the striping factor as seen in chapter 3.3. From literature data and eq.7 it was estimated that the ratio for this specific solvent evaporation process would be roughly equal to 80 (this value only applies to the organic solvent in water solute). These values were taken in account when choosing the operating values of air and water flow rate for the experiments.

The range of liquid and glass flows tested for the column and combined air-to-water ratios is presented in Table 5.2.

Table 5.2 - Air-to-Water Ratios

| | **Gas Flow Rate** | | | | | |
|---|---|---|---|---|---|---|
| **Liquid Flow Rate** | **80 (L/min)** | **160 (L/min)** | **180 (L/min)** | **240 (L/min)** | **260 (L/min)** | **320 (L/min)** |
| **1 (L/min)** | 80 | 160 | 180 | 240 | 260 | 320 |
| **2 (L/min)** | 40 | 80 | 90 | 120 | 130 | 160 |
| **3 (L/min)** | 26.7 | 53.3 | 60 | 80 | 86.7 | 106.7 |
| **4 (L/min)** | 20 | 40 | 45 | 60 | 65 | 80 |
| **6 (L/min)** | 13.3 | 26.7 | 30 | 40 | 43.3 | 53.3 |

It is important to emphasize that this stripping factor and resulting (A/W) is only valid for the organic solvent in water, and as it will be ahead discussed and verified through experimental data, the full formulated drug bulk has lower stripping factor due to reduced Henry's coefficient value.

These ratios will nevertheless be used as a base for operational flow range that will be tested in the air stripping column. Theoretically, the higher the ratio, the more mass transfer will occur, therefore if higher (A/W) don't display hydrodynamic problematics, such as flooding, it should be maximized.

## DOE Experiments and Specifications

DOE tests were executed for experiments 2/3 and 5 from Table 5.1. Due to the incongruous results of the first experiment with water-VOC solution, the data was rejected and a second replicate was necessary, improving exactitude of the trials and robustness of the design. Using the statistic JMP software, two designs of experiments (DOE) were executed as a screening experiment. Tested parameters were for both DOE, liquid and gas flow, temperature and (A/W) ratio, see Table 5.3.

Table 5.3 - Tested DOE parameters/factors

| **Tested Parameters** | **Ranges** | |
|---|---|---|
| | **Lower range** | **Upper range** |
| **Temperature** | 18°C | 30°C |
| **Liquid Flow Rate** | 1 L/min | 6 L/min |
| **Gas Flow Rate** | 80 L/min | 320 L/min |
| **Air-to-water ratio** | 41.7 | 320 |

Some factors such as pressure, stirring velocity, column height, time of sampling procedure, types of vials etc. need to remain constant during the trials. Some of these are challenging to maintain constant and process variability results in unknown noise factors which can impact the precision of data results are inevitable.

The created model runs with the solvent concentration in the liquid phase and evaporation time as responses, with aim to minimize both, and multivariate analysis of either continuous or discrete numeric factors. Based on the preliminary study, the selected variable factors are gas flow rate, liquid flow rate, temperature and (A/W) ratio. For each experiment, the model was redesigned and the process adapted to correct parameters to avoid response variability.

All detailed data table from all screening experiments with trial order (row), responses, residuals, predicted values and intervals of confidence can be found in appendix I.

**Response: Solvent concentration after stripping**

The first response is the VOC concentration in the liquid phase product after one pass through the air stripping column measured by GC analytical method and inline control with Raman probe.

**Response: Overall evaporation process time**

Overall process time in hours was added to the DOE not as a screening factor, but to minimize the time of overall evaporation process according to the studied factors and to correlate it with the solvent's concentration in the product solution. For this reason, it will only be represented as a response

in the prediction profiler matrix plot. The time value represents the time of 89% VOC removal for each trial run according to it efficiency and assuming the full-scale 500L bulk volume.

This time in hours was calculated from the solvent removal efficiency and concentration requirements through the following equations:

$$Eff = \frac{C_{Li(in)} - C_{Li(out)}}{C_{Li(in)}} \quad \text{(eq. 14)}$$

Where $C_{Li(in)}$ is the solvent's concentration in the liquid phase entering the air stripping column and $C_{Li(out)}$ is the solvent's concentration in the liquid phase exiting the column. The efficiency of the process is therefore the amount in percentage of solvent removed from the initial solution exiting through the exhaust gas.

Therefore,

$$C_{Li(out)} = C_{Li(in)} - C_{Li(in)} * Eff \quad \text{(eq. 15)}$$

$$C_{Li(out)(at\ 1\ pass)} = C_{Li(in)}(1 - Eff) \quad \text{(eq. 16)}$$

For two stripping passes:

$$C_{Li(out)(at\ \mathbf{2}\ passes)} = C_{Li(out)(at\ 1\ pass)} * (1 - Eff) \quad \text{(eq. 17)}$$

$$C_{Li(out)(at\ 2passes)} = C_{Li(in)}(1 - Eff) * (1 - Eff) \quad \text{(eq. 18)}$$

$$C_{Li(out)(at\ \mathbf{2}\ passes)} = C_{Li(in)}(1 - Eff)^2 \quad \text{(eq. 19)}$$

Therefore for $p$ number of stripping passes:

$$C_{Li(out)(at\ \boldsymbol{p}\ passes)} = C_{Li(in)}(1 - Eff)^p \quad \text{(eq. 20)}$$

Knowing the initial and desired concentration it is then possible to determine the number of passes if the efficiency is known,

$$Required\ concentration = (1 - Eff)^p \times inicial\ concentration \quad \text{(eq. 21)}$$

Where $p$ is the number of passes necessary to reach the required concentration, and time is given in hours by,

$$time\ (h) = \frac{500}{Liquid\ flow} \times p \quad \text{(eq. 22)}$$

On a side note, the overall process time was additionally used in the experiments with recirculating bulk (experiments 6, 7, 8, 9 and 10). However, the overall process time was not calculated through eq.22 but estimated through the obtained solvent profile curve equations, and extrapolated to the full-scale volume of 500L.

To monitor the process and obtain quantitative results for the studied attributes, analytical instruments where used to measure solvent concentration in liquid phase of treated product and quench solution stability through average particle size.

## Analytical Procedures

**Gas Chromatography – Solvent content**

After each experiment the sample must be tested analytically to measure its solvent content to assess the air stripping efficiency. This technology separates and analyses the compounds that can be vaporized without decomposition. The vaporized samples injected into the GC will then be carried by an inert gas (either nitrogen or helium) through a capillary column, reaching a detector which provides the quantitative measurement of VOC in the mixture.

**Raman Probe**

A Raman probe was also during trials and measured solvent content from each run as an in-line measurement control. The probe in connected to the vessel and sends the measurements to the specific software which returns the VOC peaks in a spectra plot. The area under the curves in indicative of solvent content, however it is not possible to quantify it without calibration data. It was used however as a guideline during experiments to measure concentration evolution throughout the experiment.

**Malvern Zetasizer - Particle Size and Polydispersity Index**

Mean particle size and polydispersity index are the key parameters to evaluate the physical stability of the nano-suspension; It is crucial to maintain a nano-scale particle size throughout the processual steps. Stabilization issues were already briefly described as being key to the achievement of a quality final drug product. The interaction between the API and protein is weak (Van der Waals interaction) and both substances freely dissociate in quiescent solutions. The nano-suspension is thermodynamically unstable and will tend to minimize its total energy by agglomeration [41]. This is why it is important to maintain the supply of energy to solution by continuous agitation as a mechanical stabilizer and provide steric stabilization minimizing particle-particle interaction.

Dynamic light scattering technology (DLS) is used by Malvern Zetasizer equipment to determine Z-average and PDI, due to particle Brownian motion dependence of particle size[42]. The software also allows the attainment of zeta potential and particle size distribution, these however are not critical attributes for the drug product submission, and therefore the method for this specific product will be limited only to Z-average and PID. The specifications for the drug product stable solution are a z-average between 80 and 160 and a PID lower than 0.2.

The z-average is the particle diameter cumulate mean and is defined by ISO 22412 as “harmonic intensity averaged particle diameter”. PID provides the long-term stability of nano-suspensions and should be as low as possible; it represents the width of the overall distribution assuming a single mean.

Generally for nano-suspensions, a PID value between 0.1 and 0.25 indicated a narrow size distribution, whereas values of more than 0.5 indicate a very broad distribution. [43].

**Sampling method precision**

A total of three different sampling collecting methods were performed, either due to used vessel or assembled setup. The first method used for the DOE consisted in installing a three-way valve at the bottom end of the column so that samples could be taken directly after the stripping process. This was however the less robust sampling method with precision of $\pm$50 ppm and went up to 270 ppm for a determined data set. This precision between the data gives the DOEs a rather high standard error and it is translated and considered in the model evaluation.

The subsequent experiments for the recirculating and vessel to vessel experiments sampling method was either from a tap in the middle of the vessel or with a syringe depending on the vessel. This sampling method proved to be much more robust with an average preciseness of $\pm$0.4 ppm between data sets.

## Health and Safety

During manufacture of the product, for each process step, EHS items are assessed such as, exposure to chemicals and API's, spills, machine safety, pressure, ATEX, waste and waste water.

The preparation of the nano-suspension described in experiment 10 from Table 5.1 required the biggest amount of precautions due to the API cytotoxicity and the use of a high-pressure homogenizer operating at average pressures up to 100 bar. Prior training for equipment usage and safety matters was given to assure qualified handling of equipment/excipients as well as danger awareness. The handling of the API was at all times performed inside an isolator and tyvex suit was used throughout the entire experimental routine, including cleaning procedures, which are performed using ethanol and sodium hydroxide. Care was also taken when handling the sodium hydroxide, due to its very corrosive properties.

The organic solvent used for the dissolution of API, due to its toxicity, is immediately dangerous to life at exposure via the lungs at approximately 500 ppm. The handling of the solvent must also be done following a set of safety rules. Safety gloves must be worn, and a full face mask with AX filter must be worn when opening the quenching vessel or column is mandatory. The filter gives protection at maximum concentration of 100 ppm, and the life time of the filter depends on the exposure concentration. Ventilate systems and air measurement equipment will assure the concentration of solvent in the air is below the regulated values assuring safe working environment. Additionally, MiniRae portable measurement equipment was used all times to assure safe working environment.

Regarding safety measures, an ATEX and explosive atmosphere risk assessment was completed. During the manufacturing process, ethanol is used and mixed at room temperature to a 11% (v/v) solution in non-flammable solvent. Not-flammable, solvent containers may explode if exposed to

heat, the risk of fire and explosion is little but ATEX measures are taken to mitigate any danger. The process takes place in a room equipped with ATEX control measures as an ATEX proof ventilation and extraction, monitoring and a shut-down system of electrical equipment upon 10% LEL detection.

## 5.1 Protein and PW Solution Trials

After performing the initial waterrun successfully, small tests on the foam formation of a water and carrier protein solution passing through the column were executed at different air-to-water ratios. The detailed planning of this preliminary test can be found in appendix E. This solution was set-up with the column in continuous flow and tested at several flow rates.

## 5.2 Organic Solvent and Water Solution Experiments

After the preliminary studies and experimental planning, the two evaporative experiments with purified water and organic solvent were executed, experiments 2 and 3 depicted in Table 5.1. Design of experiment, analytical and statistical studies are essential to explain the collected data, determine the critical parameters and significance of those studied factors. The detailed planning of pilot plant experimental steps can be found in appendix F as well as JMP data table with the order of experiments and corresponding responses and residues for both trials which can be found in appendix I. The equipment and material was set-up in TPCH pilot plant according to the Figure 5.3:

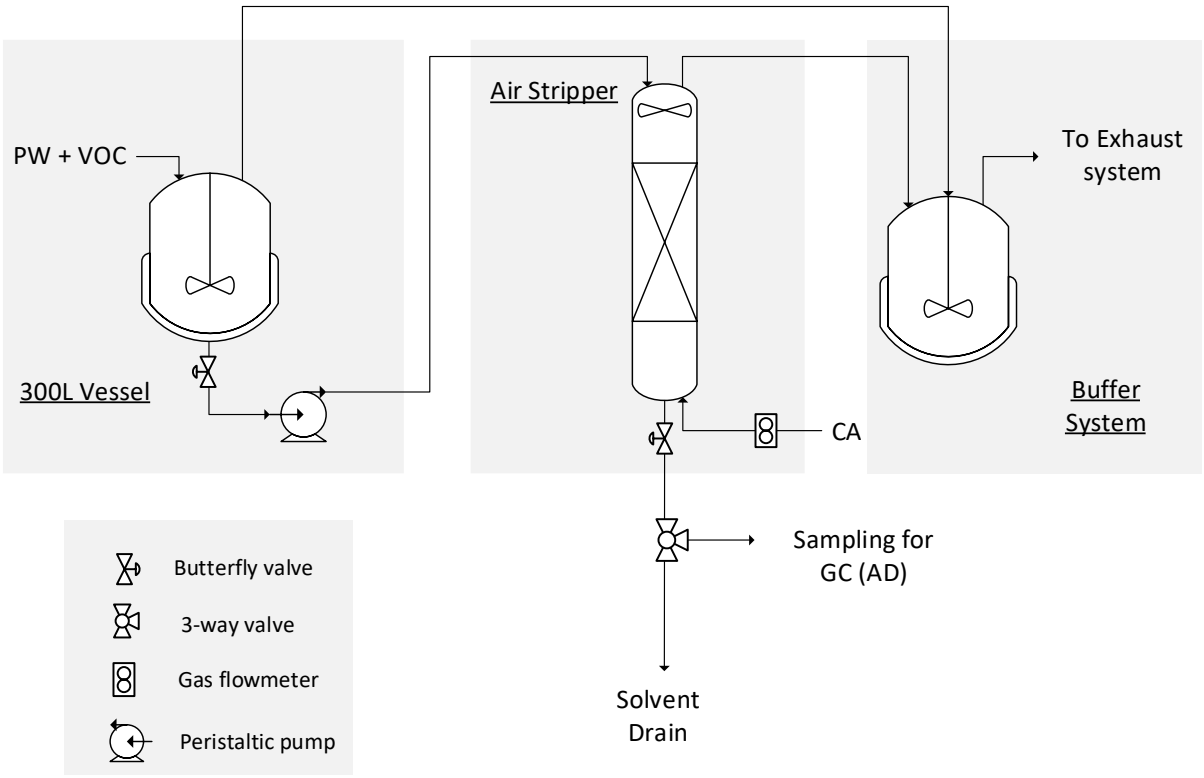


Figure 5.3 - Pilot Plant setup for water and organic solvent DOE experiments

Only water and solvent solution was used for the preliminary tests on the column's efficiency. The water utility source in the pilot plant is purified water (PW) whereas in the parenteral production area water for injection (WFI) is used to assure sterility of solution.

It is important to perform an initial simple test to assess the efficacy of the stripping column, how to operate with it and how it responds to the implemented parameter changes before testing it with the excipients and API. Figure 5.3 illustrates the setup of the equipment as well as its arrangement in the pilot plant. For this experiment, the used equipment, a 300L vessel was used, as well as a buffer system for safety reasons (avoid any overflowed liquid to enter the exhaust system), and a peristaltic pump in order to keep the liquid feed flowing (the maximum capacity of the pump is 6 L/min if connecting two tubes in parallel.

After preparations, experimental trials started and several runs (single pass) were performed through the packed column, varying liquid and gas flow rate per each run in a random order. For the second experiment temperature was also changed between 18°C and 30°C. Few trials were replicated at same conditions to verify data precision and process variability.

## First PW-VOC Experiment

The initial concentration compounded was reduced after solution transition to the vessel due to either bad solvent dissolution or due to the big headspace in the 300 L stainless steel vessel, which allowed the VOC's migration into gas phase inside the vessel. Since the organic solvent is practically insoluble in water, the solution was stirred for 30 min at 200 rpm to assure thorough solvent dissolution in water. Control samples (Control START and Control END) were taken prior and after experiment, directly from the 300L vessel in order to determine by GC analytical measurement the exact concentration in the liquid phase that is entering the air stripper. These control samples will determine the initial concentration before air stripping process, and at the end of the experiment another one is taken to make sure the initial concentration remains unchanged in time.

## Second PW-VOC Experiment

Due to results inexactitude, a second equivalent trial was executed, improving precision of trials and robustness of the design of experiment. The same concentration of solvent was used following the BMR concentration values. This approach will increase the number of trials and add a third continuous variable, temperature, which range will vary between 18 and 30°C.

Operating flows, analogously, will be introduced in JMP as continuous variables; liquid flow rate ranging from 2 and 6 L/min and gas flow rate ranging between 180 and 320 L/min. JMP model will study the full quadratic response surface, which includes all the interactions between factors and quadratic terms.

For the second experiment there was an increase of number of trials and therefore measured sample values, and due to high variability values in the previous experiment it was also decided to take at least four control samples and beginning and end of the experiment. A total of 28 samples were collected of which 24 are air stripping runs and a total of 4 runs were replicated values. The order of the runs will be random, however due to the temperature being a slow parameter to change, to not delay the experiment and perform all runs on the same day, it was set at the JMP software as a "hard to change" parameter. This will however will slightly reduce the power of analysis of temperature effect on the solvent's concentration in the design evaluation of the model.

In order to improve the experimental procedure robustness, some actions were taken. The analytical measurement by GC was performed shortly after sampling and the stoppers used for sealing the vials were replaced by evaporative resistant ones; dark blue stopper on the right featured in Figure 5.4. Additionally, parafilm was added to the vial as a seal to avoid any unwanted evaporation from the vial and they were stored in the fridge before being sent to analytical laboratory.

Figure 5.4 - Cap and stoppers used on sampling vials

Finally, the sampling process was kept as unvarying as possible and the solution would circulate through column to the drain at same conditions for at least 3 minutes before collecting a sample.

After the end of the eight sample collection, one of the inlets of the pump was opened, to retrieve a sample with no pass through the column for measurement with the Raman probe. The inlet was not properly closed after this resulting on 1 and 3 instead of 2 and 6 liquid flow rates until the end of the experiment and therefore a different design of experiments from the planned and lower number of replicated values. This DOE is however still valid, having more values for liquid flow rate variation and less replicated values.

## 5.3 Water, VOC and Protein

A smaller solution of water with the organic solvent and carrier protein (5L) was also put through the column to compare evaporation with and without the surface-active excipient; experiment 4 in Table 5.1. Same to the previous experiment with water VOC, two control samples were taken without column pass, and a total of three samples were taken after descending the air stripping column. The conditions were constant for all three samples at 2 L/min of liquid flow rate, 200 L/min of gas flow rate and room temperature.

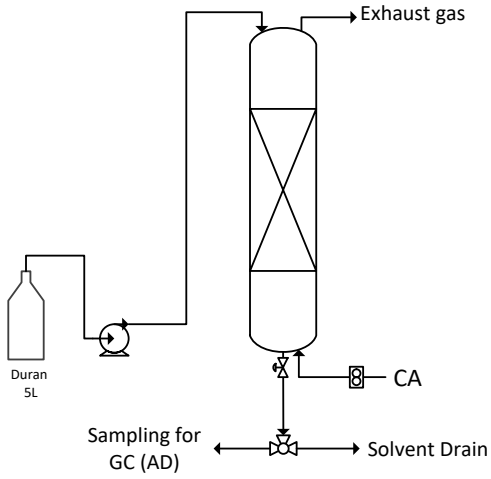


Figure 5.5 -Pilot Plant setup for PW, VOC and protein experiments

This solution required longer stirring to completely dissolve the organic solvent in suspension into a solution. The solution was in a 10 L glass bottle and was stirred for 30 minutes with an overhead stirrer, covering the opening with parafilm and using local suction point.

## 5.4 Placebo solution (without use of API)

Experiments where performed to test evaporation of the prepared placebo solution at first stages of compounding, without including the API, and the ratio of each component was once again calculated according to the formulation in the BMR. The following procedures represent experiments 5 to 9 from Table 5.1. Some additional smaller experiments using the placebo solution were performed and will also be presented as well as their purpose.

Due to some uncertainties regarding the liquid flow rate results for this experiment, additional placebo solution tests with only liquid flow rate variation was performed ahead and are presented in the results.

### DOE Experiment

The first experiment was analogous to the initial screening DOE previously executed (experiments 2 and 3 from Table 5.1) this time adding 10°C temperature level to the DOE, to get a bigger range and better understanding on mass transfer rate evolution in function of temperature.

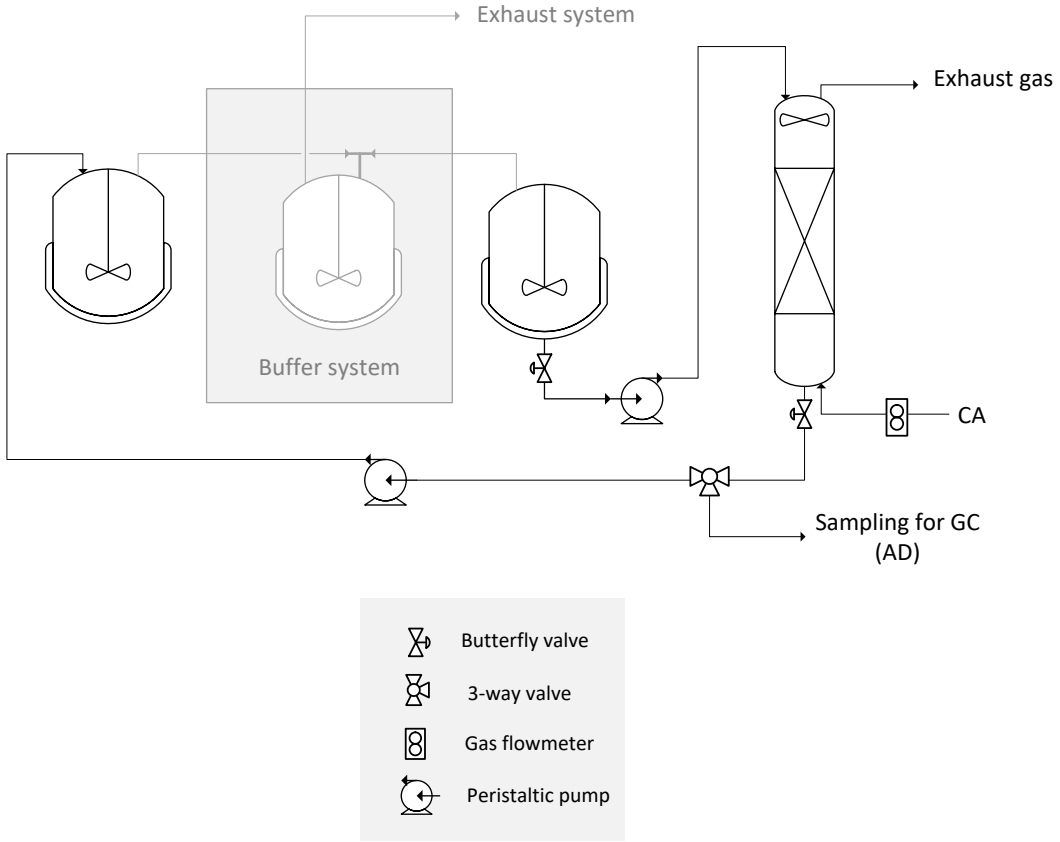


Figure 5.6 - Pilot Plant setup for placebo solution DOE experiments

This set-up, adding a second collecting vessel, allowed the reuse of the prepared solution, by only adding a make-up quantity of VOC and EtOH which has been stripped to lower concentrations in the column.

A total of 34 samples were taken in which 7 were control samples, at start, end and middle of process. A control sample was taken during the stripping process since there were some technical issues with the glycol system (chilling function was not working, interrupting the glycol system systematically). This matter resulted in an interruption of about three hours after the first six runs, making it impossible to conclude the experiment in a working day. The first six runs were repeated the day after and extra control samples were taken, since adding the VOC and EtOH as make-up would slightly alter the start values.

In average during the process, the VOC's concentration entering the air stripping column is roughly constant however due to the slight variation during the process interruption, the experiment was blocked into three and efficiency removal was also corrected accordingly.

Similarly, to experiments 2 and 3 the solution was pumped from the vessel to the air stripper and each sample was collected at the outlet of the column after one pass through the column, changing temperature, liquid and gas flow rate between runs. The main variations were the addition of the protein and other existing components in formulation and use of larger temperature range.

## Recirculating Flow Experiments

With the placebo solution (without API) several experiments at different parameters where performed. All the described experiments were performed with a recirculating flow between the vessel and the column and fixed temperature and liquid/gas flow rates and detailed procedures can be found in appendix G. Samples were taken directly from the vessel every 30 minutes to study the evolution of solvent's concentration with time. The volume of treated solution is 125 L which is a scale-down by a factor of 0.25 of the manufacturing volume of 500 L bulk.

For all the recirculating experiments, it was essential to adjust both pumps flow beforehand each experiment in a water run test, in a way that the level of the liquid in the bottom of the column does not rise above the gas distributor, and no air is entering the pump feeding the vessel. This was a crucial process design step since rise of liquid in the column decreases its efficiency and air entering the vessel would foam the solution compromising the experiment.

When equilibrium was reached, the flow was kept constant and foam in the vessel was kept to a minimum. For this the pump feeding the column was set at 2.5 L/min and the pump feeding the vessel was set at ~3.5 L/min. The air flow was also kept constant at 250 L/min throughout the experiments.

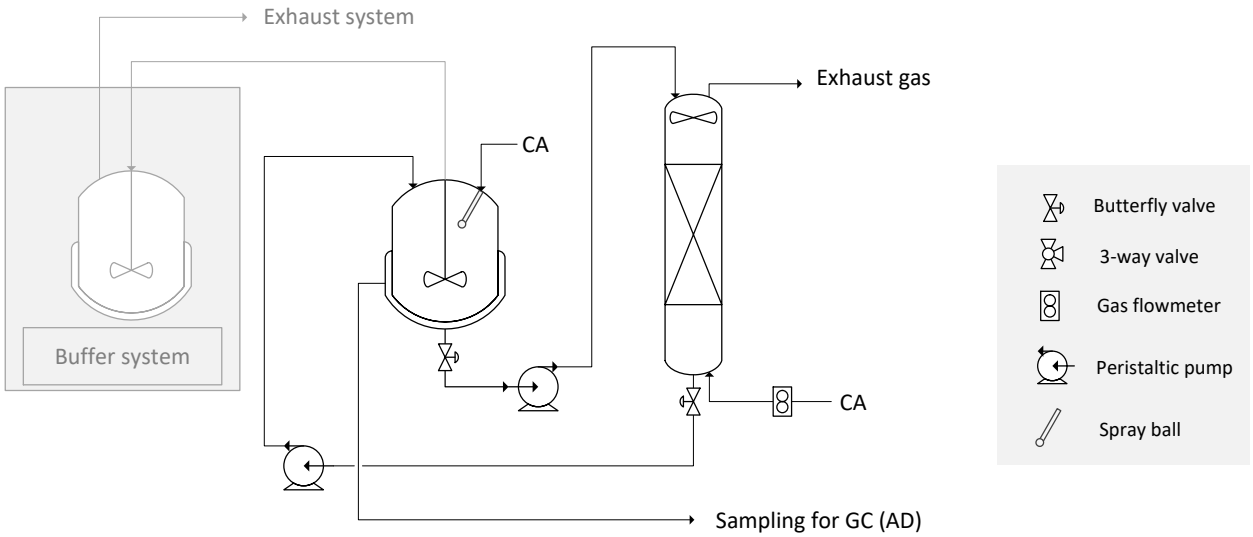


Figure 5.7 - Pilot Plant setup for recirculating placebo solution experiments

The first experiment (experiment 6 from Table 5.1) was initially supposed to operate at 25°C however due to malfunction of the glycol system, it was not used for this experiment and during the contact with the cold compressed air entering the column, the solution temperature decreased from 18.5 °C to 16.7 °C at end of the experiment.

The experiment was due to the glycol system malfunction repeated, using the same set-up, at higher temperatures. A third experiment was performed with the compressed air entering the vessel through the spray ball, in simultaneous with the solution being circulated through the air stripping column.

## Additional Placebo Solution Experiments

Some smaller experiments were performed in order to bring some clarity on particular details and linking the overall collected data together. The first important matter to determine is the impact of the process set-up and flow on the process efficiency and its duration. Therefore, an experiment was performed without recirculation on the bulk; the solution goes back and forward between two vessels while a sample is collected each time a vessel is filled. The equipment set-up depicted in Figure 5.3 was assembled, except the used vessels were of lower volume than the DOE experiment. The pumps were working at 2 L/min and waterbath equipment was used to maintain the solution at 20°C and the compressed air was entering the bottom of the air stripping column at 250L/min.

The liquid flow rate impact on the recirculating flow experiments was studied re-assembling the set-up depicted in Figure 5.7 testing it at two different liquid flow rates, 2L/min and 6L/min. The higher liquid flow rate was not reliable since it started foaming, so pump feed was reduced to 4L/min. This needs to be taken into account when designing and choosing the column internals.

## 5.5 Nano-suspension Solution

For this experiment (n°9 from Table 5.1) a full formulated nano-suspension of 50L quenched solution was prepared using a smaller scale homogenizer; the GEA PandaPlus. The amounts of components are once again scaled down according to the BMR. The detailed followed procedure can be found in appendix H. The setup used for the preparation is depicted schematically below. For this experiment, compressed air wasn't blown into the vessel; the only evaporative process unit was the air stripping column.

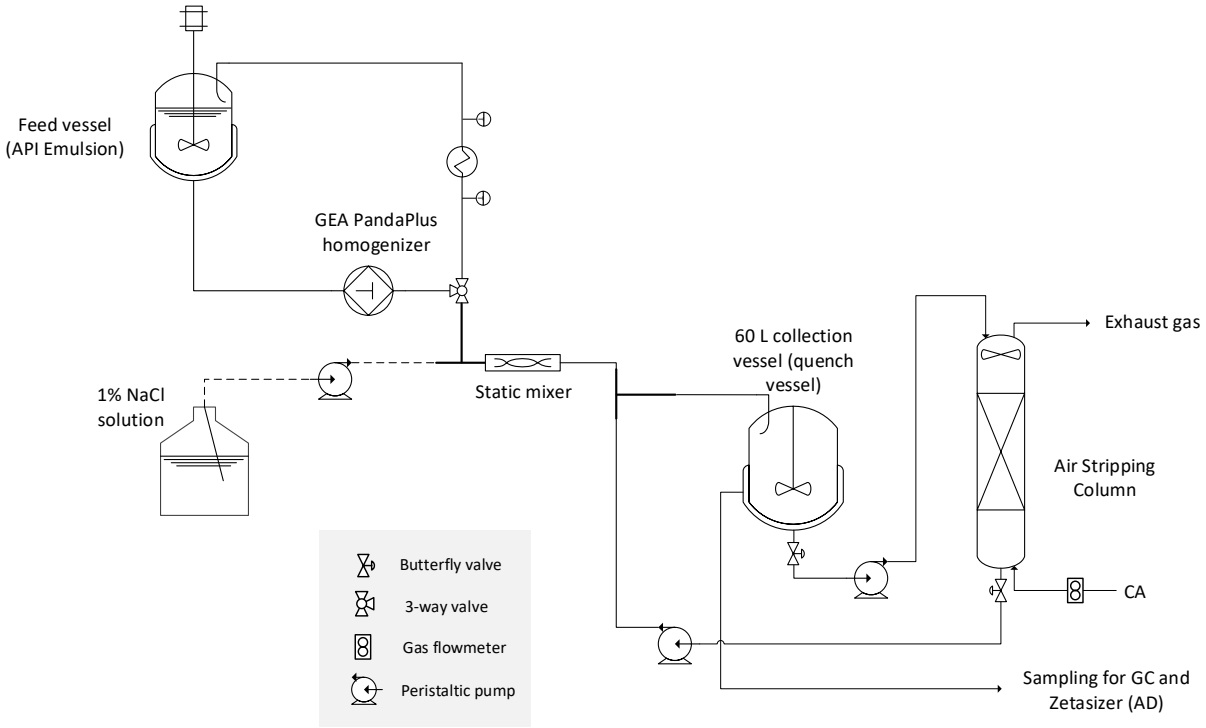


Figure 5.8 - Pilot Plant setup for nano-suspension solution experiments

This final experiment is important to make sure the column does not affect the size of the protein-API nano-particles created in the homogenizer and to assess if the nano-particles possess any impact on the evaporation of the organic solvent.

A total of ten samples were taken, five to be analyzed in solvent's content by the GC and another five to analyze the particle average size (Z-Ave) and polydispersity index (PDI) using leakage the zetasizer equipment. The stripping process was carried for a total of 3.5 hours.

# 6. Results and Discussion

The data results by GC analysis are presented as solvent concentration in the liquid phase. The results were, due to confidentiality purposes, normalized to the BMR manufacturing compounding concentration of VOC value. Between experiments, the values of initial organic solvent concentration and sampling method vary either purposely or due to difficult reproducibility being above or below the unit value which represents VOC concentration value used in drug manufacturing process. For this reason, the solvent's concentration results after stripping cannot always be evidently compared between different experiments. Therefore, the overall process time was calculated as a productivity indicator. Hence, the overall process time harmonizes the results between experiments and can be used as an indicator for comparison between all results.

For the experiments with closed loop between the vessel and the column (experiments 6, 7, 8 and 10) the overall process time was estimated through the solvent concentration profile curve equations, and extrapolated to the full-scale volume. The volume however can have additional non-measurable impact on the overall process time.

## 6.1 Protein and Water Solution Trials

As mentioned this experiment was only performed for visual control of foam formation in the column at different operation parameters and therefore no quantitative data is available. Column showed almost non-existing foam, with little formation at the bottom of the column. It was not stable foam, and was rapidly dissolved when in contact with the compressed air entering in the bottom of the column. During cleaning procedure, foam was created on the bottom of the column, which could be removed by turning on the compressed air. These favorable observations allow the continuation of the subsequent experiments, using this column and type of packing, since the solution appears to be stable in terms of foamability.

## 6.2 Organic solvent and Water Solution Experiments

### First PW-VOC DOE Experiment:

The results for each trial are presented in Table 6.1 and its values represent normalized solvent's concentration in solution.

Table 6.1 - First PW/VOC experimental data results normalized

| | Normalized solvent concentration | | | |
|---|---|---|---|---|
| GF Rate (L/min) / LF Rate (L/min) | **80** | **160** | **240** | **320** |
| **2** | 0.13 | 0.06<br>0.03 | 0.11<br>0.09 | 0.13 |
| **4** | 0.08 | 0.04<br>0.06 | 0.05<br>0.07 | 0.10 |
| **6** | 0.11 | 0.39<br>0.14 | 0.12<br>0.07 | 0.03 |

Control values are the VOC concentration in liquid phase without air stripping, in order to measure the concentration entering the column at start and end of the whole experiment and allow the calculation of column's efficiency in eq. 14. The control normalized values are 0.49 and 0.56 for control START and control END respectively. Meaning about half of the BMR theoretical initial concentration. The concentration in the stripped product results, presented in Table 6.1 by order of trial, including the START and END is graphically represented in Figure 6.1.

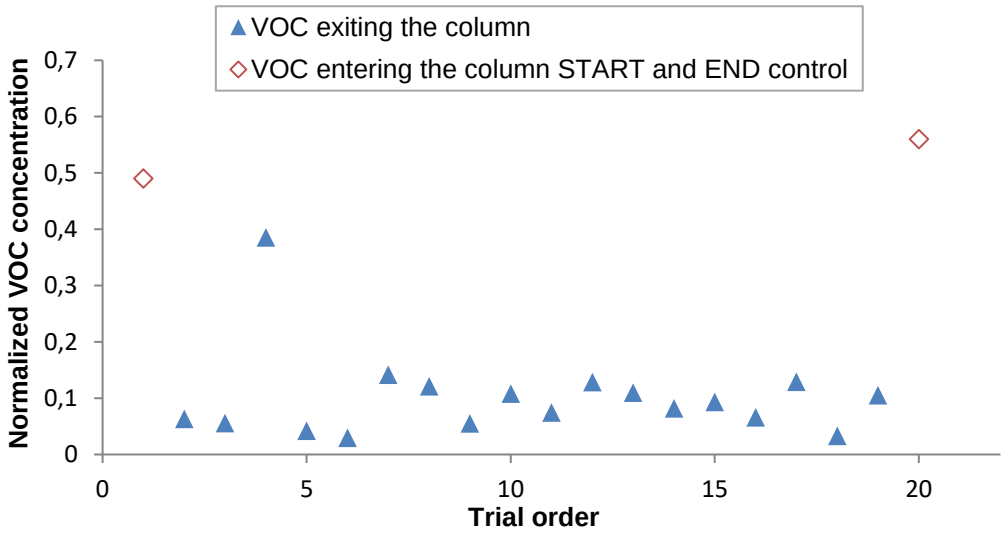


Figure 6.1 - PW/VOC first experiment – Normalized VOC concentration after stripping in trial order

The variability of results from the fist experiment suggests an introduction of non-controlled variable(s) to the responses. The START control response is smaller than the END control value, which suggests that possibly the entire group of responses, is different from the accurate values. The deviation of results performed at same conditions either implies the factors do not have significate effect on the response or a non-controlled variable impacted the samples. There is no way to determine with certainty what was the non-controlled variable(s), and if it had the same effect to all the samples. This factor can be however, presumed and if possible corrected in following experiments.

The first and most likely deviation cause were the stoppers used, which were not the most appropriate to be used when VOCs are involved. If there was evaporation still occurring in the vials, the fact that the samples took one week to be analyzed aggravated the deviation to the response results.

For the deviation between the results, two possibilities were assessed as root causes. The first source of error could have been the small circulation of bulk through column before collecting each sample. The second could have been the low precision of the peristaltic pump that feeds the liquid to the column. The pump can also introduce impreciseness in the sampling procedure, since the flow may not always be constant.

The removal of VOC by the column stripper is considerably high, averaging a total of ~81% of removal with only one pass; this value represents the efficiency of the stripping process.

Although deviation between replicated trials is significant, the mean removal of solvent in percentile does not vary much between all trials, taking values between 76 and 95%. There was one exception; an outlier value of 0.39 (LF=6 L/min and GF=160 L/min) which represents a process efficiency of only 27%. This could be explained by human experimental error in which gas flow rate was still turned off.

A distribution is said to be normally distributed if most of the observations are assembled around the mean. The density function of the normal distribution is [44]:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}}\, e^{-(x-\mu)^2/2\sigma^2} \qquad \text{(eq. 23)}$$

If the outlier value is removed the Gaussian curve for the removal of VOC fits the normal bell-shaped distribution curve, and indicates small variance for this percentile as can be seen by the high amplitude of the curve in Figure 6.2.

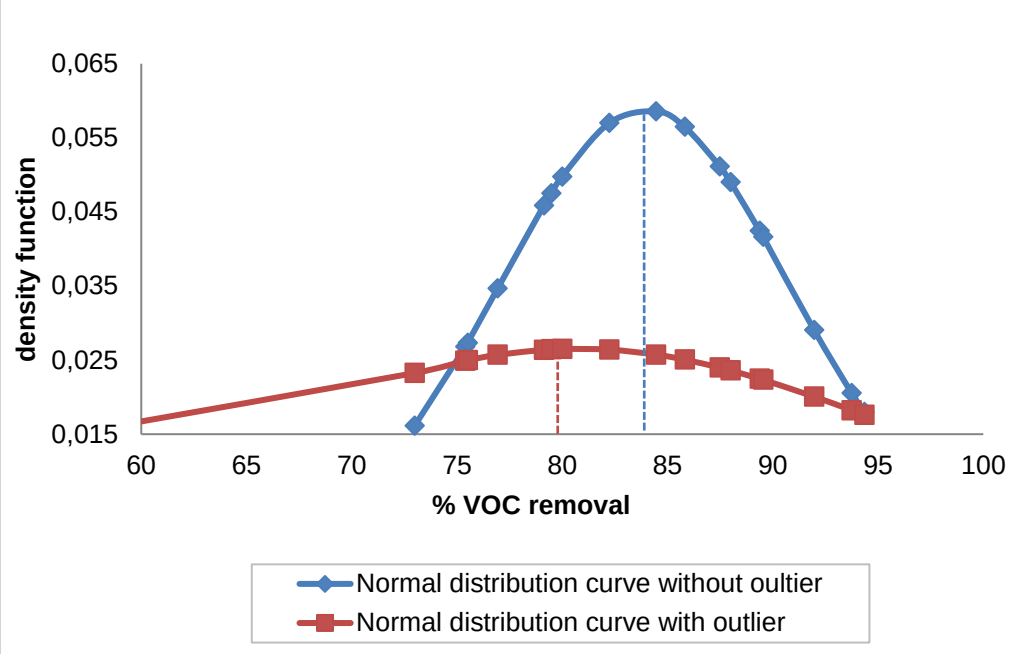


Figure 6.2 - PW/VOC first experiment distribution curves

The DOE however presents some inconsistent data, reason why the analysis of variance is erroneous and cannot lead to reliable conclusions regarding the studied factors. The JMP data for PW/VOC first experiment was therefore rejected and found inconclusive in terms of screening the statistically significant factors to the process and will not be presented in the report.

Although the DOE results were rejected due to the unknown noise factor which introduced non-controlled variability to the process, it can still be established from this first experiment, the efficacy of the column regarding the successful removal of volatile organic solvent by air stripping.

## Second PW-VOC DOE Experiment

In the repeated PW/DOE experiment (experiment 3 depicted in Table 5.1), like the previous experiment, start and end control samples were taken directly from the vessel and normalized values of VOC concentration are presented in Table 6.2

Table 6.2 - Second PW/VOC experimental control sample data results normalized

| Control Sample | Normalized VOC concentration |
|---|---|
| START1 | 0.52 |
| START2 | 0.51 |
| END1 | 0.47 |
| END2 | 0.38 |

In average during the process, the normalized concentration entering the air stripping column is roughly 0.47. This value decreases during the process due to evaporation inside the vessel; however, it was assumed an average constant concentration in terms of removal efficiency calculations.

The results for solvent concentration in solution at both temperatures for all 24 trials were registered after air stripping at different gas and liquid flow rates for both temperatures are presented in tables Table 6.3 and Table 6.4. as normalized to the BMR theoretical initial concentration value.

Table 6.3 - Second PW/VOC experimental data results at 18°C on normalized VOC concentration

| **T = 18°C** | **Normalized solvent concentration** | | | |
|---|---|---|---|---|
| **GF Rate (L/min) / LF Rate (L/min)** | **180** | **250** | **274,5** | **320** |
| **1** | 0.06 | 0.05<br>0.05 | 0.06 | 0.04 |
| **2** | - | 0.06<br>0.06 | - | - |
| **3** | 0.08 | 0.07 | - | 0.06 |
| **6** | 0.09 | - | - | 0.07 |

Table 6.4 - Second PW/VOC experimental data results at 30°C on normalized VOC concentration

| **T = 30°C** | **Normalized solvent concentration** | | | | |
|---|---|---|---|---|---|
| **GF Rate (L/min) / LF Rate (L/min)** | **180** | **226.2** | **250** | **274,5** | **320** |
| **1** | 0.03 | - | 0.02 | 0.02 | 0.02<br>0.03 |
| **2** | 0.05 | - | - | - | 0.04 |
| **3** | 0.04 | 0.04 | 0.04 | - | - |
| **6** | - | - | 0.08<br>0.05 | - | - |

The concentration in the stripped product results by order of trial, including the START and END is graphically represented in Figure 6.3.

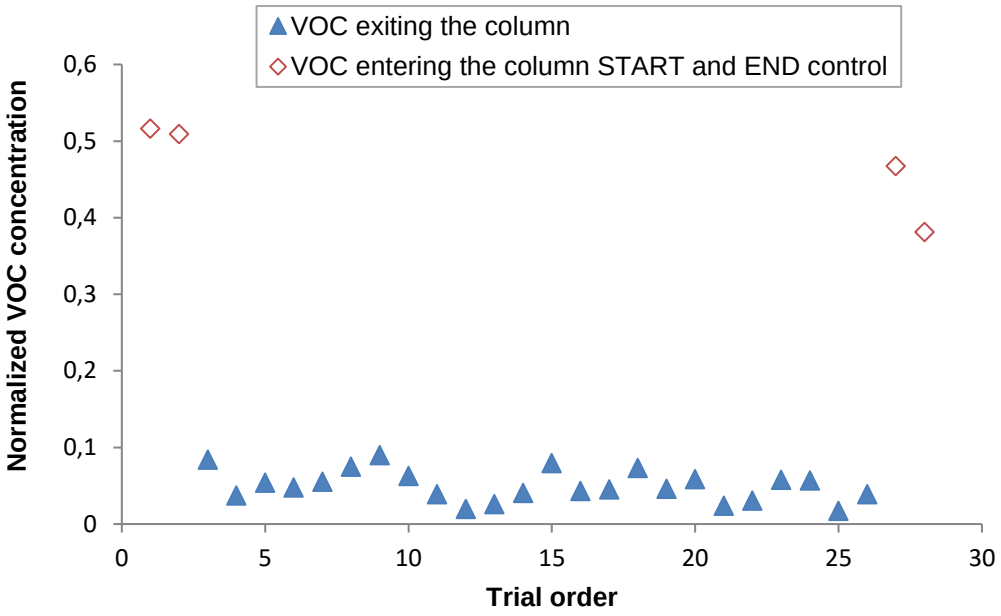


Figure 6.3 - PW/VOC second experiment; Normalized solvent concentration after stripping in trial order

Like the first experiments the percentage of removal is stable, taking values between 81 and 96%. The results from the second experience are furthermore consistent with the variables, increasing JMP model analysis reliability. The calculated average solvent evaporation efficiency in this experiment is 89%. Smaller standard deviation between replicated values compared to the first set of experiments suggests the achievement of better process robustness and/or measurement efficiency. The distribution curve of the results can be seen in the Gaussian curve in Figure 6.4:

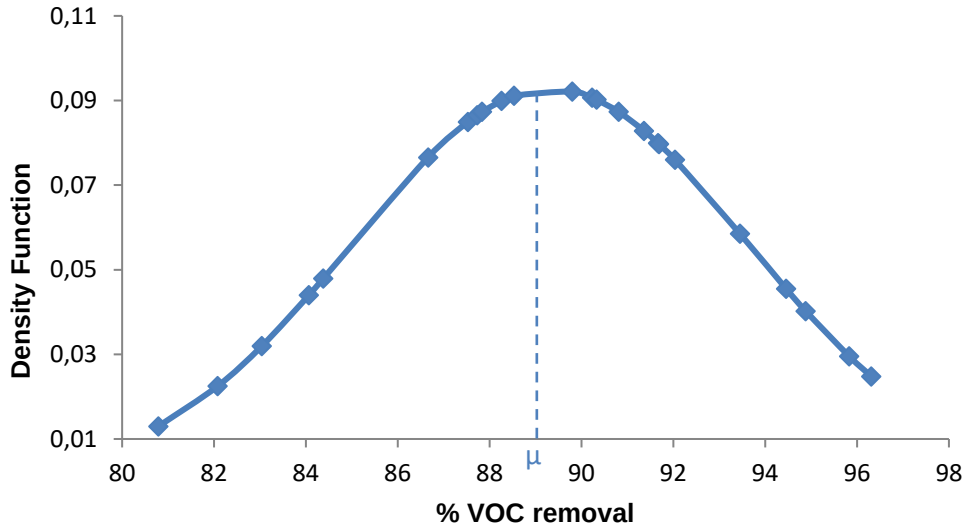


Figure 6.4 - PW/VOC second experiment; Distribution curves

## DOE Analysis

The DOE results for this experiment were analyzed, using JMP software. First run tested all main effects, including (A/W), crossed and quadratic effects. However, it is important to re-run the fit model eliminating all interactions and quadratic terms which are assessed by the model as not significant and hold therefore higher P value. Detailed information on each presented table and figure understanding is extensively described in appendix K.

Two different responses were evaluated by the DOE report; Solvent's concentration in the liquid phase exiting the column after stripping and overall process time for a 500 L solution with the initial VOC concentration to be reduced for a certain number of passes to achieve the 89% desired VOC removal. For both combined responses the effect summary with respective significance represented in the plot bars is shown in Table 6.5:

Table 6.5 - Effect Summary for combined responses

| Source | LogWorth | PValue |
|---|---|---|
| LF | 12.745 | 0.00000 |
| LF*LF | 8.134 | 0.00000 |
| Temp | 6.218 | 0.00000 |
| Temp*LF | 2.099 | 0.00795 |
| GF | 2.028 | 0.00938 |

The main response which is relevant to analyze in terms of screening effects is the solvent concentration. For this response the model is evaluated by the fit report, presented by a leverage plot see Figure 6.5, and the model's analysis of variance, see Table 6.6.

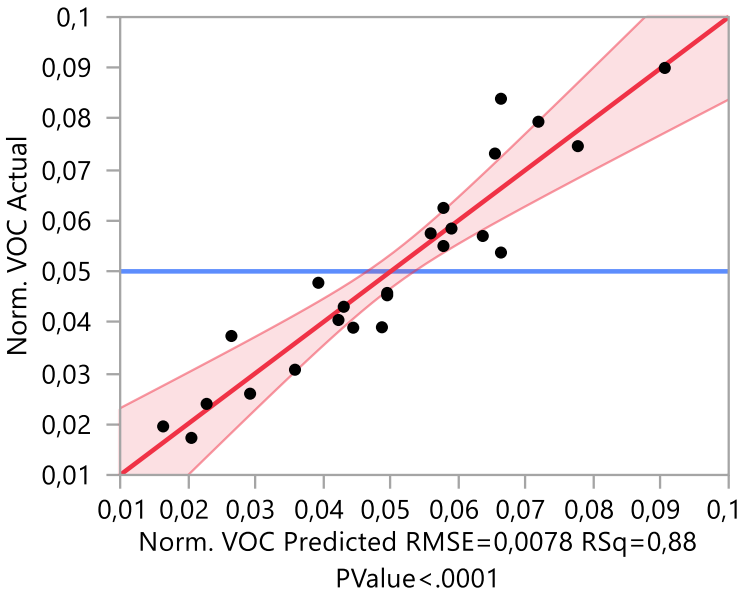


Figure 6.5 - PW/VOC second experiment – Actual by Predicted Plot for solvent's concentration response

Actual by predicted plot, provides the error of the model overview, the $r^2$ value of 0.88 and the estimate of the standard deviation of the response, RMSE=0.0078. These values suggest a trustworthy analysis.

Table 6.6 - Analysis of variance of full quadratic surface model for VOC concentration response

| Source | DF | Sum of Squares | Mean Square | F Ratio |
|---|---|---|---|---|
| Model | 5 | 0.00826424 | 0.001653 | 27.5180 |
| Error | 18 | 0.00108116 | 0.000060 | Prob > F |
| C. Total | 23 | 0.00934540 | | <.0001* |

Screening the model and analysis of variance is performed firstly for all effects and is narrowed down to significant effects, see Table 6.7.

Table 6.7 - Analysis of variance for all significant effect tests for VOC concentration response

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Temp | 1 | 1 | 0.00337998 | 56.2728 | <.0001* |
| LF | 1 | 1 | 0.00247970 | 41.2842 | <.0001* |
| GF | 1 | 1 | 0.00050798 | 8.4572 | 0.0094* |
| LF*LF | 1 | 1 | 0.00002183 | 0.3635 | 0.5541 |
| LF*Temp | 1 | 1 | 0.00005495 | 0.9149 | 0.3515 |

The JMP software assessed temperature, liquid and gas flow rate as statistically significant which is consistent with theoretical collected information. The (A/W) ratio however was not significant, which was not theoretically expected. This can be due to a not large enough (A/W) value range to impact significantly the output responses.

The p-values represented in the last column of the table with an asterisk, are small enough to indicate very convincing significance. Interaction between effects and crossed effects do not appear to have statistically relevant impact on the response. $LF^2$ and LF*Temperature effects were kept on the model to its significance in the second response analysis.

Analysis of variance in order of significance is sorted in Table 6.8.

Table 6.8 - Sorted parameter estimates for solvent concentration response

| Term | Estimate | Std Error | t Ratio | | Prob>\|t\| |
|---|---|---|---|---|---|
| Temp | -0.001978 | 0.000264 | -7.50 | | <.0001* |
| LF | 0.0083947 | 0.001307 | 6.43 | | <.0001* |
| GF | -9.185e-5 | 3.158e-5 | -2.91 | | 0.0094* |
| LF*Temp | 0.0001484 | 0.000155 | 0.96 | | 0.3515 |
| LF*LF | -0.000347 | 0.000576 | -0.60 | | 0.5541 |

Residual analysis is also consistent with distributed points in Figure 6.6, scattered randomly around zero, validating the model since homoscedasticity and independence of values seems to be respected, by random distribution of values. However, the initial row number points appear to have

slightly higher residual values as seen in Figure 6.7. This may be explained by the time of solvent dissolution which may have taken longer than expected, increasing the deviation of initial results from predicted values.

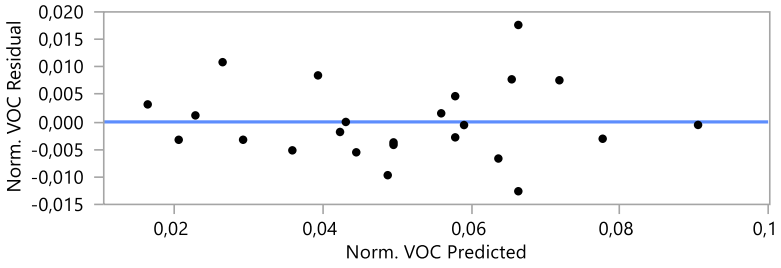


Figure 6.6 - Residual by predicted plot for solvent concentration response

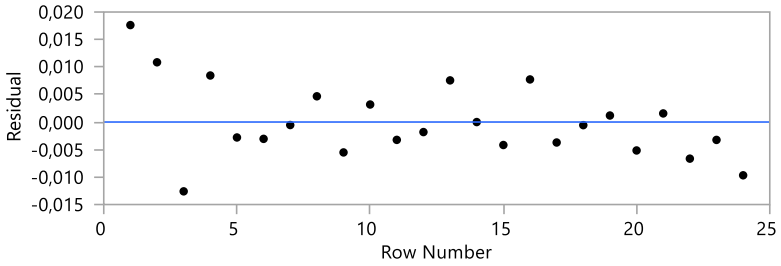


Figure 6.7 - Residual by row number plot for solvent concentration response

For the second response analyzed, the fit of the model was assessed, and significant effects were also analyzed. However, its main purpose is its analysis in the prediction profiler, which is why only the sorted parameter estimates for this response is presented in Table 6.9, showing significance of temperature, liquid flow rate both quadratic effects and cross effect.

Table 6.9 - Sorted parameter estimates for overall process time response

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| LF | -1.693909 | 0.087818 | -19.29 | <.0001* |
| LF*LF | 0.3923585 | 0.038739 | 10.13 | <.0001* |
| Temp | -0.074297 | 0.017725 | -4.19 | 0.0005* |
| LF*Temp | 0.0311276 | 0.010431 | 2.98 | 0.0080* |
| GF | -0.001524 | 0.002123 | -0.72 | 0.4821 |

The prediction profiler is useful to analyze the response surface and set the settings which produce the best response target. It is possible to define the desirability functions by setting the target to minimizing both responses to as low as possible. In JMP software, prediction profiler is an interactive chart which can be changed by moving the prediction traces (red dashed lines) for each x variable. Figure 6.8 is the prediction profiler of the performed DOE experiment with minimized values of both organic solvent concentration and process time responses.

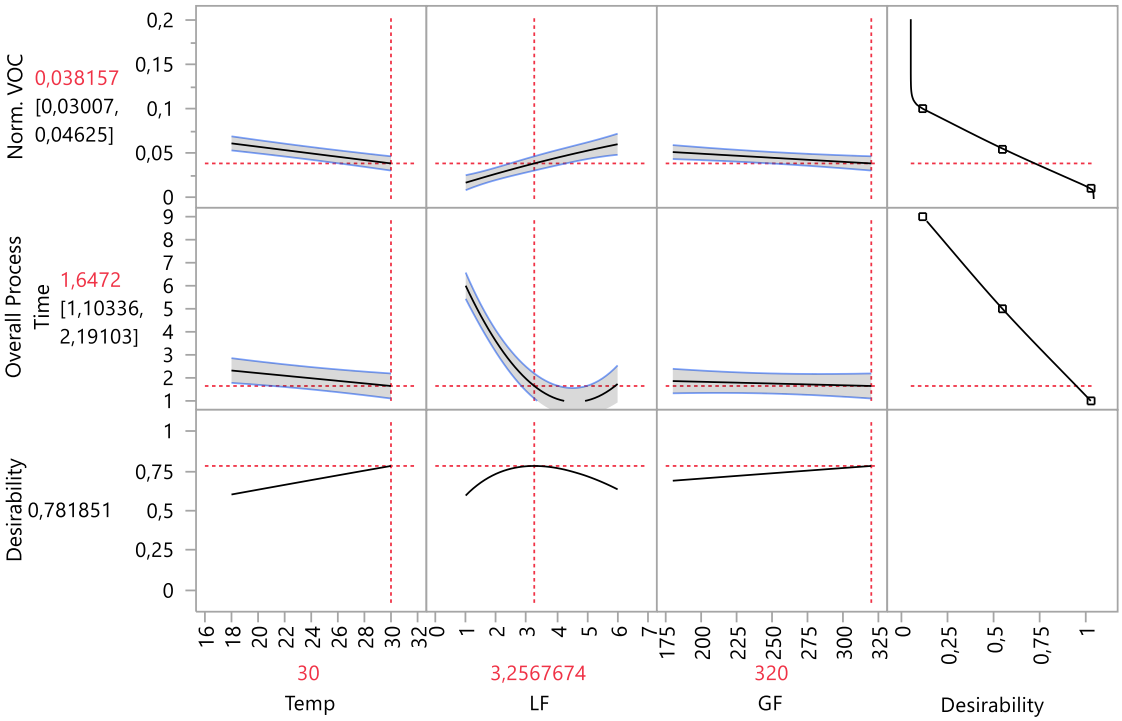


Figure 6.8 - PW/VOC second experiment; Prediction profiler matrix for overall DOE model

Prediction profiler suggests a minimized normalized solvent concentration value 0.038 still present in liquid phase when operating the column at 3.25 L/min, 320 L/min of gas flow rate and 30°C, this translates into a 91% removal efficiency per pass. In a closed system, assuming constant removal efficiency with unchanging parameters, then a 500L solution of water and solvent, could be reduced 89% in about 1,65 hours. According to the fit model of the DOE these are the optimal conditions to minimize both time and solvent concentration for this process.

For comparison purposes, seen ahead, for the parameters 20°C, 2L/min of liquid flow rate and 250 L/min of gas flow, the responses will shift to 0.05 of solvent in the liquid phase exiting the column (88% of efficiency removal per pass), and 4.6 hours of overall process time, as can be seen in appendix L.

## 6.3 Water, VOC and Protein

The initial concentration of solvent (normalized value) in the solution is roughly 0.97, and after one pass it was reduced to an average of roughly reduced to half at 0.45. The results are displayed in Table 6.10:

Table 6.10 - PW/VOC/Protein experimental control sample and experimental data results normalized

| Trial n° | VOC | Efficiency (%) |
|---|---|---|
| **1** | 0.45 | 53 |
| **2** | 0.47 | 51 |
| **3** | 0.43 | 56 |

It can be seen in this first evaporative study with the carrier protein a drop to 50% removal efficiency when comparing to the results with just PW and solvent.

As mentioned, scientific studies have been performed proving the surfactant presence reduces the VOC removal rate, due to increase of interface resistance [17].

The presence of surface active components in solution reduce the mass transfer rate in both gas-liquid and liquid-liquid systems, which is consistent with the obtained results for the solution with added protein. This reduction in mass transfer rate has been explained by the hydrodynamic and barrier mechanism, which relate to the increase of apparent viscosity of the dispersed phase and interfacial resistance, respectively [46,47].

## 6.4 Placebo solution (no presence of API)

As mentioned, experiments were performed in the pilot plant, with the placebo solution (without the API), using different setups and operating values. For these experiments the samples were directly taken from the vessel ensuring therefore the most precise way of sampling. This sampling preciseness is the closest to what would occur in parenteral production facility would the column be introduced into the manufacturing process.

Out of all experiments, the first one was a DOE screening experiment, and the three that followed were performed at fixed parameters in a continuous 5 hour air stripping process for a 250L bulk. The results will be, for all experiments using the placebo solution, presented in this chapter.

### DOE screening experiment

Table 6.11 summarizes the control sample VOC concentration normalized values. Again, these are used to make sure the solvent present in the liquid phase (before treatment) does not change significantly throughout the DOE experiment.

Table 6.11 – Placebo solution experimental control sample data results normalized

| Control Sample | Normalized concentration |
|---|---|
| **START1** | 0.73 |
| **START2** | 0.87 |
| **START3** | 0.60 |
| **MID CONTROL** | 0.76 |
| **END1** | 0.63 |
| **END2** | 0.58 |
| **END3** | 0.58 |
| **START1(30°C)** | 0.59 |
| **START2(30°C)** | 0.61 |
| **END1(30°C)** | 0.49 |
| **END2(30°C)** | 0.57 |

This was a screening design of experiments; therefore, several variables were tested. The solvent content in the liquid phase exiting the column was measured by gas chromatography and the values grouped at all temperatures, see tables Table 6.12, Table 6.13 and Table 6.14:

Table 6.12 – Placebo solution experimental normalized data results at 10°C

| T = 10°C | Normalized solvent concentration | | |
|---|---|---|---|
| GF Rate (L/min) / LF Rate (L/min) | 180 | 260 | 320 |
| 2 | 0.20<br>0.31 | 0.27 | 0.25 |
| 4 | 0.36 | 0.35 | 0.31 |
| 6 | 0.41 | 0.35 | 0.45 |

Table 6.13 – Placebo solution experimental normalized data results at 25°C

| T = 25°C | Normalized solvent concentration | | |
|---|---|---|---|
| GF Rate (L/min) / LF Rate (L/min) | 180 | 260 | 320 |
| 2 | - | 0.11<br>0.08 | 0.13 |
| 3 | - | 0.14 | 0.14 |
| 4 | 0.16 | 0.19 | - |
| 6 | 0.18 | 0.19 | - |

Table 6.14 – Placebo solution experimental normalized data results at 30°C

| T = 30°C | Normalized solvent concentration | | |
|---|---|---|---|
| GF Rate (L/min) / LF Rate (L/min) | 180 | 260 | 320 |
| 2 | 0.18 | - | 0.13 |
| 4 | - | 0.08<br>0.12 | - |
| 6 | 0.08 | - | 0.15<br>0.14 |

The organic solvent concentration in the stripped product results by order of trial, including the START and END is represented in Figure 6.9. In red are represented all the control values, which were taken in the start and end of the experimental trials. The last values were repeated the following day due to glycol system malfunction so additional control samples were taken at start and end of experiment.

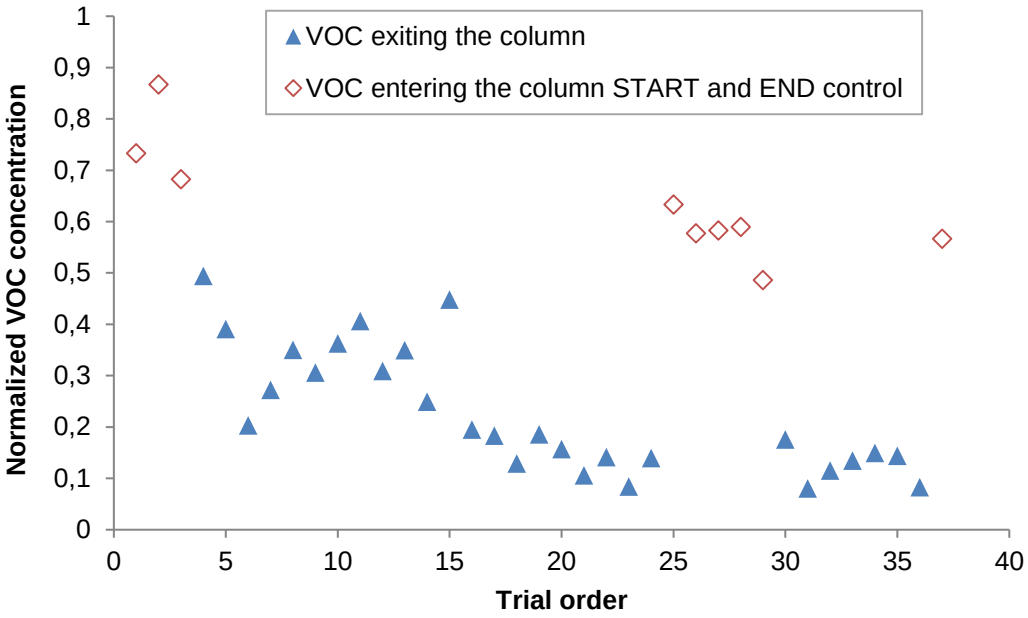


Figure 6.9 – Placebo solution DOE experiment – Normalized solvent concentration after stripping in trial order

The average removal for the whole process was 59% and standard deviation of 10%, being the higher efficiency points concentrated in the 30°C trials and the lower in the 10°C trials. For 10°C, 25°C and 30°C efficiency observed was 48%, 63% and 66% respectively.

Similarly, to the prior experiment with protein, the efficiency is considerably lower when in presence of the surface-active excipient. The efficiencies from both experiments in presence of protein are equivalent to one another, which leads to conclude, that none of the added compounds have a significant impact on mass transfer rate.

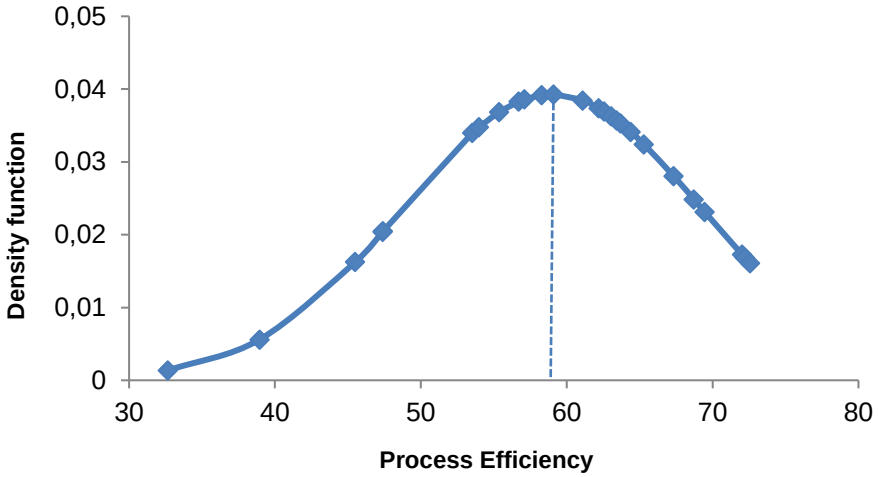


Figure 6.10 - Placebo solution DOE experiment; distribution curves

## DOE Analysis

The first model fit run tested all effects, including (A/W), crossed and quadratic effects. Some effects were removed from the analysis (holding larger P Value) for increased preciseness of results. For both combined responses the effect summary with respective significance represented in the plot bars is shown in Table 6.15:

Table 6.15 - Effect summary for combined responses

| Source | LogWorth | PValue |
|---|---|---|
| Temp | 9.284 | 0.00000 |
| LF | 4.699 | 0.00002 |
| Temp*LF | 1.691 | 0.02039 |

As in prior DOE, all not significant effects were removed, and for simplicity purposes only the final report covering significant effects is presented.

Regarding the first evaluated response, solvent concentration in liquid phase, Figure 6.11 provides the model evaluation by the actual by predicted plot.

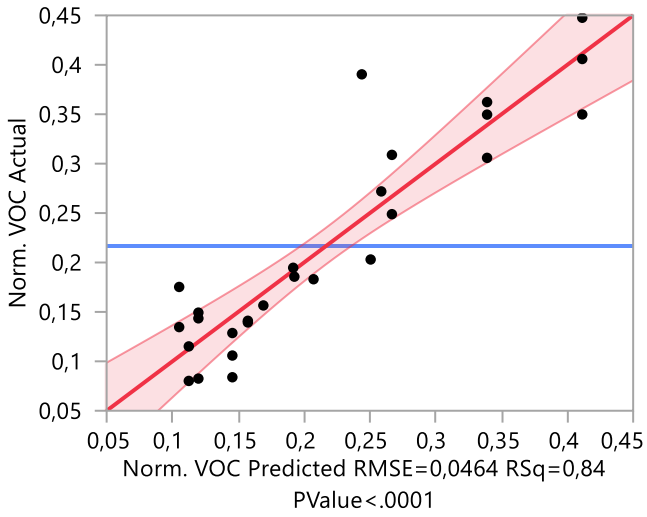


Figure 6.11 - Actual by Predicted Plot for solvent concentration response

The JMP model is reliable ($r^2$=0.84) with high power analysis and the ANOVA table indicates significance of factors, which is what is wanted to be defined with the screening analysis model.

The analysis of variance is evaluated for significant effects, see Table 6.16.

Table 6.16 - Analysis of variance of quadratic surface model for VOC concentration response

| Source | DF | Sum of Squares | Mean Square | F Ratio |
|---|---|---|---|---|
| Model | 3 | 0.26789650 | 0.089299 | 41.4210 |
| Error | 23 | 0.04958535 | 0.002156 | **Prob > F** |
| C. Total | 26 | 0.31748185 | | <.0001* |

P-values below 0.05 are indicative of existence of at least one significant value is present in the model. Significant values are identified in following tables with an asterisk.
Table 6.17 shows the terms sorted by their significance:

Table 6.17 - Sorted parameter estimates for solvent concentration response

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| Temp | -0.01109 | 0.001087 | -10.21 | <.0001* |
| LF | 0.0190938 | 0.005578 | 3.42 | 0.0023* |
| Temp*LF | -0.001625 | 0.000652 | -2.49 | 0.0204* |

The significant effects assessed by JMP model are temperature, liquid flow rate and the cross between the two. In this case gas flow rate was not influencing the responses which was assessed as significant in previous DOE, and should theoretically have an impact on process efficiency. This can be due to data accuracy or the range of values used. If using a very small gas flow rate, conceivably the efficiency would be lower. Additionally, the fact that the liquid is not being correctly distributed in the column may influence the hydrodynamic interaction between the gas and liquid. Meaning if liquid is taking preferable paths in continuous stream, the gas has more empty space to rise in the column and little interaction with liquid. The (A/W) ratio was once again not significant, which according to theoretical knowledge was not expected, this may be explained by same reasoning mentioned for previous DOE results. The gas flow rate used is possibly not large or small enough to have an impact on the stripping itself.

Residual analysis is also consistent; distributed points in Figure 6.12, appear to be scattered randomly around zero in an asymmetric pattern, being indicative of constant variability of residuals. The same applies for the residual by row plot, Figure 6.13 which validates the hypothesis of independence.

Similarly, to the first DOE the initial value also appears to have slightly higher residual value. This pattern may be explained by the time of solvent dissolution which may have taken longer than expected, increasing the deviation of initial results from predicted values.

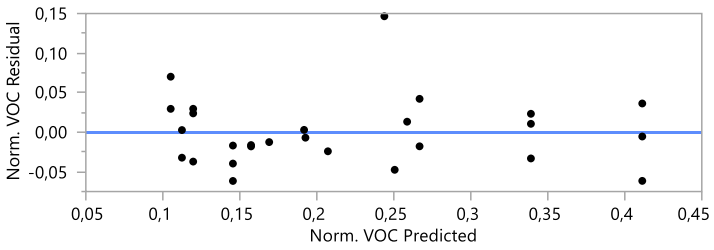


Figure 6.12 - Residual by predicted plot for solvent concentration response

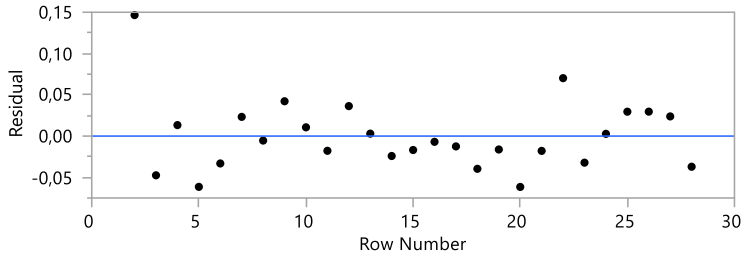


Figure 6.13 - Residual by row number plot for solvent concentration response

For the second response analyzed, the fit of the model was performed, and significant effects were also analyzed. However, its main purpose is its analysis in the prediction profiler, which is why only the sorted parameter estimates for this response are presented in Table 6.18. In this DOE report only, liquid flow rate and temperature as main effects were shown as significant opposed to the previous report with both quadratic effects as statistically significant for overall process time response.

Table 6.18 - Sorted parameter estimates for overall process time response

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| Temp | -0.01109 | 0.001087 | -10.21 | <.0001* |
| LF | 0.0190938 | 0.005578 | 3.42 | 0.0023* |
| Temp*LF | -0.001625 | 0.000652 | -2.49 | 0.0204* |

Finally, the prediction profile can maximize desirability in order to minimize both responses, since desirability functions were defined to minimize both the solvent concentration and the process time. Since gas flow rate was not determined as a significant effect buy the model, it was not included in the profiler since it change in value does not pose a change to either the responses.

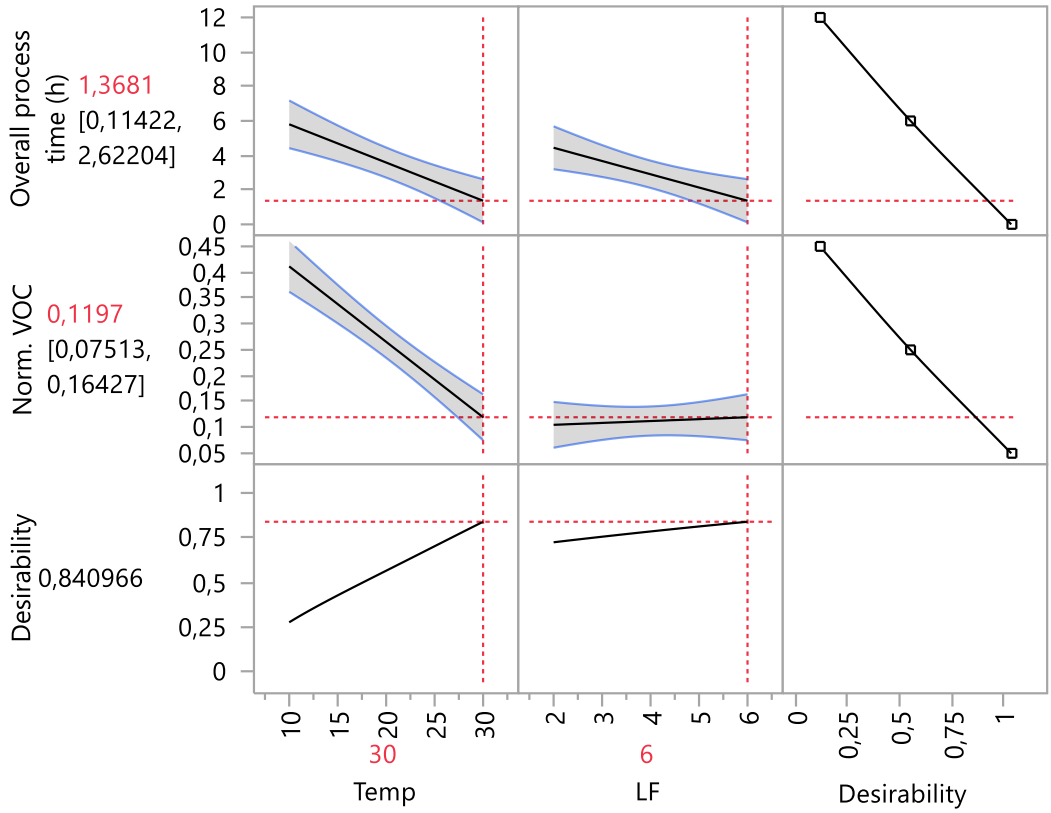


Figure 6.14 – Placebo solution DOE experiment; Prediction profiler matrix for overall DOE model

Prediction profiler suggests a minimized normalized solvent concentration value 0.12 at 6 L/min and 30°C exiting the column, translating into 80% removal efficiency per pass. At closed system circuit, if efficiency is admitted as constant with unchanging parameters, then a 500L solution of water/VOC, could be reduced 89% in about 3.16 hours. According to the fit model of the DOE these are the optimal conditions to minimize both time and solvent concentration for this process.

Changing parameters into 20°C, 2L/min of liquid flow rate and 250 L/min of gas flow rate, the responses will shift to 650.7 ppm (68% of efficiency removal per pass), and 6.9 hours of overall process time, as can be seen in appendix K.

## Liquid-Flow Rate Tests

Due to some uncertainties regarding the liquid flow rate results for the DOE collected data and its effects on the experiment, additional tests with liquid flow rate variation was performed.

The first liquid-flow rate experiment used a placebo solution with initial solvent concentration of 0.74 and passed through the column at different liquid flow rates before taking the samples. The obtained values in Table 6.19 represent the VOC concentration at liquid phase after air stripping:

Table 6.19 – Placebo solution experimental normalized data results at different liquid flow rates

| | Normalized solvent concentration | Eff (%) |
|---|---|---|
| **1L/min** | 0.16 | 78.74 |
| | 0.08 | 88.36 |
| | 0.12 | 83.72 |
| **2L/min** | 0.25 | 66.29 |
| | 0.22 | 70.69 |
| **4L/min** | 0.37 | 50.73 |
| **6L/min** | 0.52 | 30.26 |
| | 0.55 | 26.42 |
| | 0.43 | 42.86 |

Figure 6.15 represents the average concentration of solvent after one pass in the column at each flow with correspondent removal efficiency values:

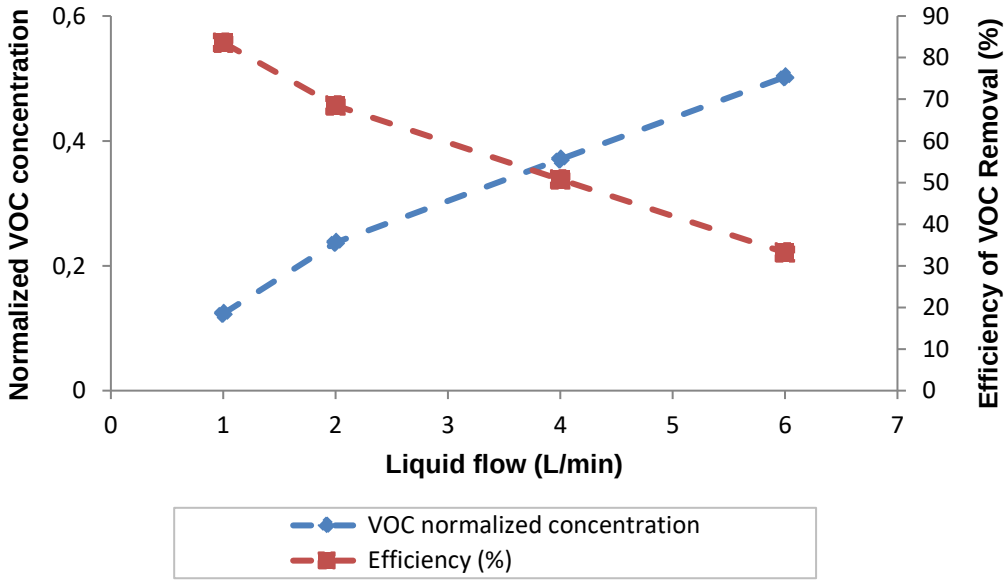


Figure 6.15 – Normalized solvent concentration after stripping and removal efficiency in function of operation liquid flow rate

The results clearly show higher column efficiency, by higher solvent evaporated per pass, when operating at lower liquid flow rates.

However, when looking at overall stripping process in a continuous flow with several passes through the column, it becomes obvious (also shown in the prediction profiler plot) that liquid flow rate has a much larger impact on the overall process time than the efficiency of removal itself. This tendency can also be observed by comparing liquid flow rate significance in the sorted parameters for both responses, VOC concentration and overall process time for reducing 89% of initial solvent concentration in liquid phase, as seen when comparing tables Table 6.20 and Table 6.21:

Table 6.20 - Sorted parameter estimates for solvent evaporation

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| Temperature | -0.011 | 0.0011 | -10.21 | <.0001* |
| **LF** | **0.019** | **0.006** | **3.42** | **0.0023*** |
| Temperature*LF | -0.0016 | 0.0007 | -2.49 | 0.0204* |

Table 6.21 - Sorted parameter estimates for overall process time

| Term | Estimate | Std Error | t Ratio | Prob>\|t\| |
|---|---|---|---|---|
| Temperature | -0.24 | 0.03 | -7.77 | <.0001* |
| **LF** | **-0.84** | **0.16** | **-5.34** | **<.0001*** |
| Temperature*LF | 0.007 | 0.018 | 0.40 | 0.6924 |

In sum, although the efficiency is lower at lower liquid flow rate, the overall process is reduced in time due to faster circulation of the solution through the column.

The proof of concept was experimentally established by re-assembling the placebo solution recirculation setup (Figure 5.7- without spray ball) and two different experiments were performed for 3 and a half hours maintaining the variables constant with exception to the liquid flow rate; temperature used was 20°C, gas flow rate of 250 L/min and solution volume of 50L.

The two tested flows were 2 L/min and 4L/min; it was not possible to increase more due to foam formation during continuous recirculation of the bulk. For result analysis purposes, the data was fitted to the same initial concentration using the calculated efficiency. The obtained results can be found in appendix 8.J and it can be verified the very similar concentration profile curve, and slightly fast solvent concentration reduction at higher flowrates in Figure 6.16.

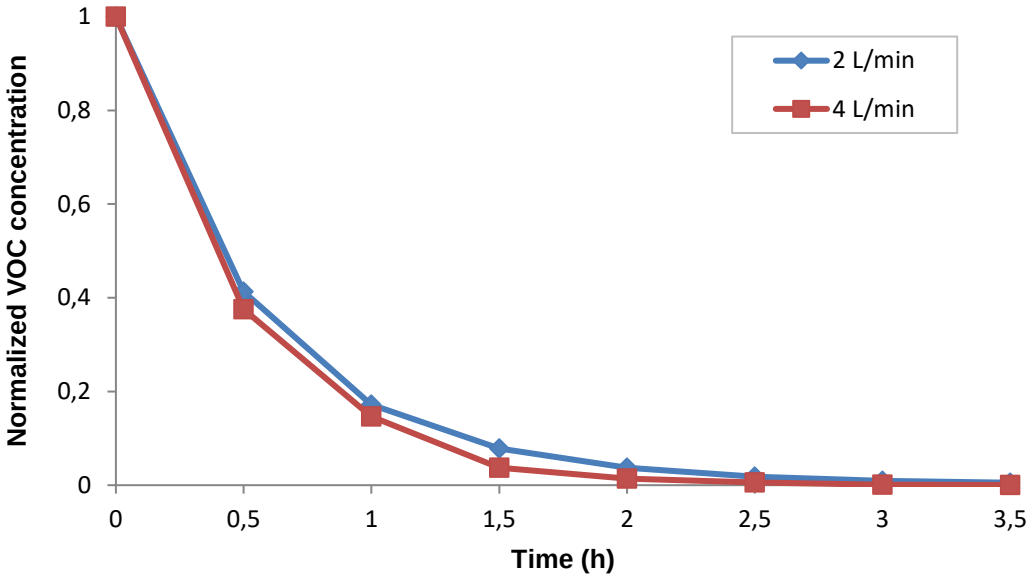


Figure 6.16 – Normalized solvent evaporative profile curve in function of time - different liquid loads

Although a bigger difference between flows would be preferential to assuredly confirm the liquid flow rate impact, the drop of overall process time with flow rate is displayed in the plotted data. From a 2 to 4 L/min liquid flow rate change, resulted in an overall 11% efficiency increase. The liquid load can therefore be increased to improve the overall process time. Although the DOE results did not show significance of gas flow rate, if liquid rate is incremented it is highly recommend adjusting the gas flow rate accordingly maintaining the (A/W) ratio.

There might be a turning point in which the column efficiency may be significantly impacted in a way the increase of flow rate would actually delay the overall process time. To study the existence of this turning point, higher flow rates would need to be tested which would require the use of higher pump capacity and ultimately change in column internals that would allow the increase of liquid loads without foam formation (random packing and different liquid distribution).

## Recirculating Flow Experiments:

The results were obtained from GC analysis and the removal efficiency for each sample was calculated for all performed trials. The measured concentrations in time and respective removal efficiency are presented for all recirculating experiments in tables Table 6.22, Table 6.23 and Table 6.24.

The first trial was performed at lower temperatures and higher initial concentration (above the theoretical BMR concentration, therefore above 1). The undesired lower temperature was due to glycol heating system and was not kept constant therefore temperature dropped two degrees during air stripping process. After three hours of stripping some foam starting to form at the top of the column, so

the process was briefly interrupted for removal of the same and restart of the process. The foam did not seem to disturb the process itself; however, it was decided to remove it, in order to maintain the process as unchanged as possible while taking the samples.

Table 6.22 – Placebo solution recirculating flow experimental normalized data results at 18°C

| Time of stripping (h) | Normalized solvent concentration | Removal efficiency (%) |
|---|---|---|
| t = 0:00 | 2.62 | - |
| t = 0:30 | 1.91 | 27.18 |
| t = 1:00 | 1.56 | 18.53 |
| t = 1:30 | 0.96 | 38.31 |
| t = 2:00 | 1.30 | -35.08 |
| t = 2:30 | 0.77 | 40.33 |
| t = 3:00 | 0.60 | 23.05 |
| t = 3:30 | 0.46 | 23.43 |
| t = 4:00 | 0.34 | 25.65 |
| t = 4:30 | 0.30 | 12.41 |
| t = 5:00 | 0.22 | 25 |
| t = 5:30 | 0.18 | 17.59 |
| t = 6:00 | 0.13 | 29.30 |

As referred, the closed-system experiment with continuous recirculation of the bulk was repeated this time using the glycol system to set and maintain the temperature at 25°C.

Table 6.23- Placebo solution recirculating flow experimental normalized data results at 25°C

| Time of stripping (h) | Normalized VOC concentration | Removal efficiency (%) |
|---|---|---|
| t = 0:00 | 1.21 | - |
| t = 0:30 | 0.77 | 36.00 |
| t = 1:00 | 0.59 | 23.57 |
| t = 1:30 | 0.47 | 21.12 |
| t = 2:00 | 0.49 | -4.23 |
| t = 2:30 | 0.39 | 20.23 |
| t = 3:00 | 0.31 | 21.01 |
| t = 3:30 | 0.26 | 13.93 |
| t = 4:00 | 0.22 | 18.06 |
| t = 4:30 | 0.18 | 17.15 |
| t = 5:00 | 0.15 | 16.83 |
| t = 5:30 | 0.12 | 19.84 |

The current process is stripping the VOC by inflowing compressed air from the top of the column through a spray ball, so the experiment was also repeated with both evaporative processes in simultaneous; air stripping column and spray ball injection of compressed air.

Table 6.24 – Placebo solution recirculating flow experimental normalized data results at 25°C with sprayball

| Time of stripping (h) | Normalized VOC concentration | Removal efficiency (%) |
|---|---|---|
| t = 0:00 | 1.02 | - |
| t = 0:30 | 0.60 | 41.28 |
| t = 1:00 | 0.35 | 42.00 |
| t = 1:30 | 0.21 | 39.28 |
| t = 2:00 | 0.19 | 8.17 |
| t = 2:30 | 0.11 | 41.23 |
| t = 3:00 | 0.07 | 35.35 |
| t = 3:30 | 0.03 | 57.85 |
| t = 4:00 | 0.02 | 39.44 |
| t = 4:30 | 0.01 | 36.70 |
| t = 5:00 | 0.01 | 27.75 |
| t = 5:30 | 0.005 | 43.97 |

Removal efficiency is not constant throughout each group trial, showing hard reproducibility of the process itself. Although the process maintains the same operating conditions the removal rate curves, in Figure 6.17 display an overall comparable tendency but its precision is quite low, increasing process variability.

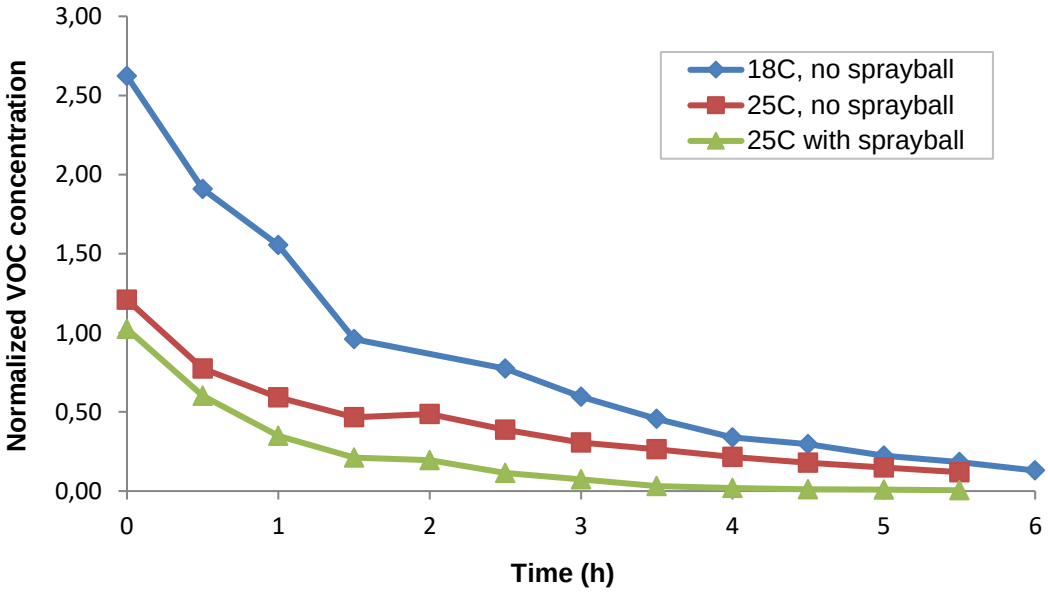


Figure 6.17 – Placebo solution recirculation experiment – Normalized solvent evaporative profile curve in function of time at different initial concentrations, temperatures and compressed air entering the vessel presence

After two hours for all three experiments big removal efficiency drop is observed, perhaps due to brief process interruption as can be seen in tables Table 6.22 and Table 6.23 in the greyed rows. If these outliers are excluded, the average removal efficiency is 23%, 20% and 40% respectively for all three experiments.

The average removal efficiency at 18°C temperature is higher than the 25°C without spray ball. This result is not coherent with the previous obtained data nor the correlation of diffusion and Henry's coefficient with temperature. This unexpected result may be result of equipment/utilities failure, as was described that the glycol system was not working properly, and/or the temperature probe was not properly measuring the temperature. The higher initial concentration could also have an influence in these results, but additional experiments would need to be performed to validate this statement.

For comparing purposes, the data from two experiments at 25°C was fitted with removal efficiency values for the initial solvent concentration to have the same start point for both experiences. Evaporation profile curves for both processes with and without compressed air entering the top of the vessel through a spray ball are shown in Table 6.23

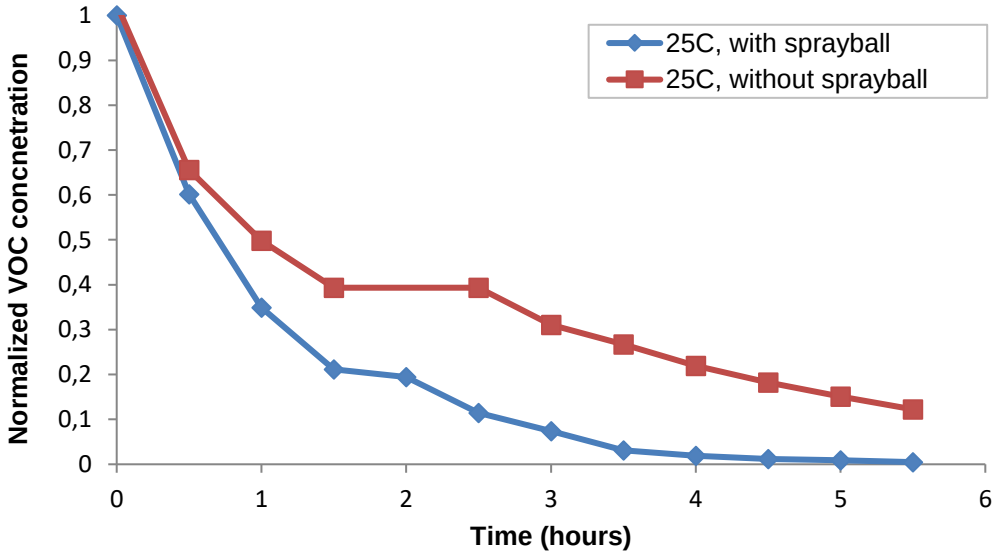


Figure 6.18 – Placebo solution recirculation experiment – Normalized solvent evaporative profile curve in function of time - with and without compressed air entering the top of the vessel

With use of compressed air evaporation takes about half the time to reach the same concentration as the experiment using only the air stripper column to solvent evaporation process. This acceleration becomes more accentuated at lower concentration gradients.

The overall process time, when using this recirculation setup (in which the solution exiting the bottom of the column is feed to the same vessel) is much lower than the DOE performed for placebo solution, as seen in the prediction profiler for the DOE experiment with the same placebo solution (experiment 5 from Table 5.1).

These observations suggest that this set-up is not ideal when comparing it to the pass by pass DOE experiment. For this reason, another experiment was performed at fixed conditions; the vessel to vessel experiment.

## Vessel to Vessel Experiment:

A different setup was tested, similar to Figure 5.6, in which the treated product coming from the bottom was not feed back to the same vessel but to a second empty one; experiment 9 from Table 5.1. The experiment was performed at 20°C and no spray ball was added to the vessels. After all volume content was transferred from the initial vessel the second vessel, a sample was taken. This process was repeated a total of five times back and forward between the two vessels. The main objective was to determine how much impact would the recirculation of treated product exiting the bottom of the column back into the feeding vessel would impact the overall process time. The represented recirculating curve was performed at same conditions of temperature and liquid/gas flow rates. Evaporative curves in time can be observed in Figure 6.19 for both recirculating and vessel to vessel set-ups.

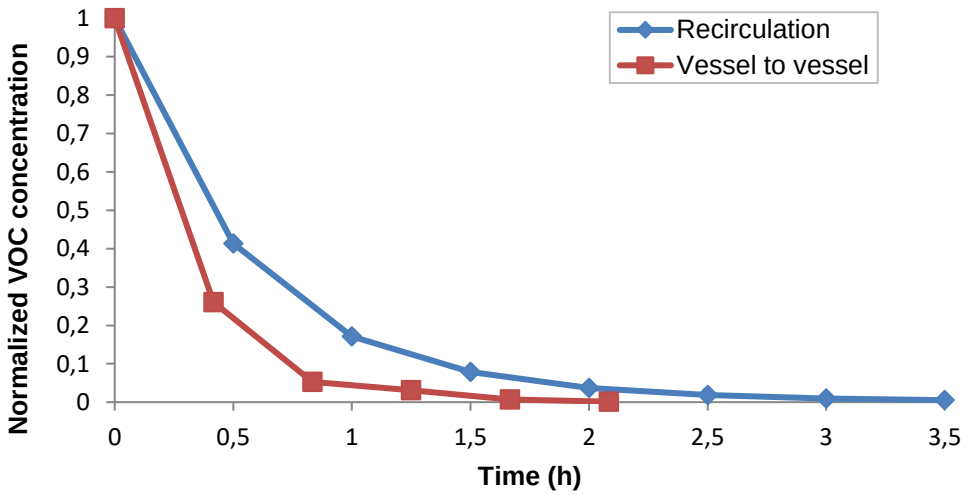


Figure 6.19- Placebo solution – Normalized solvent evaporative profile curve in function of time for the different pilot plant set-ups

The overall process time was calculated for both setups and compared with the result obtained during the placebo solution DOE (experiment 5 from Table 5.1), which can be estimated in the JMP prediction profiler by setting the parameters to 20°C and 2 L/min (appendix L). As can be seen in Table 6.25 the recirculation does have a big impact on the process duration.

Table 6.25 – Overall process time in hours for experiments 5 and 9

| Experimental setup | Overall Process time |
|---|---|
| DOE Prediction Profiler | 7 hours |
| Recirculating bulk | 12 hours |
| Vessel to vessel | 6 hours |

## 6.5 Nano-suspension Solution

This experiment main purpose was performed to assess the stability of the solution; is the air stripper column contributing to any negative impact on the stability of nanoparticles. According to the product specification values, after evaporation step, the average particle size should take values between 80 and 160 nm and the polydispersity index should not take values above 0.2. Figure 6.20 characterizes the results given by the zetasizer equipment for all taken samples.

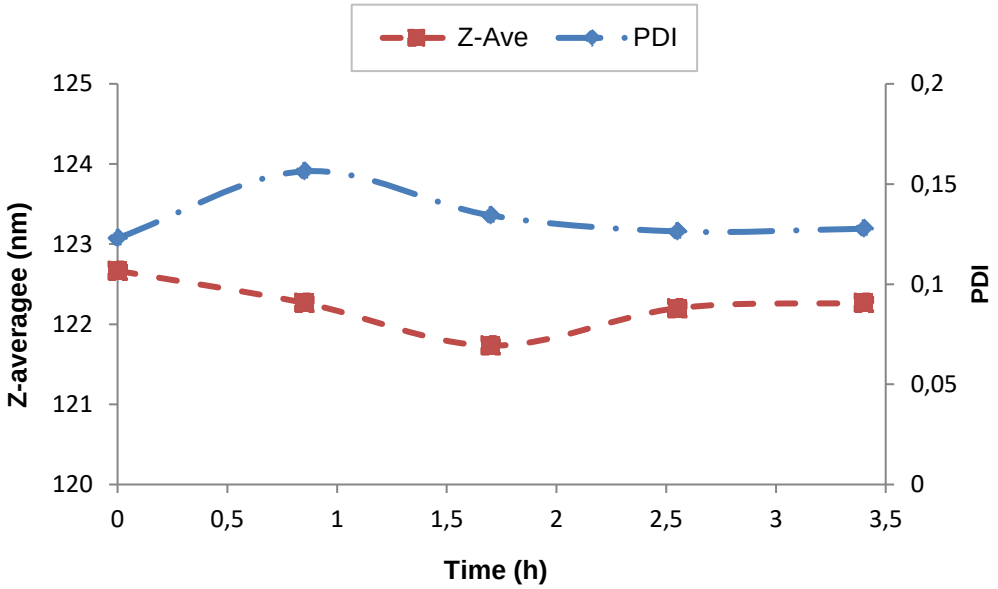


Figure 6.20 - Nano-suspension experiment; Particle size and PDI in time during air stripping operation

As can be seen from all five measurements, the average particle size shows no signs of increase from the initial values, and the PDI is below the regulated values.

Regarding the solvent evaporation of the nano-suspension solution, the obtained data was compared to an exact same operational evaporative setup and parameters using the placebo solution to determine the impact of nano-particles in the mass transfer rate as seen in Figure 6.21. The placebo curve was fitted with the obtained efficiency values to have identical start concentrations.

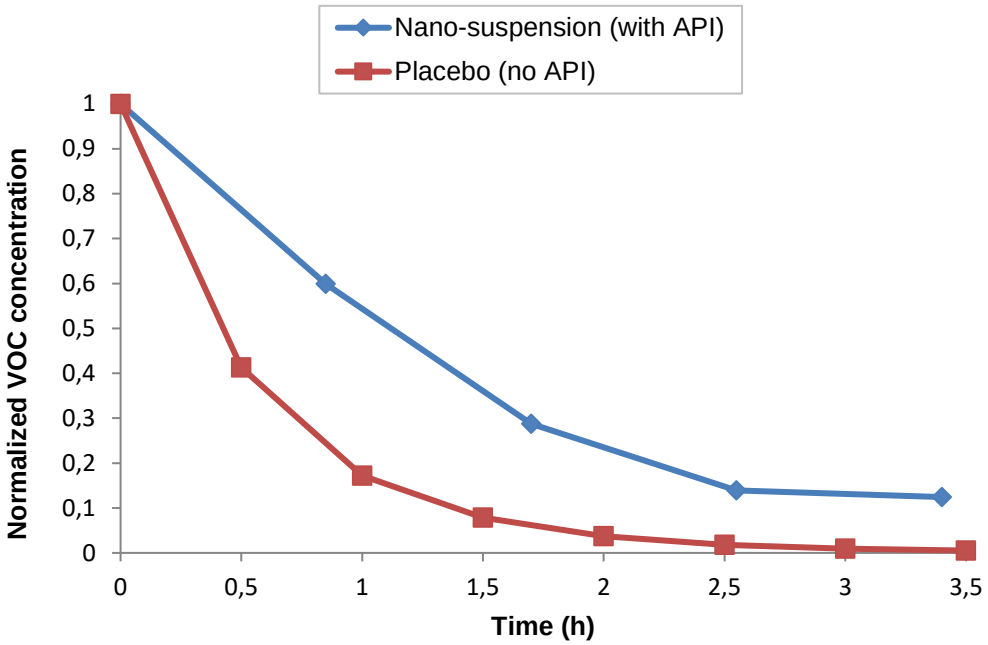


Figure 6.21 - Nano-suspension Experiment – Normalized solvent evaporative profile curve in function of time for placebo and nano-suspension solutions

The existence of the nanoparticles increased in average the process time from 1 to 3 hours to evaporate the initial concentration to the end desired value. These results are according to expected due to the presence of nano-particles which describe a kinetic mass transfer heterogeneous model. The nano-particles will therefore limit the mass transfer rate of the solvent rate during air stripping operation.

As mentioned the overall process time harmonizes the results between experiments and therefore was used for comparison purposes in Table 6.26. The overall time is the time to reduce the concentration (89% VOC removal of the initial BMR concentration) of a 500L solution at the operational parameters of each experiment. Since the DOE was performed at several parameters, the overall time is given for fixed parameters; 20°C, 2 L/min of liquid flow rate and 250 L/min of gas rate flow. These values can be estimated by the DOE analysis in the prediction profiler, see appendix K.

Table 6.26 - Result overview

| Sample ID | Description | Overall Process Time (h) | Z-Avg (nm) | PDI | Other Observations |
|---|---|---|---|---|---|
| **1 – PW & Protein** | T = Room temperature<br>Several LF/GF are tested | N.A. | N.A | N.A. | Some foam formation – not stable foam<br>Column flooding was not reached due to small liquid loads |

| Sample ID | Description | Overall Process Time (h) | Z-Avg (nm) | PDI | Other Observations |
|---|---|---|---|---|---|
| **2 – PW & VOC** | T = Constant room temperature<br>LF = 2.4 and 6 L/min<br>GF = 80, 160, 240 and 320 L/min | N.A | N.A. | N.A. | DOE data was rejected due to introduction of non-controlled variable(s) |
| **3 - PW & VOC DOE** | T = 18 and 30 (°C)<br>LF = 1 to 6 L/min<br>GF = 180, 250 and 320 L/min | 4.6 | N.A. | N.A. | - |
| **4 – PW, VOC & Protein** | T = Constant room temperature<br>LF = 2 L/min<br>GF = 200 L/min | 11.7 | N.A | N.A. | First experiment with carrier protein showed a significant decrease in the removal efficiency |
| **5 – Placebo solution DOE** | T = 10, 20 and 30 °C<br>LF = 2, 3, 4 and 6 L/min<br>GF = 180, 260, 320 L/min | 6.9 | N.A. | N.A. | - |
| **6 – Placebo solution; Recirculating flow** | T = 18 °C<br>LF = 2.5 L/min<br>GF = 250 L/min<br>Volume = 125 L | 20.7 | N.A. | N.A. | - |
| **7 – Placebo solution; Recirculating flow (no spray ball)** | T = 25 °C<br>LF = 2.5 L/min<br>GF = 250 L/min<br>Volume = 125L | 22.4 | N.A | N.A. | - |
| **8 – Placebo solution; Recirculating flow with spray ball** | T = 25 °C<br>LF = 2.5 L/min<br>GF = 250 L/min<br>Volume = 125L | 10.8 | N.A. | N.A. | - |
| **9 – Vessel to vessel setup** | T = 20°C<br>LF = 2 L/min<br>GF = 250L/min<br>Volume = 50 L | 6 | N.A | N.A | - |
| **10 – Nano-suspension solution** | T=20°C<br>LF= 2 L/min<br>GF = 250 L/min<br>Solution Volume = 50 L | 32 | 122 (±0.33) | 0.13 (±0.01) | This overall process time increase is a result of the nano-particle presence |

## 7. Design of Air Stripping Column

The column's optimal diameter relates to the hydrodynamic characteristics of the column, concretely to the gas flow rate which circulates in it [46]. Conversely, the height of the column should be proportional to the difficulty of the components to be separated; meaning the column should be taller for challenging separations and vice versa. This is logical since a bigger height will allow longer liquid hold-up in the column, therefore allowing prolonged contact between phases. The design was calculated to optimize the process to the specific product as a future recommendation.

The design is the calculation of the optimal values of height and diameter of the air stripping column necessary to reach certain removal efficiency. For the drug product manufacturing process solvent 89% removal efficiency is necessary. This will be the target point design for the stripping column removal efficiency. The fixed parameters for this calculation will be 250 L/min of gas flow rate and 3L/min of liquid flow rate for a total of 500L bulk. With proper column design and if vessel to vessel set-up is used at 3L/min of liquid load, theoretically the overall process time would be reduced to 3 hours, since solvent concentration would be reduced to the required concentration with a single pass through the column.

To estimate the required diameter of the column information on pressure drop curves, L/G ratio and packing material are required. By fixing the pressure drop it is possible in few steps the calculation of the optimal diameter by use the following diagram developed by Eckert[47], which correlates the flooding and the pressure drop in packed columns as seen in Figure 7.1.

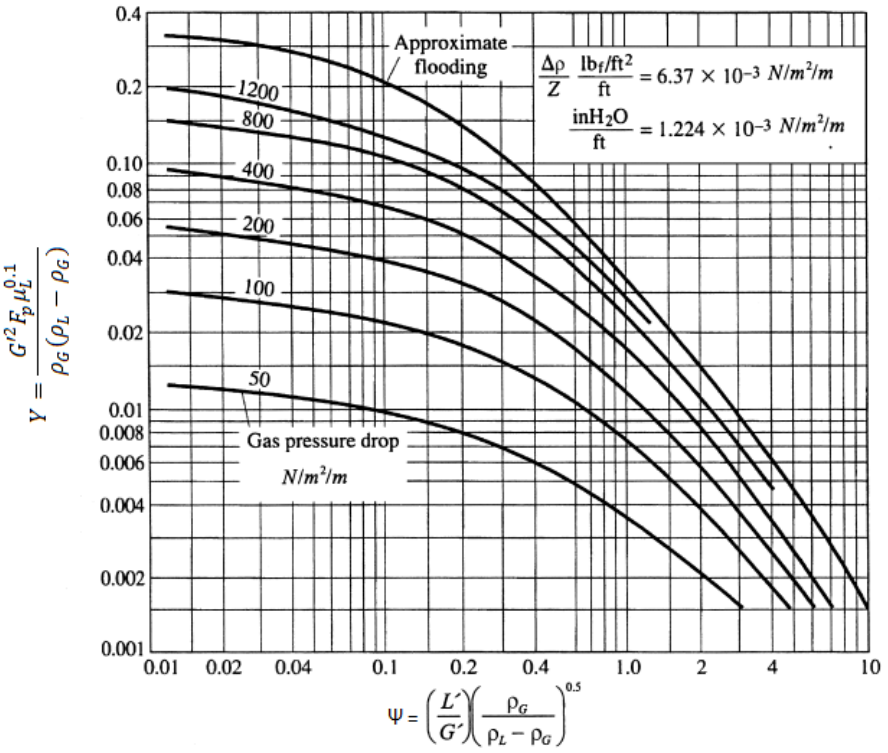


Figure 7.1 - Generalized flooding and pressure drop correlation for packed columns

The parameter Ψ represented in the abscissa of the diagram with already known data can be obtained. The ratio ($L'/G'$) represents the quotient between mass flux of both phases, which is not known since the cross-sectional area of the column is still unknown. However, this ratio will be the same if the mass flows are divided. Therefore,

$$\frac{L'}{G'} = \frac{L}{G} = 10.17 \qquad \text{(eq. 24)}$$

The solution density is available in the BMR of drug product and is equal to 1012 kg/m$^3$. By applying the values in the equation, $\Psi = 0.35$.

The pressure drop was previously discussed as ranging between 0,2 and 0,5 mbar (50 (N/m$^2$)/m), therefore the lower gas pressure drop curve. It is now possible to determine Y=0,007.

The packing factor $F_P$ is empirically determined for each packing type and size and provided by the manufacturer, the packing factor is provided for the used structured packing (Mellapak 500Y) by Sulzer [8], see Table 7.1:

Table 7.1 - Characteristics of Mellapak Structured Packing

| Name | Size or number | Area, m$^2$/m$^3$ | % voids* | Packing factor, m$^{-1}$ Normal $F_p$† | Packing factor, m$^{-1}$ Dry $F_{pd}$‡ | Vendor |
|---|---|---|---|---|---|---|
| Metals, corrugated sheets | | | | | | |
| Mellapak | 125Y | 125 | 99 | 33 | | Sulzer |
| | 170Y | 170 | 99 | 39 | | |
| | 2Y | 223 | 99 | 46 | | |
| | 250Y | 250 | 98 | 66 | | |
| | 350Y | 350 | 98 | 75 | | |
| | 500Y | 500 | 98 | 112 | | |

The liquid viscosity is 1.04 cP at protein concentration and 25°C. From the gas calculation flux value, the cross-sectional area of the column can be obtained ($A_c = G/G'$) and the diameter:

$$d_c = \left(\frac{4A_c}{\pi}\right)^{1/2} = 0.152\ m = 151.57\ mm \qquad \text{(eq. 25)}$$

For this specific drug product manufacturing specific case it was assessed to be preferable the use a packed bed, since the quench solution has propensity to foam which is suppressed by the lower gas and liquid velocities and the packing area promotes foam dispersal, especially with random packing, which possesses larger open area. The packed bed column is also preferable for small-diameter columns ($d_c$<1m), which is the case of the column necessary for the manufacture of this product due to low loading rates.

The height of the column was determined through mass transfer equations. The height is given by the product of the number of transfer units and height of transfer units:

$$h = NTU * HTU \quad \text{(eq. 26)}$$

The number of transfer units is calculated with the stripping factor, determined with the air-to-water ratio and the concentration of solvent entering and exiting the column.

$$\mathrm{N}TU = \frac{S}{(S-1)} ln\left(\frac{C_{Li(in)}(S-1)}{C_{Li(out)}} + \frac{1}{S}\right) = 2.29 \quad \text{(eq. 27)}$$

The air-to-water ratio (A/W) can also be estimated relating it to the stripping factor as seen in (eq.7). The stripping factor is the ratio of the slopes of equilibrium and operating lines and is given by the Kemser-Brown equation also used when designing absorption columns [31]. It determines the theoretical ability of the stripper to remove a specific volatile compound. However, using this equation has a big margin of error associated since as mentioned previously it is very difficult to accurately determine Henry's law coefficient in a solution.

Height of transfer units is obtained through the following equation:

$$\mathrm{H}TU = \frac{L_v}{K_L a \times A_c} \quad \text{(eq. 28)}$$

Where $\mathrm{A_c}$ is the cross-sectional area, calculated with the new obtained diameter of 0,152 m obtained from (eq.25) ($\mathrm{A_c} = 0.018\ \mathrm{m^2}$), $\mathrm{L_v}$ the volumetric liquid flow rate and $\mathrm{K_L a}$ the transfer rate constant. This last term may be estimated through the Sherwood and Holloway correlation:

$$\frac{K_L a}{D_{Li}} = 10.764\ \alpha \left(\frac{0.3048\ \times L'}{U_L}\right)^{(1-n)} \left(\frac{U_L}{\rho_L D_{Li}}\right)^{0,5} \quad \text{(eq. 29)}$$

The liquid mass flux is obtained dividing the liquid mass flow with the column cross sectional area ($\mathrm{L'} = 2.78 \frac{\mathrm{kg}}{\mathrm{m^2 s}}$). The constants $\alpha$ and $\mathrm{n}$ are specific for packing time and diameter. The reason between the diameter of the column and the packing material should be, by thumb rule 15/1, therefore the packing diameter with current column should be around 10 mm to maximize efficiency.

Data for this calculation used the information from 12 mm raschig rings, in which $\alpha = 920$ and $\mathrm{n} = 0.35$ [48].

Diffusion coefficient of the compound in liquid, $\mathrm{D_{Li}}$, needs to be determined as well using the Wilke and Chang equation [49]:

$$\frac{\mathrm{D_{Li}U_L}}{\mathrm{T}} = \frac{7.48 \times 10^{-8}(\varphi_\mathrm{B}\mathrm{M_B})^{1/2}}{\mathrm{Vb}^{0,6}} = 1.02\ \times 10^{-9}\ \mathrm{m^2/s} \quad \text{(eq. 30)}$$

Where, temperature, T, needs to come in Kelvin and liquid molecular weight, $\mathrm{M_B}$ in g/mol. Vb, represents the molal volume of solvent at normal boiling temperature and is equal to 80.7 $\mathrm{cm^3}$ [49], $\varphi_\mathrm{B}$ is the association parameter for solvent (used water as solvent where $\varphi_\mathrm{B} = 2.6$) and $\mathrm{U_L}$ is the viscosity of the solvent.

Replacing the diffusion coefficient in Sherwood and Holloway correlation equation, the transfer rate correlation was obtained ($K_L a = 9.12 \times 10^{-3} s^{-1}$) and can be used to calculate the height of transfer unit, HTU = 0.305. Finally replacing the obtained values of NTU and HTU in eq. 26 the column height equals to 0.70 m

Due to lack of physical and chemical data on the nano-suspension solution, water as solute in literature data values were used, such as henry's coefficient and both $\varphi_B M_B$ terms for diffusion coefficient calculation.

This may have a considerable impact on the diffusion and transfer rate constant calculated values, especially interfacial resistance considerations which, as seen in experimental result, is substantially reduced with addition of carrier protein.

Additionally, the nano-particle effect is not taken into consideration nor does its diffusional limitation on VOX evaporation which was also demonstrated to play a role in the performed experiments. For this reason, it is recommended to over-design the estimated value of 0.70 m of height to at least 1.2 m to comply with the desired efficiency. This average value was estimated through the experimental data and obtained removal efficiencies with the current tested column for the nano-suspension experiments.

In conclusion the column was re-designed to fit the manufacturing needs to a height of 1.2 m and internal diameter of 0.152 m.

# 8. Conclusions and Future Work

The air stripping column proved to be applicable to this drug product nano-suspension evaporative process and established a significant improvement on present process time. The process unit is practicable in terms of foam formation, if column is not operated at a liquid flow rate above 4 L/min. This liquid load value however can be increased if packing material is replaced by random packing and a liquid distributer which operates by gravity in opposition to pressure. The experimental research allowed the determination of a set of parameters which influence the solvent evaporation and quantify their impact on the mass transfer rate as well as determination of concentration profiles for VOC evaporation in time.

Two performed screening DOE - although not full factorial - experiments were successful with high model power of analysis. Results showed statistical significance of liquid flow rate and temperature as well as the combined effect of temperature*liquid flow rate. Gas flow rate was only assessed as significant for the first DOE, nevertheless marginally significant.

Increase of temperature presented a significant increase of evaporation productivity; however, it is important to maintain temperature below 30°C due to risk of protein denaturation. DOE temperature study showed an average 4.5 hours' time reduction by increasing the temperature from 10 to 30°C. Presently the used temperature during the manufacturing process is on average 20°C, and if increased to 30°C a total of two processual hours can be gained.

Liquid flow rate had opposite impact on DOE studied responses: solvent concentration after stripping and overall process time. The liquid flow rate effect on overall process time however was higher, meaning although efficiency in the column was reduced the solution was being stripped faster; with correct design of the column the efficiency is maximized for higher liquid flow rates.

Experiment with nano-suspension, including the active pharmaceutical ingredient, showed solution stability by sustaining the specified particle size and polydispersity index after 3.5 hours of air stripping through the column. The evaporation however was delayed by a factor of three, evidencing diffusional limitation on mass transfer of the solvent due to its adsorption in formed nano-particles; three phase model. Previous experiments indicated protein's surface-active properties were responsible for the increase of interfacial resistance, furthermore impacting the mass transfer rate.

Several additional experiments allowed the study of time reduction of current process using different setups which takes around 30h of evaporation time. The recirculation experiments showed a half time reduction only by adding the air stripping column to the process as can be seen when comparing experiments 7 and 8, gaining 15h time using the currently existing column model. Therefore, air stripping column should be used simultaneously with the compressed air entering the vessel through the spray ball.

If the treated product does not return to the feeding vessel but to a separate empty one the process time is additionally reduced to a total of 8 hours, however this would require additional floor space for a second 500L vessel.

With a column with proper height and internals, the process time can be reduced up to 4 hours if the two vessels setup is used. Design calculations on the optimal column height and diameter were performed to achieve the desired column efficiency. Due to low loading rates the column's diameter may be reduced to 0.152 m. The column height needs to be increased as well to attain the 89% solvent removal in one single pass, the design used the mass transfer equations, however some terms used water literature data and as described in the report, both protein and nano-particles pose limitations on VOC mass transfer. For this reason, this height was over-designed to 1.2 m which was estimated through the collected results of experiment 10 and the current column height.

Regarding the recommended internals, for the new designed column; replacement of the current structured packing by random packing material (chemically resistant, such as PVDF Raching rings) with average diameter of 15mm is needed. Change of liquid distributor to low load specific equipment, such as VKR2 liquid distributor by Sulzer, is also required. Finally, it is also recommended to add a SKP collector welded on ring or similar collector to re-distribute the liquid throughout the packing and therefore increase its efficiency.

The proposed way forward based on the outcomes of this study would be the testing of the column stripping the 500L scale nano-suspension solution and determination of volume impact on the mass transfer rate. Data suggests that at recirculating setup, the volume does have an impact on overall process time; however additional experiments are necessary to quantify this impact.

If a new column is to be used, additional pilot plant trials will be necessary, namely on efficiency improvement regarding height and impact of Rashig rings - if chosen as the new packing. The hydrodynamic data will also change depending on the new design, therefore, and studies need to be performed, particularly well defining the flooding point, since conceivably it would be easier to reach with smaller diameters.

## References


[1] M. A. Jordan and L. Wilson, "Microtubules as a target for anticancer drugs," *Nat. Rev. Cancer*, vol. 4, no. 4, pp. 253–265, Apr. 2004.

[2] L. Peltonen and J. Hirvonen, "Drug nanocrystals – Versatile option for formulation of poorly soluble materials," *Int. J. Pharm.*, vol. 537, no. 1–2, pp. 73–83, Feb. 2018.

[3] K. Göke *et al.*, "Novel strategies for the formulation and processing of poorly water-soluble drugs," *Eur. J. Pharm. Biopharm.*, vol. 126, no. 4, pp. 40–56, May 2018.

[4] A. Otte, B.-K. Soh, G. Yoon, and K. Park, "Liquid crystalline drug delivery vehicles for oral and IV/subcutaneous administration of poorly soluble (and soluble) drugs," *Int. J. Pharm.*, vol. 539, no. 1–2, pp. 175–183, Mar. 2018.

[5] D. Li, C. F. van Nostrum, E. Mastrobattista, T. Vermonden, and W. E. Hennink, "Nanogels for intracellular delivery of biotherapeutics," *J. Control. Release*, vol. 259, pp. 16–28, Aug. 2017.

[6] V. R. Patel and Y. K. Agrawal, "Nanosuspension: An approach to enhance solubility of drugs.," *J. Adv. Pharm. Technol. Res.*, vol. 2, no. 2, pp. 81–7, Apr. 2011.

[7] L. Wu, J. Zhang, and W. Watanabe, "Physical and chemical stability of drug nanoparticles," *Adv. Drug Deliv. Rev.*, vol. 63, no. 6, pp. 456–469, May 2011.

[8] D. Green and R. Perry, *Perry's chemical engineers' handbook. Section 14, Equipment for distillation, gas absorption, phase dispersion, and phase separation*. McGraw-Hill, 2008.

[9] J.-C. Huang and C. Shang, "Air Stripping," in *Advanced Physicochemical Treatment Processes*, Totowa, NJ: Humana Press, 2006, pp. 47–79.

[10] L. Rozov, L. Kudzma, H.-C. Lee, and R. Bell, "Methods for cleaning distilling columns," US9102604B1, 11-Aug-2015.

[11] R. B. Bird, W. E. Stewart, and E. N. Lightfoot, *Transport phenomena*, 2nd ed. J. Wiley, 2007.

[12] W. Henry, "Experiments on the Quantity of Gases Absorbed by Water, at Different Temperatures, and under Different Pressures," *Philos. Trans. R. Soc. London*, vol. 93, p. 249, 1803.

[13] W. Henry, “Experiments on the Quantity of Gases Absorbed by Water, at Different Temperatures, and under Different Pressures,” *Philosophical Transactions of the Royal Society of London*, vol. 93. Royal Society, p. 29-42+274-.

[14] G. Schulze and J. M. Prausnitz, “Solubilities of gases in water at high temperatures,” *Ind. Eng. Chem. Fundam.*, vol. 20, no. 2, pp. 175–177, May 1981.

[15] M. C. Annesini, L. Marrelli, V. Piemonte, and L. Turchetti, “Mass Transfer Coefficient,” in *Artificial Organ Engineering*, London: Springer London, 2017, pp. 23–31.

[16] E. Nagy, “Three-phase mass transfer: One-dimensional heterogeneous model,” *Chem. Eng. Sci.*, vol. 50, no. 5, pp. 827–836, Mar. 1995.

[17] N. Paul, P. Schrader, S. Eders, and M. Kraume, “Effects of phase behaviour on mass transfer in micellar liquid/liquid systems,” *Chem. Eng. Sci.*, vol. 115, pp. 148–156, Aug. 2014.

[18] C. L. Yaws, P. K. Narasimhan, H. H. Lou, and R. W. Pike, “Solubility & Henry’s Law constants for chlorinated compounds in water: the new correlation and data presented here are appropriate even for very low concentrations,” *Chem. Eng.*, vol. 112, no. 2, pp. 50–56, Feb. 2005.

[19] T. R. Jerry, W. K. Lawrence, H. Yung-Tse, and K. Hung Li, “Potable Water Aeration,” in *Advanced Physicochemical Treatment Processes*, Totowa, NJ: Humana Press, 2006, pp. 1–45.

[20] M. Zhao and X. Yong, “Modeling Evaporation and Particle Assembly in Colloidal Droplets,” *Langmuir*, vol. 33, no. 23, pp. 5734–5744, Jun. 2017.

[21] M. J. Rosen and J. T. Kunjappu, “Reduction of Surface and Interfacial Tension by Surfactants,” in *Surfactants and Interfacial Phenomena*, Hoboken, NJ, USA: John Wiley & Sons, Inc., 2012, pp. 235–271.

[22] M. J. Rosen and J. T. Kunjappu, *Surfactants and interfacial phenomena*. Wiley, 2012.

[23] M. A. Anderson, “Influence of surfactants on vapor-liquid partitioning,” *Environ. Sci. Technol.*, vol. 26, no. 11, pp. 2186–2191, Nov. 1992.

[24] S. Kungsanant, K. Boonyaracht, T. Rirksomboon, and S. Osuwan, “Toluene removal from nonionic surfactant coacervate phase solutions by vacuum stripping,” *Sep. Purif. Technol.*, vol. 63, no. 2, pp. 370–378, Oct. 2008.

[25] P. V. Roberts and J. A. Levy, “Energy Requirements for Air Stripping Trihalomethanes,” *J. Am. Water Works Assoc.*, vol. 77, no. 4, pp. 138–146, Apr. 1985.

[26] R. P. Borwankar and D. T. Wasan, “The effect of surfactants on interphase solute transport. A theory of interfacial resistance,” *Ind. Eng. Chem. Fundam.*, vol. 25, no. 4, pp. 662–668, Nov. 1986.

[27] L. Hitchens, L. Vane, and F. Alvarez, “VOC removal from water and surfactant solutions by pervaporation: a pilot study,” *Sep. Purif. Technol.*, vol. 24, no. 1–2, pp. 67–84, Jun. 2001.

[28] X. Wei and H. Liu, “Relationship between foaming properties and solution properties of protein/nonionic surfactant mixtures,” *J. Surfactants Deterg.*, vol. 3, no. 4, pp. 491–495, Oct. 2000.

[29] H. Cheng, Y. Hu, J. Luo, and D. A. Sabatini, “Multipass membrane air-stripping (MAS) for removing volatile organic compounds (VOCs) from surfactant micellar solutions,” *J. Hazard. Mater.*, vol. 170, no. 2–3, pp. 1070–1078, Oct. 2009.

[30] Jaeger Products inc, “Air Stripping of VOCs from Water.” [Online]. Available: http://www.jaeger.com. [Accessed: 19-Nov-2017].

[31] B. M. Jaćimović and S. B. Genić, “Tray Efficiency versus Stripping Factor,” *Ind. Eng. Chem. Res.*, vol. 50, no. 12, pp. 7445–7451, Jun. 2011.

[32] M. S. Peters and K. D. Timmerhaus, *Plant design and economics for chemical engineers*. McGraw-Hill, 1991.

[33] P. C. Wankat, *Separation process engineering : includes mass transfer analysis*. Prentice Hall, 2012.

[34] U. N. Choori, J. F. Scamehorn, J. H. O’Haver, and J. H. Harwell, “Removal of Volatile Organic Compounds from Surfactant Solutions by Flash Vacuum Stripping in a Packed Column,” *Ground Water Monit. Remediat.*, vol. 18, no. 4, pp. 157–165, Nov. 1998.

[35] K. Kumar and A. Rao, “Liquid Holdup in Concurrent Gas Liquid Upflow Through Packed Column with Random and Corrugated Structured Packing,” *World Congr. Eng. Comput. Sci.*, vol. 2, p. 6, 2012.

[36] S. Kumar, X. Xu, R. Gokhale, and D. J. Burgess, “Formulation parameters of crystalline nanosuspensions on spray drying processing: A DoE approach,” *Int. J. Pharm.*, vol. 464, pp. 34–45, 2014.

[37] Z. Pereira and J. G. Requeijo, *QUALIDADE: Planeamento e Controlo Estatístico de Processos*, 2nd ed. Lisboa: Fundação da FCT-UNL, 2012.

[38] Sulzer, "Separation Technology, Structured Packing - Mellapak™ and MellapakPlus™ | Sulzer." [Online]. Available: https://www.sulzer.com/en/shared/products/2017/03/28/13/25/mellapak-and-mellapakplus.

[39] T. Fukutake and V. Rajakumar, "Liquid hold up and abnormal flow phenomena in packed beds under conditions simulating the flow in the dropping zone of a blast furnace," *Trans. Iron Steel Inst. Japan*, vol. 22, pp. 355–364, 1982.

[40] RVT Process Equipment GmbH, "Column internals - Our complete program for optimal performance Column internals Know-how and experience." [Online]. Available: http://www.rvtpe.com/wp-content/uploads/prospekte/RVT_Column_Internals_120601.pdf. [Accessed: 20-Nov-2017].

[41] F. Ushikubo, T. Furukawa, R. Nakagawa, and M. Enari, "Evidence of the existence and the stability of nano-bubbles in water," *Colloids Surfaces A Physicochem. Eng. Asp.*, vol. 361, no. 1–3, pp. 31–37, May 2010.

[42] M. I. Limited, "Zetasizer Nano Series." [Online]. Available: https://www.malvernpanalytical.com/en/products/technology/light-scattering/dynamic-light-scattering.

[43] V. B. Patravale, A. A. Date, and R. M. Kulkarni, "Nanosuspensions: a promising drug delivery strategy," *J. Pharm. Pharmacol.*, vol. 56, no. 7, pp. 827–840, Jul. 2004.

[44] D. M. Hawkins, "A New Test for Multivariate Normality and Homoscedasticity," *Technometrics*, vol. 23, no. 1, pp. 105–110, Feb. 1981.

[45] R. P. Borwankar and D. T. Wasan, "The effect of surfactants on interphase solute transport. A theory of interfacial resistance," *Ind. Eng. Chem. Fundam.*, vol. 25, no. 4, pp. 662–668, Nov. 1986.

[46] E. Azevedo and A. M. Alves, *Engenharia de Processos de Separação*, 3rd ed. Lisbon: IST Press, 2009.

[47] J. S. Eckert, "Design Techniques for Sizing Packed Towers," vol. 79, no. 4, p. 68, 1961.

[48] Celenza and Gaetano, *Industrial waste treatment process engineering. Volume 3:*

*Specialized treatment systems*. CRC Press, 2000.

[49] J. Gosset, C. Cameron, B. Eckstorm, C. Goodman, and A. Lincoff, “Mass Transfer coefficients and Henry’s Constants for packed-tower air stripping of volatile Henry’s constants for packed-tower air stripping of volatile organics: Measurments and correlation,” Cornell University Ithaca, New York, 1985.

# Appendix

## A. Upstream Manufacturing Process

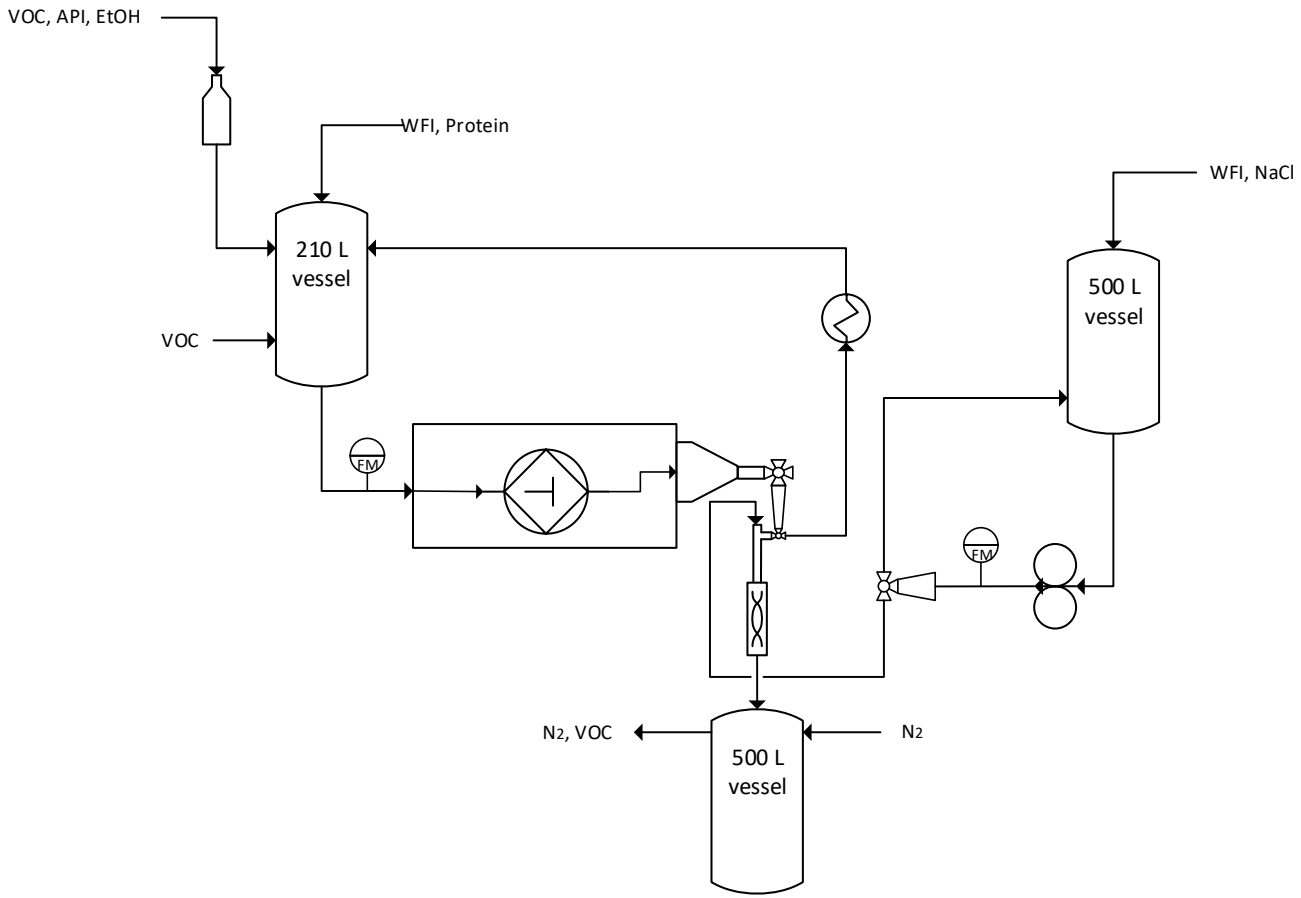


Figure - A.1 - Upstream manufacturing process

## B. Downstream Manufacturing Process

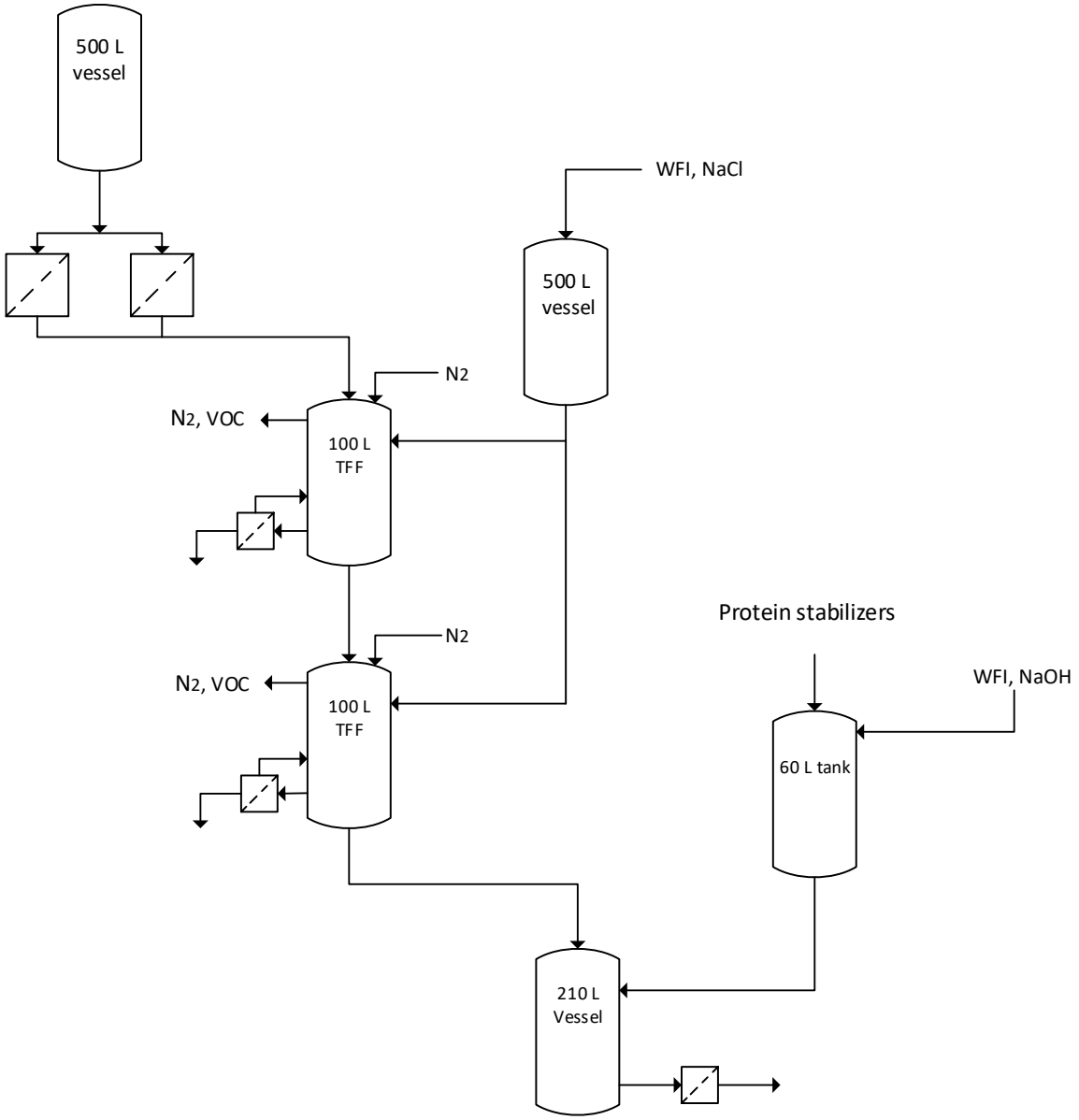


Figure - B.1 - Upstream manufacturing process

## C. Sulzer Column Design Software – Sulcol

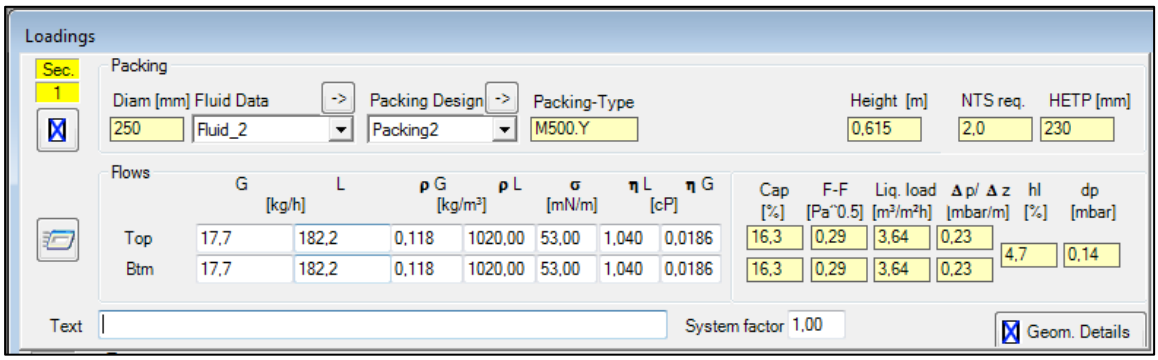


Figure - C.0.1 - Sulzer column design software resutls for air stripping column

## D. Details on Column Internals

Liquid loads are necessary to choose which liquid distributor is appropriate to the application. Liquid loads are given by the liquid flow rate by the cross section of the column:

Table - D.1 - Calculated liquid loads to tested range

| Liquid Loads* ($m^3/m^2.h$) | Liquid Flow Rate ($m^3/h$) | Liquid Flow Rate (L/min) |
|---|---|---|
| 0,1 | 0.006 | 0.095 |
| 0,5 | 0.029 | 0.477 |
| 1 | 0.057 | 0.954 |
| 2 | 0.115 | 1.909 |
| 3 | 0.172 | 2.863 |
| 4 | 0.229 | 3.817 |
| 5 | 0.286 | 4.771 |
| 7 | 0.401 | 6.680 |
| 10 | 0.573 | 9.543 |
| 50 | 2.863 | 47.713 |
| *Diameter of column= 270 mm | | |

At volumetric flow used in the air stripping column, the liquid loads will range from about 0.5 to 7 m3/m2.h

For the gas load, the F-factor must be calculated, which is the gas superficial velocity (Vg) by the gas density of the gas powered by ½. Taking into account the tubing diameter and the volumetric flow, the f-factors for the range used is the following:

Table - D.2 - Calculated F-Factor for tested range

| GF (L/min) | GF ($m^3/s$) | Vg (m/s) | F-factor $(m/s)(kg/m^3)0.5$ |
|---|---|---|---|
| 80 | 0.0013 | 0.1698 | 0.1929 |
| 160 | 0.0027 | 0.3396 | 0.3857 |
| 180 | 0.0030 | 0.3820 | 0.4339 |
| 250 | 0.0042 | 0.531 | 0.6027 |
| 360 | 0.0060 | 0.7641 | 0.8678 |

Olsson discussed the key factor for successful distributor design and operation (Chem. Eng. Progr., p. 57, October 1999). He stated that a minimum of 40 irrigation points per square meter (dip-point density) are recommended. The head-flow relationship is given by the orifice equation [11]:

$$Q = 3.96 \times 10^{-4} K_D n_D d_h^2 h^{0.5}$$

It is also advised that the liquid distributor operates as by gravity in opposition to by pressure drop, due to the tendency to foam of solution. Also due to the column's smaller diameter sulzer's options are reduced. The VKR2 liquid distributor would be an option

For the reasons above, the Sulzer's VKR2 is a good option since it does not spray, it is a circular distributer that can occupy the cross-sectional area of the packing column and it fits column specifications.

Table - D.3 - Specifications of VKR2 distributor

| | |
|---|---|
| Diameter | >0.2 m (column is 0.214 m) |
| Liquid load | 1 to 20 m3/m3.h (0.5 to 7 m3/m3.h used in the column) |
| Gas load | F-factor up to 5 Pa1/2 (gas load from 0,1 to 1 Pa1/2 in the column) |
| Construction details | Inlet calmer included, if required |
| Special note | The low-liquid load distributor |

The distributor must allow a tri-clamp connection to be used on the current column (or a self-supporting unit if used with random packing). A liquid redistributor, such as a SKP collector, would also be advised in between stages of packing to improve liquid distribution in the column.

Regarding the packing material to be used, the structured packing is more efficient than the random packing; however, the random packing is more adequate for foaming solutions. With the current liquid distributer and Mellapak packing, the limit liquid load due to foam is 5 $m^3/m^2.h$. The use of random packing, as well as VKR2 liquid distributor can be used to maximize the liquid loads.

The random packing diameter can also be chosen accordingly, knowing the smaller the diameter the more efficient the process however the easier is to foam. The reason between the diameter of the column and the packing material should be 15/1, therefore the packing diameter with current column should be around 14 mm to maximize efficiency; common packing sizes vary between 6 and 89 mm. If random packing is chosen plastic rings would be a wise choice to avoid any leachable to be introduced in the drug product due to mechanic friction.

## E. PW & Protein Test Run Experiment 1 – GR&D Pilot Plant

Table - E.1 - Material, equipment and utilities for experiment 1

| | |
|---|---|
| Air Stripping Column | Viton tubing |
| 10L Duran Bottle | Purified Water (PW) |
| Peristaltic pump | Compressed air |
| Air Flow Meter | 7 bottles of carrier protein solution |

Table - E.2 - Preparation for experiment 1

| No. | Action | Settings |
|---|---|---|
| 1. | Calibrate peristaltic pump before start use. | |
| 2. | Make sure protein bottle solution is available | |
| 3. | Build the set-up according to Figure 5.5 | |
| 4. | Perform water-run to wet the packing (for first run). | |
| 5. | Connect the flow meter to the system. Connect the compressed air to the air inlet on bottom of the column. | |
| 6. | Connect column inlet to the TEVA general DRAIN. | |

Table - E.3 - Start of operation for experiment 1

| No. | Action | Settings |
|---|---|---|
| 7. | Tare the empty 10L Duran Bottle, record the weight.<br>***Weight (kg):*** | |
| 8. | Add PW of 15–25 °C to the 10L bottle. | |
| 9. | Add protein directly from the vials (using rod), slowly via the wall of the vessel, to the Duran 10 L Bottle. | |
| 10. | Add slowly, via the wall of the vessel, PW of 20–25 °C to the required weight. Record the weight.<br>***Weight (kg):*** | |
| 11. | Open air flow valve, set flow rate in flow meter and record it.<br>***Gas flow rate (L/min):*** | |
| 12. | Activate pump, set and record liquid flow rate<br>***Liquid flow rate (L/min):*** | |
| 13. | Continue process until solution is in continuous circulation. Stop pump. Observe foam record process and take picture. | |

| No. | Action | Settings |
|---|---|---|
| 14. | Cleaning: Close air stripping valve and fill the column with 15-20˚C PW. Leave for 20 minutes and empty the column to TEVA Drain. Note: If column still has foam repeat the procedure until no more visible foam is present. | |
| 15. | Blow compressed air through the column for about 2 hours to dry the packing material | |

**Following trials:**

Repeat the steps 8. to 14. with different air flow and liquid flow rate values, and determine if feasible to production implementation. Assess best operational flows regarding foaming formation. It too much foam is formed, lower down the inlet distributer to reduce head space, using sight glass or similar equipment.

## F. VOC & PW Test Run Experiments 2&3 – GR&D Pilot Plant

Table - F.1 -Equipment, material, utilities for experiments 2 and 3

| | | | |
|---|---|---|---|
| Air Stripping Column | Stainless steel funnel | PW | Sample collecting vials |
| 300L Vessel | Stainless steel recipient | Compressed air | 3way valve |
| 60 L Vessel (for buffering) | Duran Pure Bottle | Organic solvent (VOC) | Viton/BP Tubing |
| Peristaltic pump | Duran PTFE Cap | Face mask | 100 ml syringe |
| Botlek container | Raman Probe | AX filter | Tee piece (x2) |

Table - F.2 - Preparation for experiments 2 and 3

| No. | Action | Settings |
|---|---|---|
| 1. | Calibrate pump before start use. | |
| 2. | Make sure VOC is available. | |
| 3. | Make sure all equipment listed above is available. | |
| 4. | Build the set up according to Figure 5.3 | |
| 5. | Connect with BP tubing, the outlet of 300L Vessel to column inlet, with pump. | |
| 6. | Connect the compressed air to the air inlet of column. Connect the flow meter to the system. | |
| 7. | Connect 3 way valve in liquid outlet for sample collection before draining it to solvent drain | |
| 8. | Set the buffering system before connecting air outlet to exhaust system. | |

Table - F.3 - Start of operation for experiments 2 and 3

| No. | Action | Settings |
|---|---|---|
| 9. | Rinse outside the isolator one 1L Duran Pure bottle, Duran PTFE Cap, and the stainless steel funnel with alcohol absolute. | |
| 10. | Place the VOC recipient, the rinsed 1L Duran Pure bottle, one Duran caps for bottle, the 1L Duran Pure bottle filled with alcohol, and the stainless steel funnel in the isolator.<br>Note: VOC compounding can alternatively be performed under suction point if protective mask is used. | |

| No. | Action | Settings |
|---|---|---|
| 11. | Tare the empty 10L Duran Bottle, record the weight.<br>***Weight (kg):*** | |
| 12. | Add VOC to the 1L Duran Pure bottle via the stainless steel funnel.<br>***Weight B (kg):*** | |
| 13. | Close the 1L Duran Pure bottle using a Duran Premium Cap. Transfer the closed 1L Duran Pure bottle to outside the isolator, label the bottle, and put it in identified protective transportation bucket. | |
| 14. | Tare the empty vessel 300L Noorden vessel in control room. | |
| 15. | Add PW of 15–25 °C to the 300L Noorden vessel. Make a weighing print and record the weight.<br>***Weight A (kg):*** | |
| 16. | Start the GMP mixer on 100 ± 5 rpm (req. 25–35 rpm)**.** | |
| 17. | Add VOC from the 1L Duran Pure bottle to the 300L Noorden vessel. Close the 300L vessel. Record total 300 L vessel weight<br>***Weight B (kg):*** | |
| 18. | Close the lid and all valves of the 300L Noorden vessel. Confirm the vessel is closed. | |
| 19. | Set the GMP mixer to 200 ± 15 rpm for at least 30 minutes. | |
| 20. | Take initial control sample from 300L vessel to assess initial VOC content in vessel. | |
| 21. | Open air flow valve, set flow rate in flowmeter according to DOE and record it.<br>***Gas flow rate (L/min):*** | |
| 22. | Activate pump, set flow rate according to DOE and record it.<br>***Liquid flow rate (L/min):*** | |
| 23. | Continue process until about 5kg have left the vessel. Close the 300 L vessel valve and stop pump. | |
| 24. | Turn slightly the 3 way valve to sample side. | |
| 25. | Collect two samples, one to sample bottle to be sent to GC analysis and on in stainless steel recipient to measure VOC content using Raman Probe. | |
| 26. | Repeat steps 21. to 25. until all DOE trials have been performed | |
| 27. | Take final control sample from 300L vessel to assess initial VOC content in vessel. | |
| 28. | Measure VOC content with GC (and Raman Probe) in final solution | |

| No. | Action | Settings |
|---|---|---|
| 29. | Cleaning: Close air stripping valve and fill the column with 15-20˚C PW. Leave for 20 minutes and empty the column to TEVA Drain.<br>Blow compressed air to dry column as much as possible. | |
| 30. | Cleaning: Clean vessel and mobile parts rinsing two times with PW. | |

## G. Recirculating bulk experiments – Experiments 5 to 8 (component weight for 50L solution – scale-up for 125L used in experiments 5 to 7)

**Equipment/material/utilities:**

Table - G.1 - Equipment, materials and utilities for experiments 5 to 8

| | | | |
|---|---|---|---|
| Air Stripping Column | Stainless steel funnel | Org. Solvent | Sample collecting vials |
| 60L Vessel | Stainless steel recipient | Carrier Protein bottles | Butterfly valve (x2) |
| 10 L Vessel (for buffering) | Duran Pure Bottle (x2) | NaCl | Viton Tubing |
| Peristaltic pump (x2) | Duran PTFE Cap (x2) | EtOH | BP tubing |
| Air flowmeter | Weighing scale | Compressed air | Tee piece (x2) |
| Spray ball | Face mask + AX filter | Purified water | Waterbath |

Table - G.2 - Preparations for experiments 5 to 8

| No. | Action | Settings |
|---|---|---|
| 1. | Calibrate both pumps before start use. | |
| 2. | Make sure all excipients are available. | |
| 3. | Make sure all equipment listed above is available. | |
| 4. | Build the set up according to Figure 5.7 Add waterbath (60L vessel does not possess glycol system entrance) | |
| 5. | Add purified water to the 60L vessel and record the weight<br>**Weight (kg):** | |
| 6. | Perform initial waterrun and check system for leakages | |
| 7. | Set the pump re-feeding to the vessel to a liquid flow rate which maintains the liquid level at the column constant and below the air distributor. Record the liquid-flow in pump number two<br>***Liquid flow rate (L/min):*** | |

Table - G.3 - Compounding of VOC and Protein solutions for experiments 5 to 8

| No. | Action | Settings |
|---|---|---|
| 9. | Rinse outside the isolator two Duran Pure bottle, Duran PTFE Cap, and the stainless steel funnel with alcohol absolute. | |
| 10. | Place the VOC, the rinsed Duran Pure bottle, one Duran caps for bottle, the Duran Pure bottle filled with alcohol, and the stainless steel funnel in the isolator. Note: VOC compounding can alternatively be performed under suction point if protective mask is used. | |
| 11. | Tare the empty Duran Bottle, record the weight.<br>***Weight (g):*** | |

| No. | Action | Settings |
|---|---|---|
| 12. | Place the Duran glass bottle on the scale, place the stainless steel funnel in the bottle, and tare the balance. Add *VOC* to the Duran Pure bottle via the stainless steel funnel.<br>***Weight (g):*** | |
| 12. | Close the Duran Pure bottle using a Duran Premium Cap. Transfer the closed Duran Pure bottle to outside the isolator, label the bottle, and put it in identified protective transportation bucket. | |
| 13. | Tare the second empty Duran Bottle, record the weight.<br>***Weight (g):*** | |
| 14. | Add protein solution directly from the protein solution vials (using rod), slowly via the wall of the vessel, to the bottle and record the weight.<br>***Weight (kg):*** | |
| 15. | Close the Duran Pure bottle using a Duran Premium Cap. and label the bottle. | |
| 16. | In stainless steel container weigh of sodium chloride with help for a spatula. Record the weight.<br>***Weight (g):*** | |
| 17. | In stainless steel container weigh of alcohol absolute. Record the weight.<br>***Weight (g):*** | |

Table - G.4 - Start of operation for experiments 5 to 8

| No. | Action | Settings |
|---|---|---|
| 18. | Add contents of both Duran bottles and stainless steel to the 60 L vessel and record total weight<br>***Weight (kg):*** | |
| 19 | Close the lid and all valves of the 60L Noorden vessel. Confirm the vessel is closed. | |
| 20. | Stir the solution sat 150 rpm for at least 30 min<br>***Rpm:*** | |
| 21. | In the meantime connect the waterbath and set to the experiment plan required temperature | |
| 22. | Stop stirrer, collect control sample for VOC concentration with aid of a 100 ml syringe. | |
| 23. | Turn back stirrer to 80 rpm.<br>***Rpm:*** | |
| 24. | Open air flow valve, set flow rate in flowmeter to 250L/min and record it.<br>***Gas flow rate (L/min):*** | |

| No. | Action | Settings |
|---|---|---|
| 25. | Activate pump feeding the column, set flow rate according to the experiment plan and record it.<br>***Liquid flow rate (L/min):*** | |
| 26. | Activate the pump re-feeding the column at the flow rate recorded in step 7 and confirm the required liquid level in column. | |
| 27. | In necessary perform adjustments to the second pump do maintain liquid level below air flow distributor. If so record new liquid flow rate.<br>***Liquid flow rate (L/min):*** | |
| 28. | Check regularly for any foam formation both in air stripping column and 60L vessel. | |
| 29. | Every 30min of stripping, interrupt the process and collect sample with aid of 100 ml syringe.<br>Note: after each sample collection restart process at same conditions | |
| 30. | Send samples to GC analysis for measurement | |
| 31. | Interrupt process, turn off both pumps and gas flow rate and water bath equipment | |
| 32. | Drain the 60L contents to solvent drain. | |
| 33. | Cleaning: Fill 60L vessel with PW and circulate bulk between vessel and column for 30 min. | |
| 34. | Drain vessel to Teva general drain and repeat step 31. | |
| 35. | Blow compressed air for about 2h in order to dry packing material before storage of the equipment | |
| 36. | In the meantime disassemble set-up and collect mobile parts to be cleaned. | |
| 35. | Clean vessel and mobile parts rinsing two times with PW. | |

## H. Full formulation nano-suspension

Table - H.1 - Preparation of experiment 10

| No. | Action | Settings |
|---|---|---|
| 1. | Build the HPH setup and place the collection vessel on the floor balance. | |
| 2. | Flush the Panda with at least 1L purified water and 1L alcohol 96% or absolute. Drain the system. | |
| 3. | Add purified water. Recirculate the water for a minute at 1000 bar on the 1st stage and 100 bar on the 2nd stage, and switch the 3-way valve to collect the water.<br>- Determine the water flow rate.<br>- Determine the void volume in the HPH-loop (HPH, 3-way valve, heat exchanger) by calculating the difference between the collected volume and the initial volume. | Water flow rate:<br><br>Void HPH volume: |
| 4. | Calibrate the peristaltic pump with purified water and set the rate to 1202 mL/min | |
| 5. | Prepare the 1% NaCl solution for the quenching in a 60L bucket | Weight NaCl:<br><br>Final weight: |
| 6. | Test the isolator by closing it and performing a glove test. | |
| 7. | Bring the following items into the isolator:<br>o Large balance<br>o Waste beaker/bucket<br>o Cleaning wipes<br>o API, in a bottle<br>o VOC, in a bottle<br>o Alcohol abs., in a bottle<br>o 1L glass Duran bottle with cap<br>o Powder funnel<br>o Spoon/spatula for the API<br>o Alcohol spray bottle<br>o plastic bag | |
| 8. | Activate the cooling bath for the heat exchanger in the HPH setup at 2°C<br>***Setpoint:*** | |

Table - H.2 - Preparation of Protein solution saturated with VOC (partly in isolator) for experiment 10

| No. | Action | Settings |
|---|---|---|
| 9. | Place the 10L jacketed pressure vessel on the balance. Add:<br>- Protein solution<br>- Purified water | |
| 10. | Stir the protein solution for 5 min with an overhead stirrer and place it back in the isolator.<br>**Rpm:** | |
| 11. | **Use a face mask with AX filter.**<br>Weigh VOC in the 100 mL glass beaker and pour it immediately into the Protein solution and cover the opening with parrafilm. | |

| No. | Action | Settings |
|---|---|---|
| 12. | Stir the protein solution for 30 min with an overhead stirrer, covering the opening with parafilm and using a local suction point.<br>***Rpm:*** | |

Table - H.3 - Preparation of the API-VOC solution (isolator) for experiment 10

| No. | Action | Settings |
|---|---|---|
| 13. | **Close the isolator.**<br>Place the 1L Duran bottle on the balance and place the funnel on the bottle.<br>Add:<br>- API (slowly to minimize spreading of powder)<br>- VOC<br>- alcohol abs<br>Remove the funnel and close the bottle. | |
| 14. | Swirl the bottle a few times during a period of approx. 30 min until the API is dissolved. | |
| 15. | In the meantime, clean and empty the isolator | |

Table - H.4 - Mixing (isolator)

| No. | Action | Settings |
|---|---|---|
| 16. | Bring the following items into the isolator:<br>○ Large balance<br>○ Ultra Turrax with 18mm shaft on a lab stand<br>○ Waste beaker/bucket<br>○ Cleaning wipes<br>○ Alcohol spray bottle<br>○ Stainless steel funnel<br>○ 10L pressure vessel with protein solution<br>○ 1L duran bottle with API solution<br>○ Overhead stirrer<br>○ Plastic bag | |
| 17. | Pour the API-VOC solution into the protein solution and immerse the turrax shaft into the mixture. | |
| 18. | Disperse the mixture for 5 min at approx. 5.000-10.000 rpm (mixture should become completely turbid within 30 s).<br>***Rpm:*** | |
| 19. | Take the disperser out of the mixture. Flush the disperser shaft with water and ethanol. | |
| 20. | Place a overhead stirrer in the solution stir the solution with 150 rpm.<br>***Rpm:*** | |
| 21. | Cover the opening of the 10L vessel with parafilm | |
| 22. | Connect a waterbath to the jacket of the vessel with a setpoint of 2°C<br>***Setpoint:*** | |

Table - H.5 - Preparation of the nano-suspension for experiment 10

| No. | Action | Settings |
|---|---|---|
| 23. | Wear a Tyvek suit and face mask with AX filter. | |
| 24. | Connect the diptupe of the 10L vessel to the inlet of the Panda HPH, Connect the outlet of the heatexchager to the inlet of the 10L vessel. | |
| 25. | Start the overhead stirrer in the 10L vessel<br>***Rpm:*** | |
| 26. | Start the homogenizer in recirculation mode (do not apply pressure yet). Wait until the inlet temperature of the heat exchanger reaches 2-8°C. | Start temp.:<br>End temp: |
| 27. | Activate the 2nd stage pressure to 90-110 bar. Activate the 1st stage pressure to 900-1100 bar.<br>Keep these conditions for at least 125 min and fill in the table below.<br>Keep the water bath of the heat exchanger at 2°C by regularly adding ice. | |

The vessel is then to be taken to the pilot plant and air stripping process performed according to recirculation experiment described in annex H. Double the number of samples are required in order to send for particle size and polydispersity index measurement.

## I. Experimental data table plus JMP tables:

Table - I.1 - Experimental data and JMP tables for experiment 3

| **Trial number** | **Temperature** (°C) | **LF** (L/min) | **GF** (L/min) | **Normalized VOC** | **(A/W)** | **% Removal** | **Overall Process Time** (h) | **Residual VOC** | **Predicted VOC** |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 30 | 6 | 250 | 0.084 | 41.7 | 82.1 | 1.75 | 0.018 | 0.066 |
| 2 | 30 | 2 | 320 | 0.037 | 160 | 92 | 3.57 | 0.011 | 0.026 |
| 3 | 30 | 6 | 250 | 0.054 | 41.7 | 88.5 | 1.39 | -0.01 | 0.066 |
| 4 | 30 | 2 | 180 | 0.048 | 90 | 89.8 | 3.96 | 0.008 | 0.039 |
| 5 | 18 | 2 | 250 | 0.055 | 125 | 88.3 | 4.22 | -3e-3 | 0.058 |
| 6 | 18 | 6 | 320 | 0.075 | 53.3 | 84.1 | 1.64 | -3e-3 | 0.078 |
| 7 | 18 | 6 | 180 | 0.09 | 30 | 80.8 | 1.83 | -5e-4 | 0.091 |
| 8 | 18 | 2 | 250 | 0.062 | 125 | 86.7 | 4.49 | 0.005 | 0.058 |
| 9 | 30 | 3 | 226,2 | 0.039 | 75.4 | 91.7 | 2.42 | -0.01 | 0.044 |
| 10 | 30 | 1 | 320 | 0.02 | 320 | 95.8 | 5.69 | 0.003 | 0.016 |
| 11 | 30 | 1 | 180 | 0.026 | 180 | 94.5 | 6.25 | -3e-3 | 0.029 |
| 12 | 30 | 3 | 250 | 0.04 | 83.3 | 91.4 | 2.46 | -2e-3 | 0.042 |
| 13 | 18 | 3 | 180 | 0.079 | 60 | 83 | 1.47 | 0.008 | 0.072 |
| 14 | 18 | 1 | 320 | 0.043 | 320 | 90.8 | 7.57 | -7e-6 | 0.043 |
| 15 | 18 | 1 | 250 | 0.045 | 250 | 90.3 | 7.74 | -4e-3 | 0.049 |
| 16 | 18 | 3 | 250 | 0.073 | 83.3 | 84.4 | 3.25 | 0.008 | 0.065 |
| 17 | 18 | 1 | 250 | 0.046 | 250 | 90.2 | 7.77 | -4e-3 | 0.049 |
| 18 | 18 | 3 | 320 | 0.058 | 107 | 87.5 | 2.89 | -6e-4 | 0.059 |
| 19 | 30 | 1 | 250 | 0.24 | 250 | 94.9 | 6.08 | 0.001 | 0.023 |
| 20 | 30 | 3 | 320 | 0.031 | 107 | 93.4 | 2.21 | -0.01 | 0.036 |
| 21 | 18 | 1 | 180 | 0.057 | 180 | 87.7 | 8.63 | 0.002 | 0.056 |
| 22 | 18 | 3 | 269,6 | 0.057 | 89.9 | 87,8 | 2.86 | -0.01 | 0.064 |
| 23 | 30 | 1 | 274,5 | 0.017 | 275 | 96.3 | 5.48 | -3e-3 | 0.021 |
| 24 | 30 | 3 | 180 | 0.039 | 60 | 91.7 | 2.42 | -0.01 | 0.049 |

Table - I.2 - Experimental data and JMP tables for experiment 5

| Trial number | Temperature (°C) | LF (L/min) | GF (L/min) | Normalized VOC | % Removal | (A/W) | Overall Process Time (h) | Residual VOC | Predicted VOC |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 19 | 4 | 320 | 0.494 | 25.6 | 80 | 15.22 | 0.147 | 0.236 |
| 2 | 18.4 | 4 | 260 | 0.39 | 41.3 | 65 | 8.49 | -0.05 | 0.244 |
| 3 | 12 | 2 | 180 | 0.203 | 69.5 | 90 | 7.62 | 0.013 | 0.251 |
| 4 | 11 | 2 | 260 | 0.272 | 59.1 | 130 | 10.11 | -0.06 | 0.259 |
| 5 | 10 | 6 | 260 | 0.35 | 47.4 | 43,3 | 4.69 | -0.03 | 0.411 |
| 6 | 10 | 4 | 320 | 0.306 | 54 | 80 | 5.82 | 0.023 | 0.339 |
| 7 | 10 | 4 | 180 | 0.362 | 45.5 | 45 | 7.44 | -0.01 | 0.339 |
| 8 | 10 | 6 | 180 | 0.406 | 39 | 30 | 6.11 | 0.042 | 0.411 |
| 9 | 10 | 2 | 180 | 0.309 | 53.5 | 90 | 11.79 | 0.011 | 0.267 |
| 10 | 10 | 4 | 260 | 0.35 | 47.4 | 65 | 7.03 | -0.02 | 0.339 |
| 11 | 10 | 2 | 320 | 0.249 | 62.6 | 160 | 9.2 | 0.036 | 0.267 |
| 12 | 10 | 6 | 320 | 0.448 | 32.7 | 53,3 | 7.62 | 0.003 | 0.411 |
| 13 | 23 | 4 | 260 | 0.195 | 55.4 | 65 | 3.68 | -0.02 | 0.192 |
| 14 | 24 | 6 | 180 | 0.183 | 57.1 | 30 | 2.34 | -0.02 | 0.207 |
| 15 | 25 | 2 | 320 | 0.129 | 65.3 | 160 | 5.5 | -0.01 | 0.146 |
| 16 | 25 | 6 | 260 | 0.186 | 56.7 | 43,3 | 2.36 | -0.01 | 0.193 |
| 17 | 25 | 4 | 180 | 0.157 | 61.1 | 45 | 3.12 | -0.04 | 0.169 |
| 18 | 25 | 2 | 260 | 0.106 | 68.7 | 130 | 4.92 | -0.02 | 0.146 |
| 19 | 25 | 3 | 260 | 0.141 | 63.4 | 86,7 | 3.89 | -0.06 | 0.157 |
| 20 | 25 | 2 | 260 | 0.084 | 72 | 130 | 4.37 | -0.02 | 0.146 |
| 21 | 25 | 3 | 320 | 0.139 | 63.7 | 107 | 3.86 | 0.07 | 0.157 |
| 22 | 30 | 2 | 180 | 0.175 | 58.3 | 90 | 6.78 | -0.03 | 0.105 |
| 23 | 30 | 1 | 260 | 0.08 | 72.6 | 260 | 2.14 | 0.003 | 0.112 |
| 24 | 30 | 1 | 260 | 0.115 | 67.3 | 260 | 2.58 | 0.03 | 0.112 |

| Trial number | Temperature (°C) | LF (L/min) | GF (L/min) | Normalized VOC | % Removal | (A/W) | Overall Process Time (h) | Residual VOC | Predicted VOC |
|---|---|---|---|---|---|---|---|---|---|
| 25 | 30 | 2 | 320 | 0.135 | 64.4 | 160 | 5.66 | 0.03 | 0.105 |
| 26 | 30 | 6 | 320 | 0.149 | 62.2 | 53.3 | 2.02 | 0.024 | 0.12 |
| 27 | 30 | 6 | 320 | 0.144 | 63.1 | 53.3 | 1.96 | -0.04 | 0.12 |
| 28 | 30 | 6 | 186 | 0.083 | 72.2 | 31 | 1.44 | 0.147 | 0.12 |

## J. Results of liquid flow rate experiments – Placebo solution (without API)

Table - J.1 - Normalized results of liquid flow rate experiments and efficiencies

| | 2L/min | | 4L/min | |
|---|---|---|---|---|
| Time (h) | Normalized VOC conc. | Efficiency | Normalized VOC conc. | Efficiency |
| 0 | 1.19 | - | 0.84 | - |
| 0,5 | 0.49 | 58.7% | 0.31 | 62.5% |
| 1 | 0.20 | 58.4% | 0.12 | 61.0% |
| 1,5 | 0.09 | 54.3% | 0.03 | 74.8% |
| 2 | 0.04 | 52.9% | 0.01 | 61.8% |
| 2,5 | 0.02 | 50.4% | 0.004 | 58.9% |
| 3 | 0.01 | 48.2% | 0.001 | 79.4% |
| 3,5 | 0.006 | 44.4% | 0.0005 | 48.6% |

## K. Prediction profilers of both DOE used to estimate overall process time at 20°C and 2L/min of liquid flow rate operating parameters

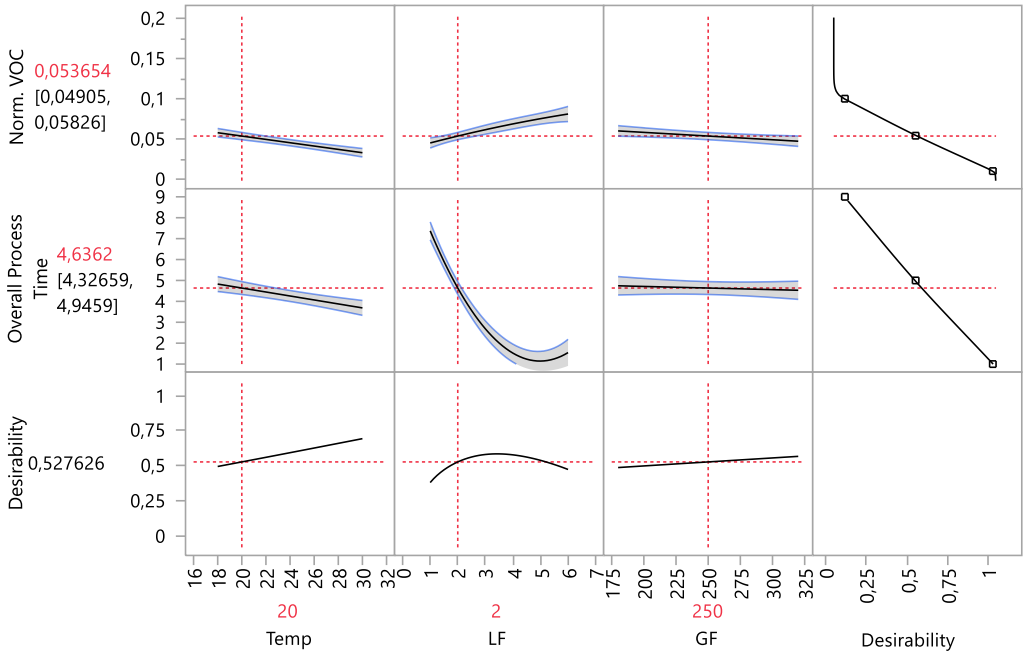


Figure - K.1 - Prediction profiler at 20°C and 2L/min for experience 3

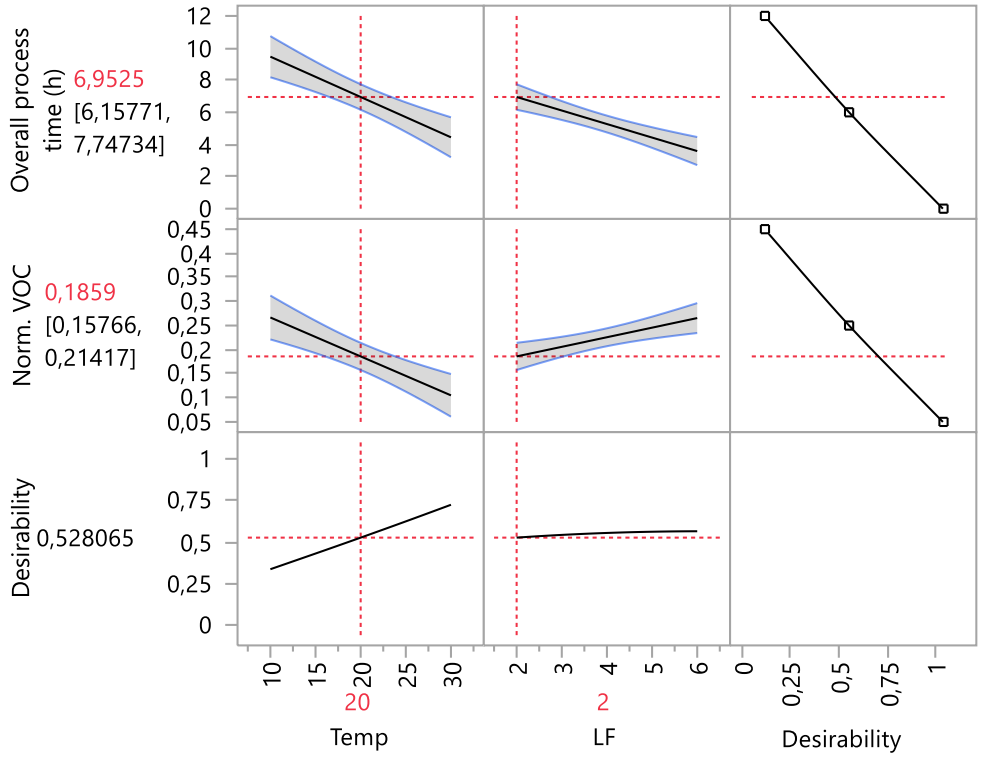


Figure - 0.2 - Prediction profiler at 20°C and 2L/min for experience 5

## L. DOE Analysis Description

The analysis results from the DOE report will be presented in the results and will include:

- Model fit evaluation – Leverage plot and analysis of variance
- Effect tests evaluation – Leverage plot, analysis of variance and sorted parameter estimates
- Leverage plot of significant effects
- Residual analysis
- Prediction profiler

**Model Fit Evaluation**

The plot of the actual by predicted values represent the leverage plot for the whole model, giving insight into how the fit carries the data. These plots have confidence curves (dashed red curves) indicating if the test is significant at the 5% level. It's important to refer the significance value is, by convention 0,05 (5%). If both curves cross the horizontal line, the effect is significant. Since the residuals are the difference between the observed response values and predicted values, the distance of a point to the line of fit is the actual residual for each observed result.

The model is evaluated by a fit report, which provides $r^2$ indicative of the model's error, i.e, proportion of variation in the response that can be attributed to the model instead of to a random error. The root mean square (RMSE) is the given standard deviation of the value to the model.

Analysis of variance of the model is also calculated by the sum of squares and mean square of each source of variation. Fisher distribution is used for this statistical test in order to list the p-value (Prob>F). P-values below 0,05 are indicative of existence of at least one significant value is present in the model. Significant values are identified in the following tables with an asterisk.

**Effect Tests Evaluation**

Screening of factors and analysis of variance is performed for all defined factors and cross factors. The software will screen all the factors and assess which ones possess the most statistically significant effect on the responses. The p-values represented in the last column of the ANOVA will give information on the significance and impact of all effects and interactions. The model generally needs to be run second time eliminating all interactions and quadratic terms which are assessed by the model as not significant.

The sorted parameters estimates table is useful for the screening experiments since it sorts the terms in decreasing order of significance. The vertical dashed line in the tRatio bar chart represents the critical value for the 0,05 significance value.

**Residual Analysis**

When performing ANOVA runs, variance of the residual terms must be constant and uncorrelated with past inputs. To test these assumptions, residual analysis should be performed, for homoscedasticity, i.e homogeneity of covariance and independence. These tests can be simply be performed by plotting the residuals with the predicted responses and residuals by order of run and interpreting the graphs. Note that other statistical and more objective tests for residual analysis exist such as Kolomogorov-Smirnov test or Hawkins test.

Deviation from non-random pattern of residuals can possibly be indicative of missing variable or missing interaction between the effects. This interpretation is based on the knowledge that the residuals should not contain any predictive information.

**Prediction Profiler**

The prediction profiler is useful to analyze the response surface and set the settings which produce the best response target. It is possible to define the desirability functions by setting the target to minimizing both responses to as low as possible, since for this particular experiment the goal is to minimize two different responses. This is an interactive chart which can be changed by moving the prediction traces (red dashed lines) for each $x$ variable.