Continuous Manufacturing of Liposomal Drug Products

A Thesis submitted to University College London

for the degree of Doctor of Philosophy (PhD) in Biochemical Engineering

By

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I, Robert David Worsham, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

To my wife and kids

Abstract

Over the last several years, continuous manufacturing of pharmaceuticals has evolved from bulk APIs and solid oral dosages into the more complex realm of biologics. The development of continuous downstream processing techniques has allowed biologics manufacturing to realize the benefits that come with continuous processing (e.g. improved economics, more consistent quality). If relevant processing techniques and principles are selected, the opportunity arises to develop continuous manufacturing designs for additional pharmaceutical products including liposomal drug product formulations. Liposome manufacturing has some inherent aspects that make it favorable for a continuous process. Other aspects such as formulation refinement, materials of construction, and aseptic processing need development, but present an achievable challenge. This thesis aims to explore the feasibility, challenges and economic benefits of continuous manufacturing of liposomal drug products.

Liposomal drug product manufacturing has evolved into a commercial scale batch process. A commercial liposomal drug product batch process for a formulation delivering amikacin through a nebulized inhalation suspension (Arikayce®) was assessed for specific considerations and requirements in order to convert the design to a continuous process. Continuous process options for specific unit operations (liposome generation, diafiltration) were evaluated with detailed characterisation of the impact of key process parameters on the performance. The most significant of these was the impact of retentate ethanol concentration on hollow fiber permeability and overall diafiltration efficiency. Retentate ethanol concentration directly impacted the morphology of the hollow fiber filters, decreasing their permeability and permeate flux. The findings determined that

dilution of the retentate offset the impact to hollow fiber permeability allowing for a continuous diafiltration design with minimal stages (5% retentate ethanol concentration requiring 7 ILDF stages) and buffer consumption that is competitive with the batch process. These experimental findings fed into an economic assessment of the continuous process as compared to the batch showing the continuous option to be advantageous in drivers such as cost of goods when demand surpasses a certain threshold (<5M annual doses/vials). A convertible process is proposed to leverage both batch and continuous economic and capacity advantages as a function of demand.

The work in the thesis demonstrates that using a continuous process for the manufacture of liposomal drug products is feasible, can be significantly optimized, and has benefits that allow the design to compete with or surpass a batch process design.

Impact Statement

The application of continuous manufacturing principles to liposomal drug product manufacturing is a novel concept and can be implemented to increase production capacity of many liposomal formulations including vaccines and gene delivery products. The understanding derived around the impact of organic solvents (ethanol) on diafiltration efficiency and continuous diafiltration designs is an important discovery that helps to optimize the production capability of liposomal drug products. In understanding this impact, inputs to the process such as buffer consumption, can be minimized, making the continuous design not just competitive, but advantageous as compared to the batch design. The economic assessment of this impact/design further illustrates the benefits of this understanding and employment of a continuous process design, particularly when product demand is significant.

The impact has been reinforced by the VP of Tech Ops of Insmed; “For a liposomal product with significant annual demand (doses/yr), implementation of a continuous design and principles could save tens of millions (USD) per year and yield a positive ROI (return on investment) within the first year.” For liposomal based products projecting high annual demand, the benefit is clear.

Acknowledgements

I would like to thank my supervisors Dr. Suzanne Farid and Dr. Vaughan Thomas for their support and willingness to take on an unorthodox approach to a doctoral program. Additionally, I would like to thank Insmed for allowing to pursue this project/program and particularly Tim Gillespie, Devin McDonald, Kyle O'Connell and Justin Varvar for their support in the laboratory.

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Abbreviations

ACI	Annual Capital Investment
ADME	Adsorption, Distribution, Metabolism, and Elimination
API	Active Pharmaceutical Ingredient
ATF	Alternating Tangential Flow
CAGR	Compound Annual Growth Rate
CAPEX	Capital Expenditures
CIP	Clean-in-Place
COG(s)	Cost of Good(s)
CWDF	Constant-Weight Diafiltration
CVDF	Constant-Volume Diafiltration
DOE	Design of Experiments
DPPC	Dipalmitoylphosphatidylcholine
DV	Diavolumes
EtOH	Ethanol
FCI	Fixed Capital Investment
FDA	United States Food and Drug Administration
GC	Gas Chromatography
HF	Hollow Fiber
HPLC	High Performance Liquid Chromatography
ILDF	In-line Diafiltration
LNP	Lipid Nanoparticle
PAT	Process Analytical Technology
PES	Polyethersulfone
POD	Podbielniak Contactor
PSF	Polysulfone
QC	Quality Control
RMU	Relative Monetary Unit

mRNA	Messenger Ribonucleic Acid
SIP	Steam-in-Place
SPTFF	Single Pass Tangential Flow Filtration
TFF	Tangential Flow Filtration
TMP	Transmembrane Pressure
VHP	Vaporized Hydrogen Peroxide
WFI	Water for Injection

Chapter 1

Introduction

Liposome-based drugs are a growing market. Multiple market research firms have shown the sector growing 8-9% Compound annual growth rate (CAGR) (Astute Analytica 2023, Data Bridge Market Research 2022, Transparency Market Research 2023). These same reports have shown market value of upwards of $10B by 2031 driven by at least 23 approved products and over 40 liposomal drugs in clinical trials as of 2022. The numbers are impressive, but may still be underestimated. The reports focus mainly on small molecule drug families such as oncology products (Janssen's Doxil®, 1995 [US] and Jazz Pharmaceuticals's Vyxeos®, 2017 [US]) and anti-infection products (Gilead's AmBisome®, 1997 [US] and Insmed's Arikayce®, 2018 [US]), but neglect areas such as biologics derived liposome-based vaccines and gene therapy related applications (Liu et al, 2022, Tenchov et al, 2021, Krasnopolsky et al, 2022). For example, the mRNA vaccine market has been projected as 9.6% growth to $27.7B by 2032 with the liposome-based Pfizer-BioNTech/Moderna COVID-19 vaccines positioning for a significant share.

Based on the breadth of products/indications and the scale of certain and potential demand, manufacturing processes for liposome-based products will need to increase capacity while decreasing costs. Continuous manufacturing presents a means of providing for both.

This introductory chapter provides an overview of continuous manufacturing, liposome manufacturing techniques and the considerations that arise from merging the two. **Sections 1.1 & 1.2** describes the concept of continuous manufacturing implementation with the pharmaceutical industry. **Section 1.3** covers the principles of liposome

manufacturing and description of the various manufacturing methods. **Section 1.4** covers the considerations of converting a specific liposome manufacturing method to a continuous process including tangential aspects such as product contact materials of construction and sterility assurance. The conclusions from these sections are reviewed in **Section 1.5**. Finally, the aims and objectives of the thesis are presented in **Section 1.6**.

1.1 Continuous Manufacturing

Continuous manufacturing is a processing concept whereby raw materials constantly flow into a process and intermediates or final product constantly flow out. This concept has a long history in many non-pharmaceutical industries and has been adopted in some types of pharmaceutical processes such as the synthesis of active pharmaceutical ingredients (API) and generation of solid oral dosage forms (tablets, etc.) (Kleinebudde et al. 2017, Subramanian 2015, Domokos et al., 2020, Suzuki et al. 2021). The potential benefits of implementing such a concept include economic advantages (lower capital expenditures, smaller facility footprint, lower overall cost of goods (COG)), as well as improved consistency and quality of product (Kleinebudde et al. 2017, Subramanian 2015, Domokos et al., 2020, Suzuki et al. 2021). As success and acceptance are realized, the concept is being adapted into more complex aspects and types of pharmaceutical manufacturing.

In recent history, continuous manufacturing has progressed into the production of biologics. The manufacture of biologics has continued to develop the requirements and

aspects to consider surrounding operating upstream and downstream unit operations in a continuous fashion such as cell culture, chromatography, viral inactivation and tangential flow filtration (TFF) as well as integrated continuous upstream and downstream processes (Pollock et al. 2013a and b, Warikoo et al. 2012, Mahajan et al. 2012, Godawat et al. 2012, Bisschops et al. 2013, Orozco et al. 2017, Parker et al. 2018, Pollock et al. 2017, Walther et al. 2015, Castilho 2015, Whitford 2015). For continuous perfusion cell culture, the biologics sector has moved from internal spin-filters to external retention devices such as alternating tangential flow (ATF) or TFF systems for media exchange (Pollock et al. 2013, Castilho 2015, Whitford 2015). TFF systems support continuous filtration by clearing the membrane surface with tangential fluid flow while ATF uses a cyclical backflush. Single pass tangential flow filtration (SPTFF) (Subramanian 2015, Elich et al. 2019) has been evaluated for cell culture harvest concentration and for protein concentration allowing this process step to happen in a continuous fashion instead of the batch mode required by traditional TFF (Arunkumar et al. 2017, Casey et al. 2011, Brower et al. 2015, Jungbauer 2013, Dizon-Maspat et al. 2012, Coffman et al. 2021, Jabra et al. 2022). Traditional TFF concentrates product through multiple passes of a recirculating loop while SPTFF concentrates in an inline fashion with a single pass through multiple TFF cassettes in series. SPTFF enables product to be continuously fed to the next unit operation or process step with the additional benefits of lower system hold-up volumes. These efforts towards continuous filtration operations are of particular interest when considering lessons learnt that may translate to applications in liposomal drug product formulations, which have had little to no exploration of application of continuous process techniques and typically uses TFF in batch mode as a unit operation.

Other aspects for commercial implementation of continuous manufacturing such as the need for process analytical technology (PAT) and the potential advantages provided by single-use componentry have been previously explored. The consensus is that PAT around critical process measurements is a requirement for continuous processing as this replaces the off-line testing at intermediate stages in a batch process, but often specifics of implementation are left to the end user (Callener 2017, Rathore et al. 2009, Haneef et al. 2021, Clegg 2020). The implementation of single-use technology provides the same conceptual benefits as it would for a batch process, but increased in magnitude as more product is generated per single-use item. The evaluation of these methods/aspects have led to the conclusion that implementing continuous manufacturing in biologics can provide potentially similar advantages as shown in the processing of more conventional pharmaceutical products (Brower et al. 2015, Bisschops et al. 2015, Novais et al. 2001, Farid et al. 2014).

Given these conclusions, it becomes prudent to explore application to other product families including the production of liposomal drug products. Manufacturing of liposomal products has some common aspects to the precedent of other continuous pharmaceutical manufacturing process and some unique aspects that require further exploration. Frequently, liposomal products are reformulations of compendial APIs designed to alleviate adverse clinical side effects and/or provide a more targeted delivery as compared to systemic dosages (Maurer et al. 2001, Lian et al. 2001). Thus, liposomal products have some elements of solid oral products (API manufacturing/sourcing/supply chain), some from biopharma (Mixing vessels, TFF, filtration, etc.) and some unique

elements, such as liposome generation and use of organic solvents, which will be examined further here.

1.2 Definition of Continuous Manufacturing for Liposomal Drug Product

Continuous manufacturing has been defined in many ways. Some feel that the term should only apply to processes capable of running 24 hours a day, 7 days a week and 50 weeks per year (Badman et al. 2015). Others state that the term should also include restrictions around intermediate surge vessels or processing breaks between API and drug product (Hernandez 2017). In many ways, terms such as continuous, semi-continuous or others are irrelevant. Each process and product should be individually assessed to determine which concepts of continuous manufacturing are beneficial and which are not. Converting to continuous manufacturing is not always practical and should only be implemented after thorough evaluation (Stanton 2017).

With respect to liposomal drug product manufacturing, it will be assumed here that the end-product is a reformulation of a compendial API and, therefore, the API is available from many sources on a more cost-effective basis when compared to the complexity associated with combining drug substance and drug product manufacturing into the same process. The focus here will be on outlining the manufacturing processes involved in preparation of liposomal formulations and how implementing continuous manufacturing can be achieved and provide benefit to the liposomal drug products.

1.3 Liposome Manufacturing

Liposomes were first discovered in the early-1960s. Since that time, a number of strategies have been demonstrated for their manufacture (Mozafari 2005, Maherani et al. 2011). Until recently, the application of liposomal products in pharmaceutical development has suffered from a lack of reliable manufacturing methods with sufficient throughput to enable commercial scale-up (Table 1.1). Generally, strategies for liposome synthesis focus on addressing and optimizing one or several of the key driving forces of vesicle assembly including the component solubilities, concentrations, and process thermodynamic parameters (i.e., temperature, pressure, etc.) (Mozafari 2005, Maherani et al. 2011). Manufacturing methods can be designed to fine-tune liposomes with various properties and, in doing so, can lend both advantages and disadvantages amenable to large-scale processing. In addition, selection of the manufacturing method often depends on the end product requirements for clinically efficacy including liposome size and size distribution, lipid composition, and the drug release characteristics, together, which dictate the pharmacokinetic demonstration of adsorption, distribution, metabolism, and elimination (ADME).

Table 1.1 Liposome formation methods

Method	Mechanism	Suitability for Continuous Manufacturing	References
Bangham	Rehydration of thin lipid film	Not practical means of continuous dehydration/rehydration steps	(Bangham et al. 1965, Bangham et al. 1965, Deamer et al. 1976)
Sonication	Sonication of an aqueous lipid suspension	Requires small scale batch operation for sonication to be practical	(Perret et al. 1991)
Reverse phase evaporation	Aqueous phase added to organic phase and evaporated to form liposomes	Overly complex to regulate continuous solvent evaporation, sterile boundary hard to establish	(Meure et al. 2008, Szoka et al. 1978)
Detergent depletion	Liposomes formed through detergent lipid interaction	Slow process with difficult to establish sterile boundary, detergent use general disadvantageous	(Brunner et al. 1976, Lasch et al. 2003)
Microfluidic channel	Intersection of lipid/API solutions in micro-channels	Very small scale, not a practical manufacturing process with existing technology	(Jahn et al. 2007)
High pressure homogenization	Liposome formation through high pressure mixing	Very high pressures required, difficult to sterilize equipment	(Barnadas-Rodriguez et al. 2001, Carugo et al. 2016)
Heating	Heating of a lipid aqueous/glycerol solution to form liposomes	Hydration step and high temperatures make continuous production impractical	(Mozafari 2005)
Supercritical fluid	Use of supercritical fluids as solvent for lipids instead of organic solvents	High pressures required for feed vessels make resupply/continuous operation impractical	(Meure et al. 2008, Santo et al. 2015, Santo et al. 2014, Campardelli et al. 2016, Frederiksen et al. 1997, Otake et al. 2001)

Method	Mechanism	Suitability for Continuous Manufacturing	References
Dense Gas	Use of dense gas as solvent for lipids instead of organic solvents	High pressures required for feed vessels make resupply/continuous operation impractical	(Meure et al. 2008, Otake et al. 2001, Anton et al. 1994)
Ethanol/Ether injection	Precipitation of liposome from organic phase into aqueous	Simple process with inherently continuous liposome formation step, very suitable	(Jaafar-Maalej et al. 2010, Santo et al. 2015, Batzri et al. 1973, Deamer et al. 1976)
Crossflow	In-line Precipitation of liposome from organic phase into aqueous	Simple process with inherently continuous liposome formation step, very suitable	(Wagner et al. 2006, Wagner et al. 2002, Wagner et al. 2002, Wagner et al. 2011, Wagner et al. 2002)

The most basic and earliest methods for liposome formation began with multistep synthetic strategies involving the rehydration of thin phospholipid films in aqueous media which resulted in the spontaneous formation of lipid structures of varying sizes, shapes, and lamella (Bangham et al, 1965, Bangham et al. 1965, Deamer et al. 1976). For uniform product generation, these suspensions required post-formation mechanical size manipulations strategies (Barnadas-Rodriguez et al. 2001, Carugo et al. 2016). The combination of these methods, although effective and well-understood, has proven to be inconvenient for large-scale manufacture. More recently, efforts have been dedicated towards investigating the possibility for single-step scalable techniques that involve programmable online flow-based strategies to arrive at the controlled precipitation and subsequent self-assembly of phospholipids into uniform structures, which is ideal for processing in a regulated pharmaceutical environment (Wagner et al. 2006).

The most successful examples of scaled methods for liposome manufacture to date have followed the principles of alcohol injection (Figure 1.1) or crossflow techniques (Figure 1.2), wherein dissolved lipids are precipitated from an organic solvent into an aqueous solution (anti-solvent) by means of reciprocal diffusion of the alcohol and aqueous phases (Jaafar-Maalej et al. 2010, Wagner et al. 2002, Wagner et al. 2002, Wagner et al. 2011, Wagner et al. 2002). A change in the local solubility of the lipids during this process ultimately leads to the spontaneous formation of liposomes that encapsulate a small volume of the aqueous solution. Depending on the chemical nature of the API, which can range from a small molecule to mRNA, it can be encapsulated in the aqueous core or embedded in the lipid layer (D'Mello et al. 2017, Webb et al. 2022). The critical parameters for the formation of liposomes by this method are residence time and geometry of the

mixing/intersection of organic-solvated lipid and the antisolvent which are dictated by programmed flow conditions. After liposome formation, the mixture containing undesired organic solvent and unencapsulated API can then be refined to the desired formulation strength and composition using TFF or similar methods (Wagner et al. 2002, Kim et al. 2012, Li et al. 2011).

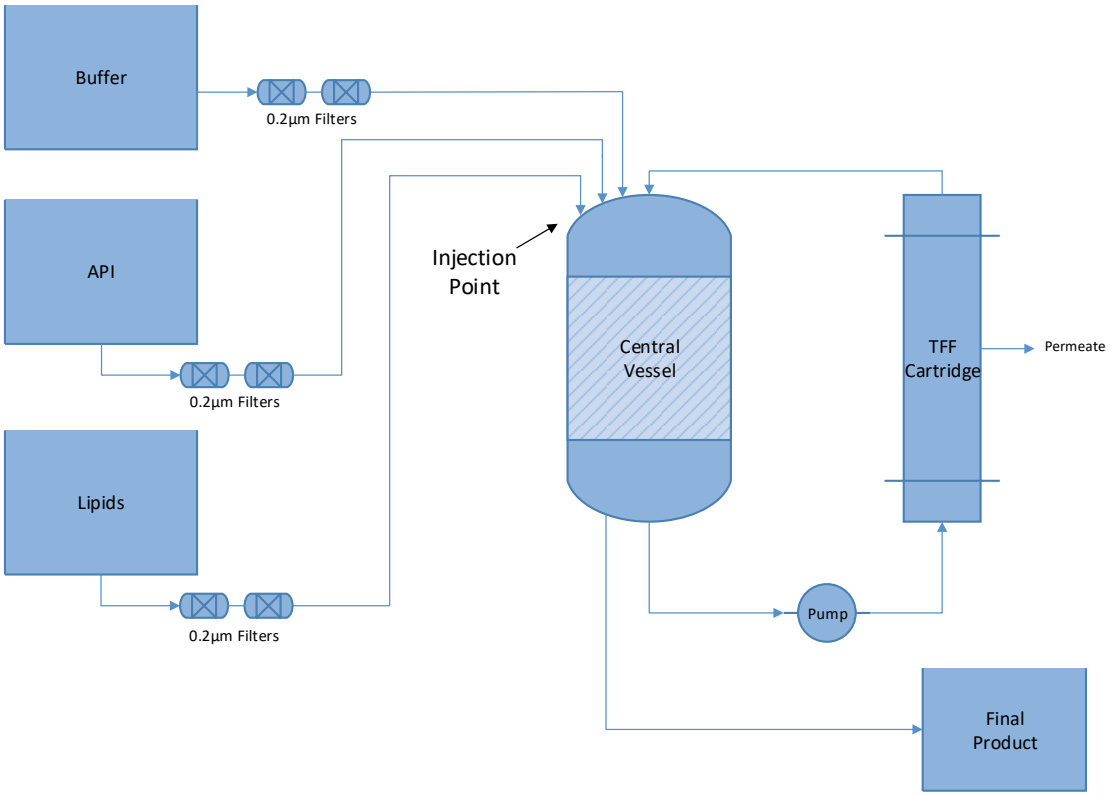

Figure 1.1 Liposomal Drug Product Manufacturing Process Flow Diagram – Batch Design. Ethanol/Ether Injection Method: Lipid/Solvent solution is directly fed into the central vessel. Formulations are refined in multi-step buffer exchange diafiltration and concentration steps.

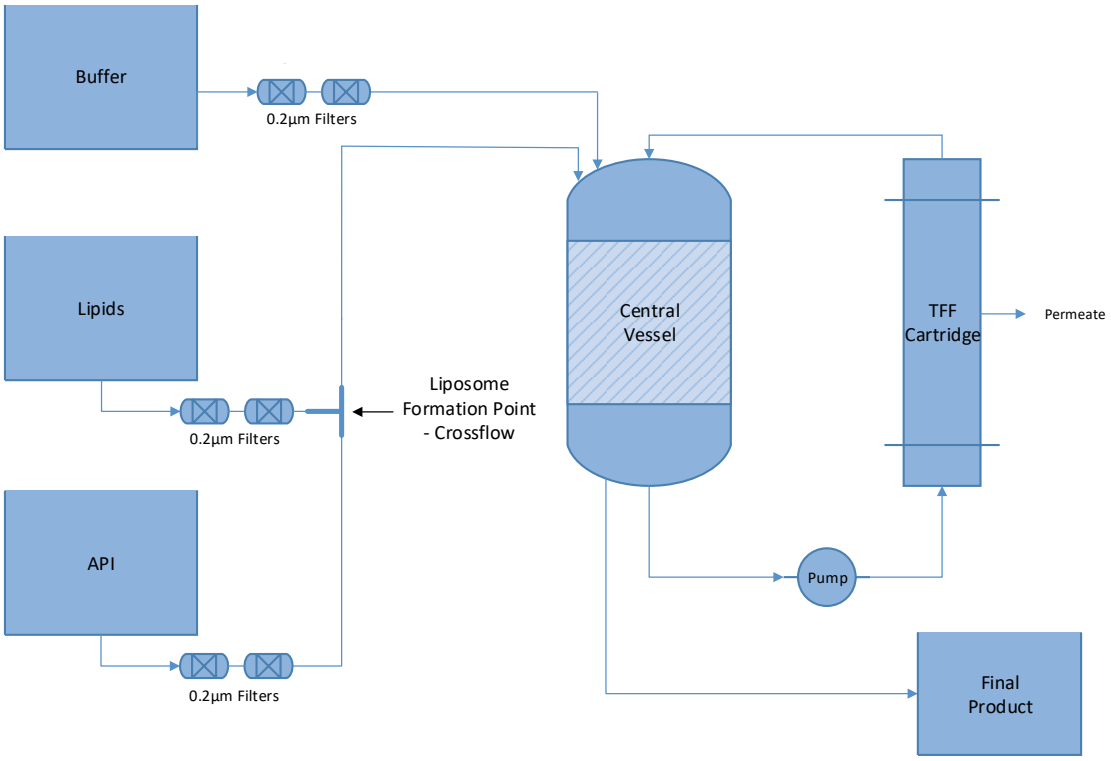

Figure 1.2 Liposomal Drug Product Manufacturing Process Flow Diagram – Batch Design. Crossflow Method: Solvent/anti-solvent mix in-line at an intersection point. Formulations are refined in multi-step buffer exchange diafiltration and concentration steps.

All of the aforementioned production methods were designed to operate as a batch process, but the injection and crossflow methods are based on a liposome formation step which is continuous in its natural mechanism (Figure 1.1 and 1.2). So long as each feed stream is continuously fed, liposomes will be continuously generated. It should be noted that the supercritical fluid and dense gas methods use their namesakes as the solvent for the lipid solution while the injection and crossflow methods use organic solvents. While similar in principle, supercritical and dense gas feed solutions require high pressure that would be difficult to adapt to a continuous design (Meure et al. 2008, Santo et al. 2015, Santo et al. 2014, Campardelli et al. 2016, Frederiksen et al. 1997, Otake et al. 2001,

Anton et al. 1994). Injection and crossflow methods, which are formulated under close to ambient conditions, present the most practical methods to adapt to continuous operation. (See Table 1.1 for comments on suitability for continuous manufacturing for each method.) With continuous formulation of the feed solutions, the liposome formation step can proceed indefinitely. By adding continuous steps, similar to those explored in biologics processing, which support refinement of the drug product to the desired end formulation, continuous manufacturing of liposomal drug products is a feasible concept.

1.4 Challenges for Continuous Liposome Production

While the central aspect (liposome formation) of liposomal drug product manufacturing is conducive to continuous manufacturing, there are special nuances in the areas of formulation refinement, materials of construction, and sterility assurance that need to be addressed for adaptation to a regulated pharmaceutical environment.

1.4.1 Formulation refinement

The unit operations downstream of liposome formation are used to refine the drug product formulation to the desired specification. Frequently, unit operations such as TFF are used to remove undesired elements, such as non-encapsulated API or organic solvent, and concentrate the drug product to a final desired strength. In this case, the retentate contains the drug product and the permeate acts as a waste stream. This is not dissimilar from downstream unit operations in biologics manufacturing (Jungbauer 2013). TFF for the buffer exchange and concentration in liposomal drug product manufacturing would

need to be properly balanced to support continuous operation. A batch mode design for this operation would entail a TFF step where the liposome-containing retentate is returned to the central vessel and the permeate/waste stream is made up with a feed of fresh buffer (constant-weight diafiltration, CWDF, or constant-volume diafiltration, CVDF), facilitating the buffer exchange. Once buffer exchange is complete, the product is concentrated to the desired strength by ceasing buffer addition. A continuous design would allow for continuous buffer exchange and a concurrent concentration step. Arrangements such as these are not unfamiliar in the world of biologics, but unique aspects of liposomes would need to be considered and experimentally tested for such an operation (Jungbauer 2013). Depending on the composition of the incoming feeds and specification of the desired end formulation, this could be facilitated by various arrangements. A single vessel buffer exchange TFF system with single stage concurrent concentrating SPTFF serves as the base case for a continuous design (Figure 1.3). If steady state diafiltration or single pass concentration are not able to achieve the required rate of buffer exchange or concentration with a single stage, additional stages may be added (Figures 1.4 and 1.5). Additionally, more compact and elegant designs for continuous buffer exchange, such as the Pall Cadence™ In-line Diafiltration Module (ILDF), are becoming available and should be explored (Gjoka et al. 2017). An ILDF design concluding with SPTFF would eliminate the need for multiple vessels to support continuous buffer exchange (Figure 1.6).

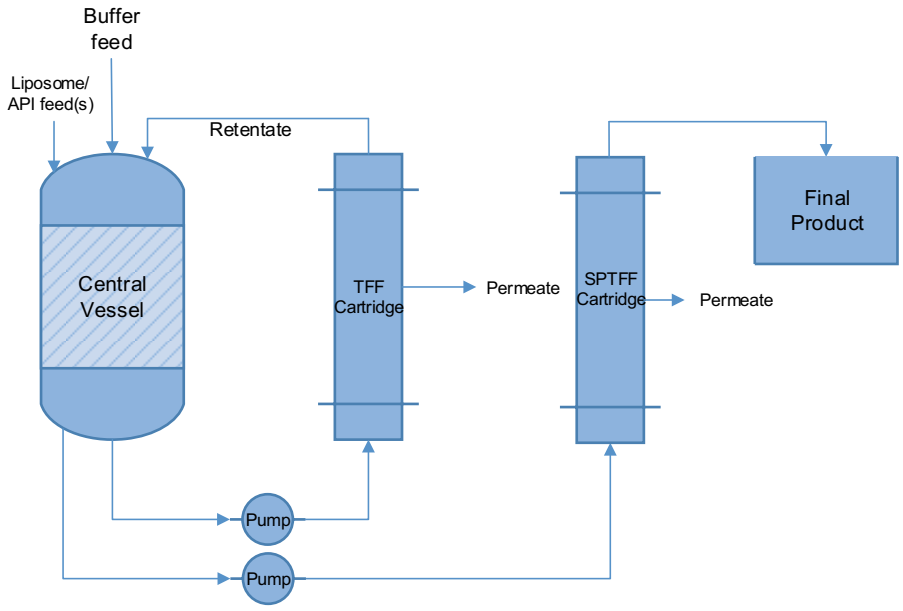

Figure 1.3 Proposed novel process designs for continuous liposome drug product manufacturing. Single tank buffer exchange TFF and single stage concurrent concentrating SPTFF.

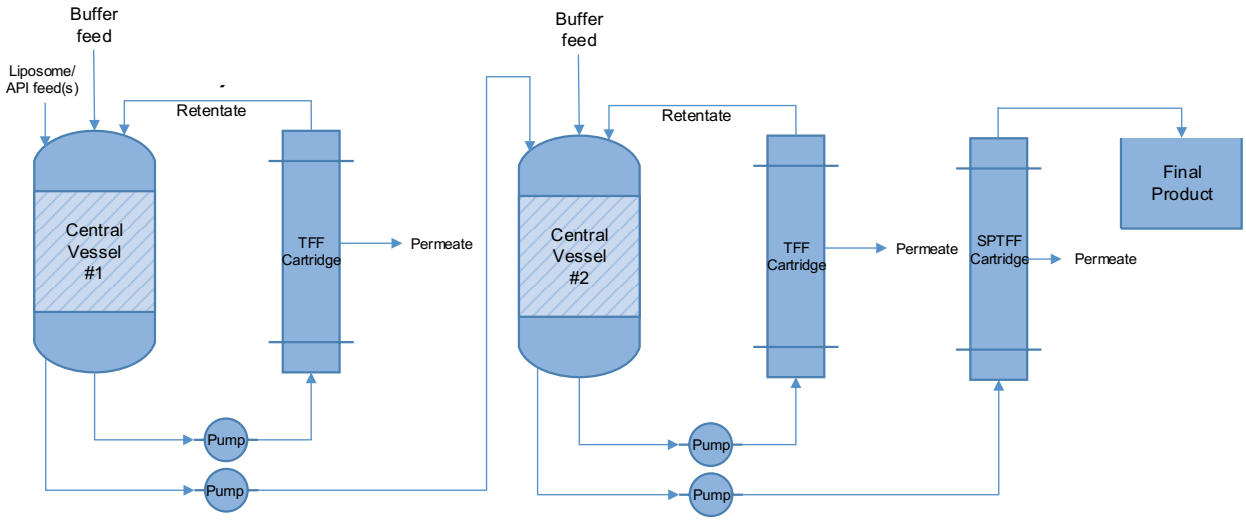

Figure 1.4 Proposed novel process designs for continuous liposome drug product manufacturing. Continuous multistage (multi-vessel) buffer exchange TFF and single stage concurrent concentrating SPTFF.

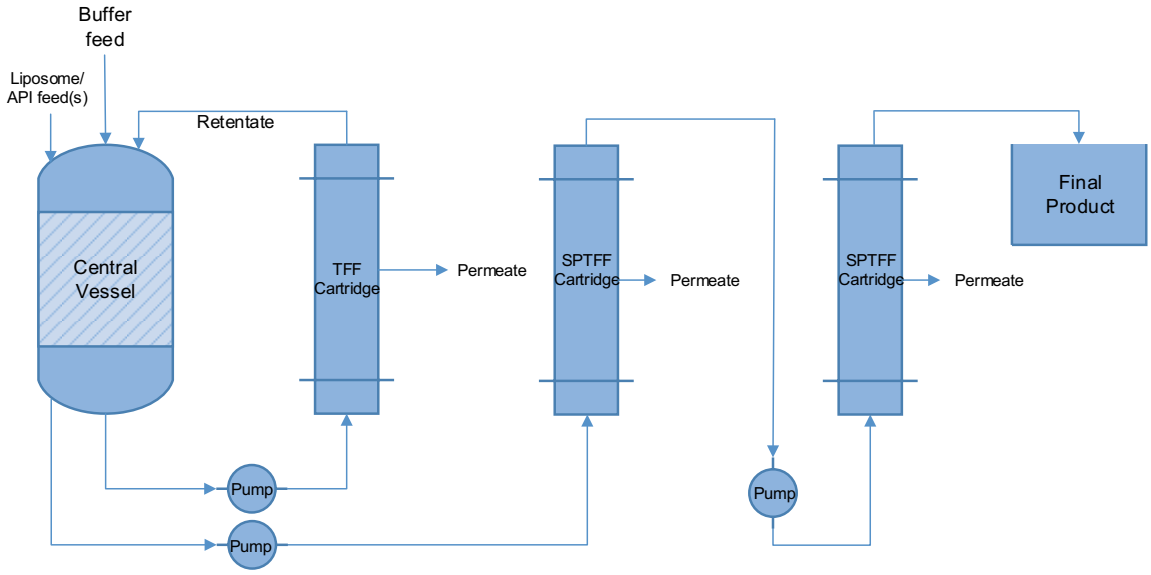

Figure 1.5 Proposed novel process designs for continuous liposome drug product manufacturing. Single tank buffer exchange TFF and multistage concurrent concentrating SPTFF.

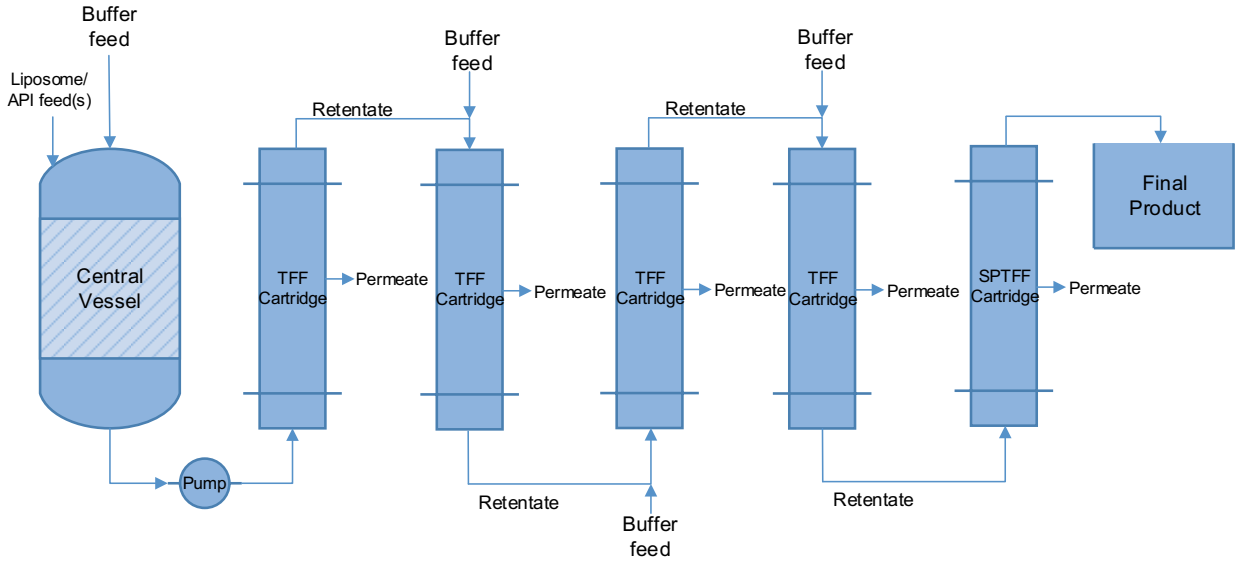

Figure 1.6 Proposed novel process designs for continuous liposome drug product manufacturing. Multistage buffer exchange and concurrent concentrating SPTFF.

SPTFF is an additional unknown for liposomal formulations, but data has been generated for use of SPTFF for concentration of cell culture harvest or for protein concentration (Arunkumar et al. 2017, Casey et al. 2011, Fuchs et al., 2023, Jabra et al. 2022, Malladi et al. 2023). It cannot be assumed that liposomes will behave the same as cells or protein, but similar to cell suspensions and protein solutions, liposome formulations increase in viscosity exponentially during concentration. Since final concentration specifications often have a narrow tolerance, a high level of control and accuracy would be required for such an operation. This raises and re-enforces another canonical requirement of continuous manufacturing, process analytical technology (PAT).

During manufacturing of liposomal formulations, there is allowable and expected variability in capture efficiency of the active ingredient. In a batch process, this is compensated for by offline in-process measurement of active ingredient concentration prior to the concentration step. While basic measurements such as flow rates, mass, and density provide a level of control and are easily implemented in a continuous operation, a greater level of assurance would be provided by a real-time concentration measurement such as in-line High Performance Liquid Chromatography (HPLC) (Callener 2017, Rathore et al. 2009).

In-line HPLC methods are available but would require significant development to overcome assay requirements such as lysing of liposomes to determine concentration, rendering it a destructive test method. Given the feedback delay can be overcome by the consistency of the other process controls, Rapid HPLC, which reduces off-line testing time from 60 minutes to 4 minutes is a more likely candidate (Kumar et al. 2013). Other

in-line measurements, such as particle size, may be applicable, given they can be correlated to concentration.

1.4.2 Materials of Construction

Many of the benefits of continuously manufacturing biologics are leveraged from the incorporation of single-use systems and componentry (Whitford 2015, Bisschops 2015, Novais et al. 2001, Hammerschmidt et al. 2014). This eliminates the need for expensive capital equipment, simplifies cleaning and sanitization/sterilization, and can provide additional flexibility for multi-product operations. However, with liposome manufacturing, single-use componentry presents several issues. Since the manufacturing of liposomes requires the use of organic solvents, use of single-use components such as tubing and bags, can present issues around extractables/leachables (Ferrante 2017, Hernandez 2017). Additionally, if single-use components are pre-sterilized through gamma-irradiation, there can be issues with free-radical generation and incorporation into the drug product. Ultimately, these can cause degradation of some liposome components and/or a need for significant characterization of previously undetected impurities in the final product (Schnitzer et al. 2007, Toh et al. 2013). Another issue with single-use componentry is the risk in their ability to maintain a sterile boundary, which leads to perhaps the most specific nuance of liposome manufacturing: aseptic processing.

1.4.3 Sterility Assurance / Aseptic Processing

Commercial scale manufacturing of liposomes, in the vast majority of cases, will require aseptic processing. This is due to liposomes typically having a particle size greater than 0.2μm (unable to be terminally sterile filtered) and their instability in the presence of excessive heat, aggressive chemicals, or radiation (i.e. autoclave, vaporized hydrogen peroxide (VHP), e-beam, gamma) (Jan Zuidam et al. 2003, Toh et al. 2013). With aseptic processing comes the need to establish and defend a sterile boundary around the process. Use of single-use componentry can increase the risk to the integrity of that boundary as bags and tubing assemblies can have a higher probability of leaks (especially if custom) than more robust reusable systems such as stainless steel (Stock 2014). Additionally, extended use of flexible tubing in pumping systems can lead to spalling and breaches as well (Bahal et al. 2002).

Beyond building in sterility assurance through designing a durable integral boundary, the ability to maintain an aseptic process must be demonstrated through simulations and validations. Assuming the process is set up using pre-sterilized componentry and/or steam-in-place (SIP) equipment, any feed solutions (API containing aqueous solution, lipid containing organic solution, or buffer) must enter the system through sterilizing filters containing a pore size of typically 0.2 μm or less. The capability (ability of the filter to remove given concentrations of organism) and duration (time of use before grow-through of an organism compromises the filter) of the sterile filtration step must be validated. For a continuous design, the duration is most concerning as the general rule of thumb for use of a sterile filter is less than four hours. Overcoming this would require either massively redundant filtration designs or sequential use of parallel filtration pathways. Sequential

use of parallel pathways is a more viable solution since multiple redundant pathways would cause significant pressure drop issues. (Note: Most regulatory authorities require redundant filtration containing two filters as standard practice.)

The requirement of aseptic process validation or growth media simulations further builds on the foundation of sterility assurance measures such as pre-sterilized componentry, SIP, and sterile filtration validation. Aseptic process validation involves processing growth media in place of feed solutions and product to further establish the ability to maintain an aseptic process. These simulations should encompass the anticipated duration of the continuous operation, which causes the re-visitation of the continuous manufacturing definition. Ultimately, simulating a multiple month process is not practical from an operational standpoint. Simulations compete with production and the risk to the sterile boundary increases directly with duration of the process. The risk of growth media simulation failure should be considered when determining the duration of a continuous design. Conversely, FDA guidance views a continuous operation as advantageous due to the reduction of start-up and shut-down operations, where most breaches occur, as compared to quantity of product produced (Yu 2016). Ultimately, there is a risk/benefit inflection point for each process that should be determined.

Another consideration is the sterility sampling plan of the bulk and/or filled final product (FDA 2014). With PAT for microbiology still in its infancy (NIST Rapid Microbial Testing Methods Consortium), sterility is assured through the design and validations mentioned above, coupled with a statistically sound sampling plan. For a batch process, a single bulk sample is taken to assure sterility prior to proceeding with filling. If continuous filling is integrated into the process, the bulk will be continuously flowing to the filling operation,

preventing a representative bulk sample from being taken. This could be compensated for by taking additional samples during the filling operation to represent both real-time bulk and filled units. At present, the sterility assurance requirement and lack of microbiology PAT prevent real-time release as individual units could not be released without passing microbiological results from all bulk and all final product samples. This is another element to the risk/benefit profile that should be considered.

1.5 Conclusion

Continuous manufacturing is a concept that has clear benefits to many industries. Biologics manufacturing has taken the lead for applying this concept to the pharmaceutical industry, but now its application can be expanded to pharmaceutical liposomal drug products. At current, there are no evaluations of continuous manufacturing of liposomal drug products. An examination should be undertaken to determine applicability of continuous manufacturing options to liposomal drug product manufacturing while considering PAT, materials of construction, and sterility assurance.

1.6 Thesis Aims and Objectives

Previous work has covered initial assessments of continuous manufacturing options as they apply to API, tablet and biologics manufacturing, but not liposomal drug products. This thesis aims to address whether a design and process requirements can be determined for a continuous liposomal drug product manufacturing process and whether

that design would provide economic benefit. In order to realize this aim, the following objectives were established for the proceeding chapters.

Chapter 2 contains a description of the Materials and Methods that will be standard for the various experimental chapters. More specific Materials and Methods will also be contained in each chapter as pertains to the experiments.

Chapter 3 covers aspects of a specific commercial liposomal drug product batch process and how they may impact a continuous process design. This particular batch process is used to create a liposomal amikacin suspension (Arikayce ®) that has been formulated to a required strength in a NaCl buffer solution. The process will serve as a baseline for comparison to various options for continuous processing. This chapter endeavors to elucidate aspects of the batch process that may impact the continuous design options.

Chapter 4 examines a pilot/commercial scale configuration mimicking in-line diafiltration (ILDF) in place of constant volume diafiltration (CVDF) using the same hollow fiber cartridges as the batch design. A series of experiments based on the findings in Chapter 3 were executed to further determine the optimal conditions for use of ILDF with a liposomal formulation. The objective of this chapter was to determine recommendations on process conditions for continuous diafiltration and how the process performance compared to the batch process.

The objective of Chapter 5 was to perform an economic analysis and comparison of the example batch process and the recommended continuous process and determine when each option presents an advantage. Aspects such as COG, capital investment, risk

were determined and compared. The objective of the chapter was to determine when the process design options are favorable and factors/strategy in making option selection.

Chapter 6 provides summary conclusions of the work contained in the thesis and future plans for additional work. Publications and patents are also listed.

Chapter 2

Materials and Methods

This chapter includes the materials and methods that are generally applicable to all the subsequent chapters. More specific materials and methods sections will be contained within each corresponding chapter. The materials and methods are directly related to those used for manufacturing and testing of Arikayce® product. **Section 2.1** describes the composition of the feed solutions used in the liposome formation and buffer exchange process steps. These feed solutions are used in all the subsequent chapters/experiments/modelling. **Section 2.2** provides an overview description of the batch process targeted for conversion to a continuous design. **Section 2.3** describes the arrangement used in Chapter 4 to study the ILDF concept for the continuous process design. The analytical methods used to test the composition of the process output are described in **Section 2.4**. The calculations used in economic modelling and comparison of the batch and continuous scenarios are described in **Section 2.5**.

2.1 Feed Materials

The amikacin solutions were prepared by dissolving amikacin sulfate in water-for-injection (WFI) or deionized water at 45 mg/mL amikacin base and pH adjusted to 6.7 using NaOH.

The lipid solution was prepared by dissolving Dipalmitoylphosphatidylcholine (DPPC) and cholesterol at a 2:1 weight ratio in 100% ethanol at 20mg/mL.

The buffer solution consists of 1.5% NaCl in water-for-injection (WFI) or deionized water.

2.2 Batch Processing

The batch process for manufacturing liposomal drug product, as depicted in Figure 1.2, involves mixing/infusing streams of lipid solution and amikacin solution at the crossflow point in an approximate 1:2 ratio. The magnitude of these flow rates is dependent on the scale of the process. The output of the crossflow point ("liposome mixture") is fed into a central vessel concurrently with the buffer solution. Constant volume diafiltration is performed using a 50kDa MaxCell hollow fiber cartridge (Cytiva, Marlborough, MA, USA) (Model# UFP-500-E-85MSM or UFP-500-E-152M) until six diavolumes have been processed. The product is then concentrated using the hollow fiber membrane(s) until the retentate is 70mg/mL amikacin base. The product is then transferred to a final product vessel and filled into 10mL glass vials.

2.3 Simulated ILDF System

The simulated ILDF system consists of equipment similar or the same to that used in the batch process as described in Section 2.2. The equipment was arranged in a manner supporting multiple independent concentration/buffer exchange passes with opportunity for sampling and dilution with buffer in between each pass (Figure 2.1).

The starting solutions and liposome mixture as described in Section 2.1 and 2.2 were used as a starting material. The buffer solution was used to pre-dilute the liposome mixture as described in Chapter 4.

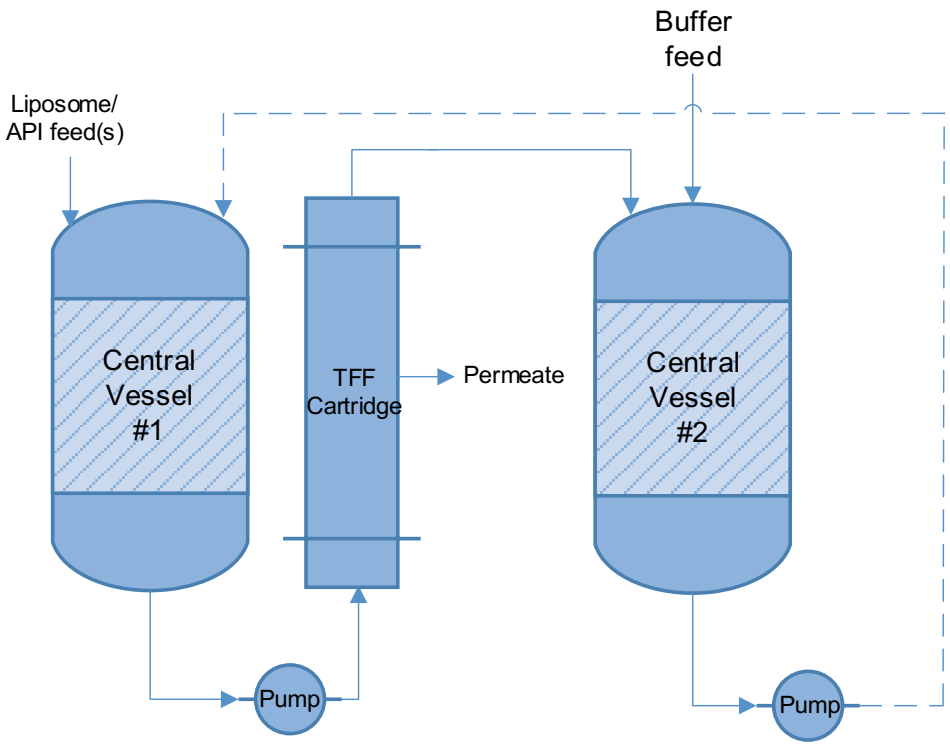

Figure 2.1. Simulated ILDF Set-up: Process Flow Diagram of the single-pass concentration/buffer exchange, which can be used to simulate ILDF with the ability to analysis retentate/permeate in between passes/stages. Solution vessels and liposome formation equipment not shown. The dashed line indicates the bulk is not returned to Central Vessel #1 until after analysis/dilution of its entirety.

2.4 Analytical Methods

Total amikacin concentrations were measured by high-performance liquid chromatography (HPLC) using a Hypersil GOLD (Thermofisher, Waltham, MA, USA) C18 column (175 Å, 3 µm, 150 mm × 4.6 mm) with a mobile phase of 65% methanol, 35% water, and 0.3% pentafluoropropionic acid (PFPA). An aliquot of each liposome suspension was centrifuged in an Amicon Ultra-0.5 centrifugal filter unit (EMD Millipore, Burlington, MA, USA) with Ultracel-30 Kda membrane to separate free amikacin, and the amount of unencapsulated amikacin was then determined by HPLC.

Lipid concentrations were measured by HPLC using an Xbridge (Waters Corp., Milford, MA) C8 column (130Å, 3.5µm, 150mm × 4.6mm) with a mobile phase A consisting of 49.9% acetonitrile, 49.9% water, 0.1% acetic acid, and 0.1% triethylamine and mobile phase B consisting of 44.9% acetonitrile, 45% isopropyl alcohol, 10% water, 0.1% acetic acid, and 0.1% triethylamine.

Residual ethanol or ethanol concentrations were measured by using gas chromatography (GC) using a Duraguard (Agilent) DB-624 column (30m x 0.53mm x 3.0 µm) and a 100 µg/mL ethanol working standard.

2.5 Economic Comparison

The economic evaluation and modeling assessed the impact on cost of goods, taking into consideration product output, components and starting material consumption of batch and continuous process designs. This included a comparison of capital investment and a qualitative risk assessment.

2.5.1 COG Model and Cost Data

Cost of goods (COG) was calculated using an in-house model created in Microsoft Excel (version 2311). The calculation for COG involved annual direct (variable) and indirect (fixed) costs divided over the annual production demand. Annual production demand is calculated as total number of filled vials per year (1 vial = 1 dose). The direct costs include Reagents (chemical raw materials for production, cleaning, etc.),

Consumables (e.g. filters, tubing, vials), and Labor (variable labor for production, in-process and release testing, waste disposal, utilities generation, etc.). The indirect costs include Depreciation of initial fixed capital investment (FCI) and ongoing annual capital investment (ACI) as well as Other (annual maintenance, calibrations, requalifications). The annual direct costs are dependent on the batch size/run time and number of batches required to fulfill the annual production demand. The annual indirect costs are fixed with activities such as maintenance being dictated on schedule related to equipment usage. Table 2.1 summarizes the calculation.

Table 2.1 Summary of COG model

COG calculation		
Cost Category		Equation
Direct	Reagents	Per batch raw materials x Annual batches
	Consumables	Per batch consumables x Annual batches
	Labor	Tiered per dose cost x Batch size x Annual batches
Indirect	Depreciation	10% x (FCI + ACI) annually
	Other	Fixed annual costs to maintain production and compliance
COG		(Direct + Indirect)/annual production in vials

Direct and indirect costs for the batch process design scenario were based on a real-world example. These include real-world pricing data, resource planning, and facility utilization. For the scenarios involving the continuous process design, the batch process design scenario costs were extrapolated or manipulated as needed. The process design scenarios dictate the production capabilities/capacity.

2.5.2 Capital Investment

Capitalized spend includes all process and utility equipment, build out of an existing facility (not greenfield), and tech transfer activities to achieve a validated state ready for commercial production. The capital investment for the batch design is also based on a real-world example. The other design options are normalized to the batch design capital cost.

2.5.3 Qualitative Risk Assessment

As a means of comparing the more intangible aspects of the process designs, a qualitative risk assessment was performed. Risk around aspects such as implementation, operation, and economics were relatively assessed at various demand levels for the batch and continuous process designs. The risk levels are shown qualitatively using a three-level scale (Low, Medium, High). The separate aspects were evenly weighted and an overall risk comparison was generated.

Chapter 3

Liposomal Amikacin Batch Processing

3.1 Introduction

As indicated in the previous chapters, liposomal products are typically made using batch processes and there is interest in exploring continuous designs. In this chapter, the batch process used to make liposomal amikacin (Arikayce ®) will be described. The process will serve as a baseline in the evaluation of potential continuous process options in Chapters 4 and 5. Various aspects of the batch process need to be considered in a continuous process design.

Liposomal amikacin batch processing has historically been performed using a variant of the cross-flow liposome formation method as shown in Figure 1.2. The process involves intersection/in-line mixing of two fluid streams in order to create liposomes. In the baseline process, this involves an aqueous amikacin solution and an ethanolic lipid solution. As the fluids mix, the presence of the aqueous solution causes the lipids to precipitate out of the ethanol and spontaneously form liposomes. The liposomes encapsulate a core of the aqueous solution containing the amikacin. The liposome containing mixture is then processed through constant volume diafiltration (CVDF) in order to exchange the undesired components (ethanol, unencapsulated amikacin) with a NaCl buffer. The buffer containing the liposomes is concentrated to the desired amikacin strength and filled into the primary packaging (vials).

The process contains various steps that must be completed sequentially. Therefore, the only way to produce more product with the batch process is to scale up the size of the supporting equipment or add additional processing lines. Additionally, the process is

performed aseptically and is designed to support accepted practices for sterility assurance.

The batch process will be examined and discussed further for the applicability and considerations for the adaptation to a continuous process. Each unit operation will be described and the design and historical process performance will be assessed for conversion to a continuous design or operation. **Section 3.2** describes the batch process method in greater detail. **Section 3.3** outlines the sequential unit operations of the batch process and aspects to consider in conversion to a continuous process design. The most significant consideration is discussed in **Section 3.3.2**, which surrounds the impact of ethanol concentration on diafiltration. Conclusions for the Chapter are contained in **Section 3.4**.

3.2 Batch Process Methods

The methods used involve the feed materials, batch processing and analytical methods described in Chapter 2.

3.2.1 Feed Materials

The amikacin solutions were prepared by dissolving amikacin sulfate in water-for-injections (WFI) or deionized water at 45 mg/mL amikacin base and pH adjusted to 6.7 using NaOH.

The lipid solution was prepared by dissolving Dipalmitoylphosphatidylcholine (DPPC) and cholesterol at a 2:1 weight ratio in 100% ethanol at 20mg/mL.

The buffer solution consisted of 1.5% NaCl in water-for-injections (WFI) or deionized water.

3.2.2 Batch Processing

The batch process for manufacturing liposomal drug product, as depicted in Figure 1.2, involves mixing/infusing streams of lipid solution and amikacin solution at the crossflow point in an approximate 1:2 ratio. The magnitude of these flow rates is dependent on the scale of the process. The output of the crossflow point ("liposome mixture") is fed into a central vessel concurrently with the buffer solution. Constant volume diafiltration is performed using 50kDa MaxCell hollow fiber cartridge (Cytiva, Marlborough, MA, USA) (Model# UFP-500-E-85MSM or UFP-500-E-152M) until six dia-volumes have been processed. The product is then concentrated through the hollow fiber membrane until the retentate reaches 70mg/mL amikacin base. The product is then transferred to a final product vessel and filled into 10mL glass vials. The overall process has various instrumentation including pressure, temperature, and flow meters with various capabilities.

3.3 Results and Discussion

In this section, various unit operations of the batch process will be described and how their current design or performance may impact a continuous design. This could include the need for physical changes in process flow or how an aspect of the liposomal

amikacin drug product processing may impact optimization of continuous process conditions.

3.3.1 Liposome generation

Liposome generation is the key step of any liposomal drug product process. The standard batch process involves the formulation of amikacin and lipid solutions followed by feeding those solutions at prescribed flow rates into a cross-flow mixing arrangement where liposomes are formed and flow from the outlet. This step in the process is inherently continuous and the main motivation or inspiration for converting liposomal drug product processing to a continuous process.

Liposome generation could be maintained indefinitely given a continuous supply of feed solutions. Due to the need for aseptic processing, all solutions feeding the process need to pass through a 0.2μm sterilizing filter (most regulatory bodies require passing through two sterilizing filters). Additionally, sterile filtration is only recommended for use over a limited timeframe on each filter given the risk of “grow-through” for any microbiological contamination (Kaushal et al. 2013, Lutz et al. 2013).

Depending on the anticipated duration of the process, continuous liposome generation would require either large solution vessels or an array of vessels to support mixing/formulation of the starting solutions and then feeding of the solutions to the cross-flow intersection. Also, based on the anticipated duration, parallel circuits of sterilizing filters would need to be employed in order to support sterility assurance requirements.

As this step in the process is inherently continuous, the greater challenge is not the supply into the liposome generation unit operation, but how to handle the outflow. This is discussed more in the next section.

3.3.2 Diafiltration

In the batch process, the outflow from liposome generation is fed into a recirculating diafiltration arrangement (Figure 1.2) using hollow fiber cartridges. Depending on the size/scale of the central vessel, diafiltration will start prior to liposome generation ending. This is primarily done to keep the central vessel from overflowing/minimizing the necessary size of the vessel. Once liposome generation ends, constant volume diafiltration (CVDF) can begin. The liposome generation mixture exiting that unit operation contains approximately 36% ethanol prior to entering the central vessel. The standard batch process feeds the NaCl buffer solution into the central vessel concurrently with the liposome generation mixture. The standard batch process begins constant volume diafiltration (CVDF) with a mixture that contains approximately 30% ethanol in the central vessel due to the dilution from the added NaCl buffer solution. Through the prescribed six diavolumes, the ethanol concentration decreases to an end concentration of less than 1.0%, along with the removal of the unencapsulated API (amikacin). Upon completion, the liposomes containing amikacin are suspended in the NaCl solution.

The batch process diafiltration operates with constant inlet pressure to the hollow fiber. Permeate flow is fully open with no applied back pressure. The buffer stream flow matches the permeate flow to maintain constant volume in the central vessel.

Detailed evaluation of the batch process diafiltration performance showed an unexpected phenomenon. Regardless of the constant inlet pressure, the permeate flow showed an increasing pattern as the diavolumes progressed as opposed to a constant flow. Shown in Figure 3.1, permeate flow increased and leveled off through the run. Additionally, transmembrane pressure (TMP) behaved in the opposite fashion, decreasing and leveling off.

TMP is a measurement of the hollow fiber hydrostatic pressure gradient, which is the driving force in diafiltration (Equation 3.1). TMP is calculated as

$$Transmembrane\ Pressure\ (TMP) = \frac{(P_i - P_r)}{2} - P_p \qquad \text{(Equation 3.1)}$$

where P_i is the hollow fiber inlet pressure, P_r is the hollow fiber return/retentate pressure and P_p is the hollow fiber permeate pressure.

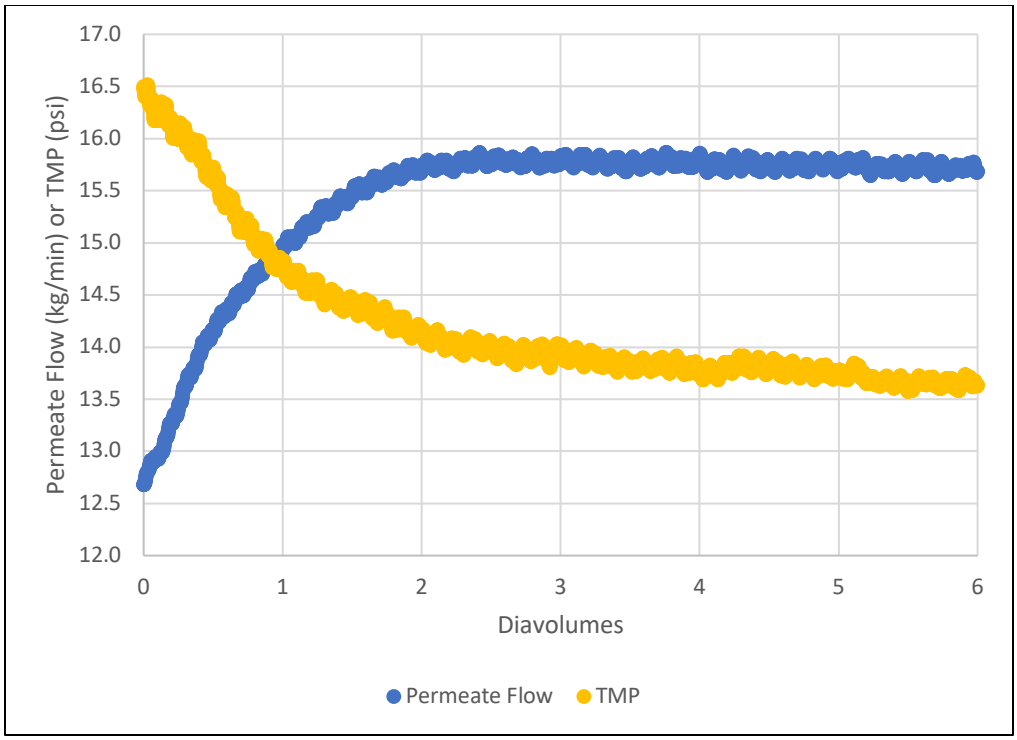

Figure 3.1 Permeate Flow Rate and TMP vs. Diavolumes. Permeate Flow Rate increases and levels off as diafiltration progresses while TMP correspondingly decreases and levels off. Data was generated using a commercial scale system measuring permeate flow rate and system pressures every five seconds.

The permeate flow rate and TMP patterns indicate permeability of the hollow fiber membrane improves as the diavolumes progress. The expectation would have been that the permeate remains generally consistent across the diavolumes.

The permeate flow was assessed further by using the density measurement feature on the permeate flow meter. As diafiltration proceeds, the density of the permeate approaches that of the buffer solution (1.003 g/cc), showing that the ethanol containing mixture has been exchanged for the buffer (Figure 3.2).

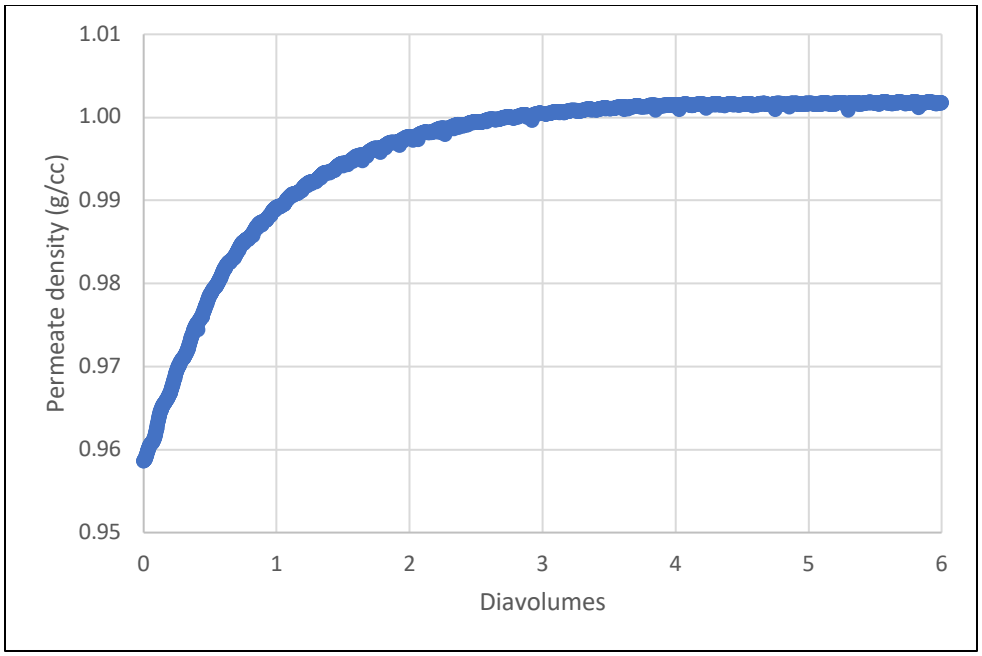

Figure 3.2 Permeate Density vs. Diavolumes during Constant Volume Diafiltration. Permeate Density increases and levels off as diafiltration progresses. Data was generated using a commercial scale system measuring density of the permeate every five seconds.

3.3.2.1 Ethanol Dependent Permeate Flow

Using the known densities for the amikacin and buffer solutions (1.003 g/cc) and the lipid solution (0.789 g/cc), the real-time composition of the permeate stream can be calculated (Figure 3.3). Further, the permeate ethanol concentration can be plotted against the permeate flow (Figure 3.4) to show a linear increase in flow as ethanol decreases. This shows that permeate flow and ethanol concentration are interrelated.

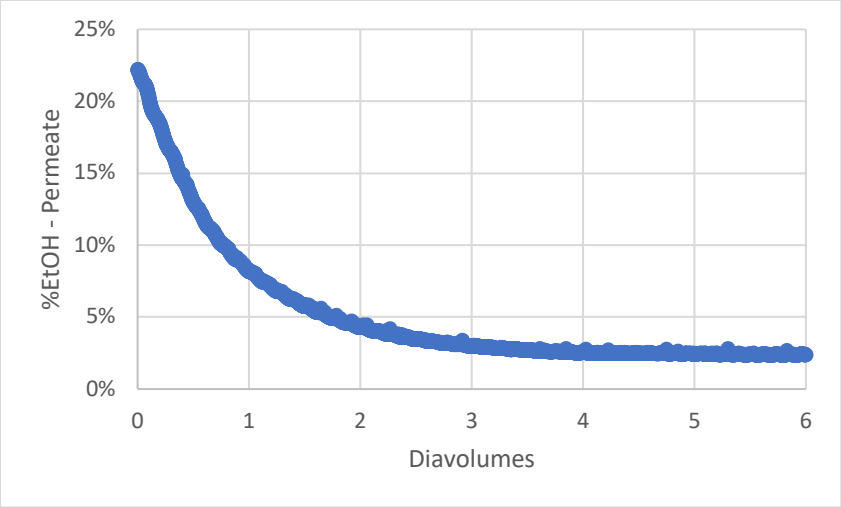

Figure 3.3 %EtOH in the permeate vs. Time during Constant Volume Diafiltration. The concentration of ethanol in the permeate stream was calculated based on the density data as shown in Figure 3.2 and shows the removal of ethanol as diafiltration progresses.

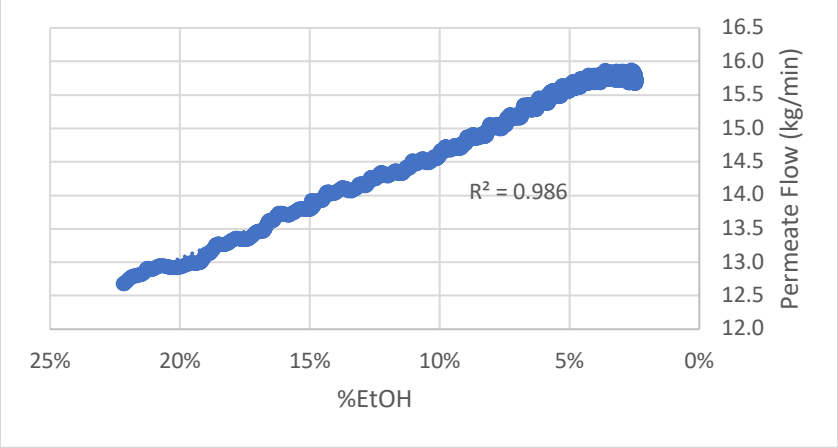

Figure 3.4 Permeate flow rate vs. Ethanol concentration. Permeate flow vs. %EtOH in the permeate during Constant Volume Diafiltration. The flowrate increases linearly as the concentration of ethanol decreases. The ethanol concentration was measured by converting density as measured by flow meter to ethanol concentration and confirmed by analytical method. Note the reversal of the x-axis to match chronology of the diafiltration. (Note the decreasing x-axis in order to maintain the left-to-right chronology of the data.)

Diafiltration would be expected to remove a target component, such as ethanol, in a manner following the standard diafiltration equation (Equation 3.2).

$$c = c_0 e^{-(1-\sigma)\frac{V_b}{V_s}} \quad \text{(Equation 3.2)}$$

where c_0= initial component concentration, c= final component concentration, V_b = the volume of the buffer added, V_s = the constant volume value, and α = the component rejection coefficient (Foley 2016). The ratio $\frac{V_b}{V_s}$ is the number of diavolumes. The rejection coefficient ranges from 0 to 1. A value of 0 represents a component that is free flowing through the diafiltration membrane or hollow fiber (i.e. water). A value of 1 represents a component that cannot pass through the diafiltration membrane or hollow fiber and thus $c=c_0$ (i.e. lipids/liposomes). It is common to assume the component targeted for removal is free flowing and has a rejection coefficient of 0. The results in Figure 3.4 suggest that the rejection coefficient for ethanol may have an ethanol concentration dependency.

To assess the ethanol rejection coefficient, the batch process was analyzed using the Equation 3.2 model. With the inline density measurement from the flow meter on the permeate line, the ethanol concentration was calculated across the diavolumes. Applying mass balance for the system, the retentate ethanol concentration was calculated, and the curve fitted to Equation 3.2 to determine the ethanol rejection coefficient. As shown in Figure 3.5, the rejection coefficient (α) calculated to approximately 0.5 as opposed to the zero coefficient that would have been assumed. Additionally, the fit showed the rejection coefficient to be greater than 0.5 early in the process and less than 0.5 later in the process, supporting the notion of an ethanol

concentration dependency to the rejection coefficient and/or an ethanol related impact to permeability.

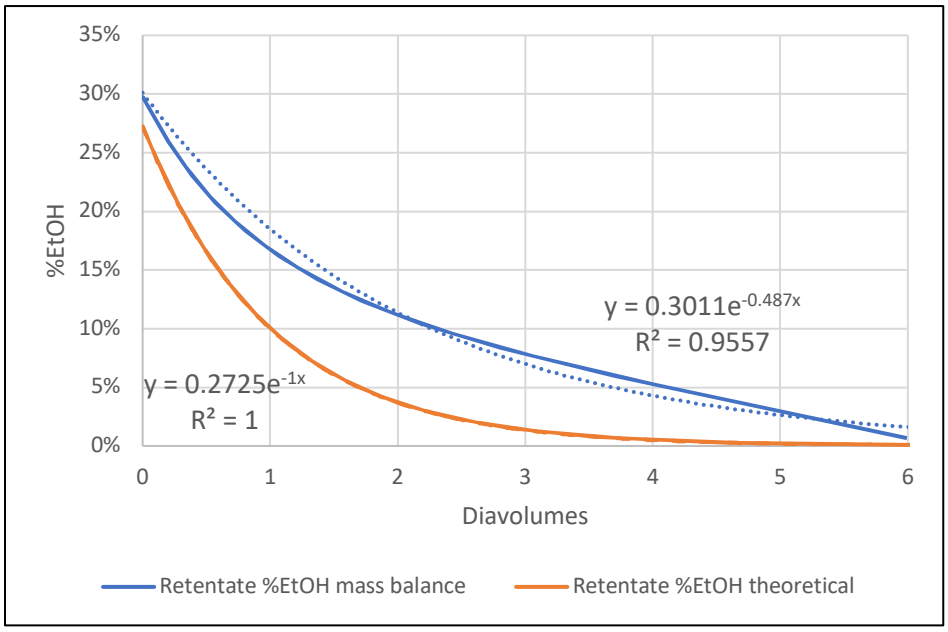

Figure 3.5 Ethanol (EtOH) concentration of batch retentate vs. diavolumes as compared to a theoretical diafiltration curve with α=0 rejection coefficient. The actual batch process rejection coefficient for EtOH calculates to approximately 0.5.

Previous studies have found that the presence of ethanol can impact the performance of diafiltration and that dilution of ethanol increases permeate flow (Jaffrin et al.1994, Paulen et al. 2011). The specific cause in these cases was not determined, but additional studies from other industrial applications have shown that ethanol can cause hollow fiber swelling, negatively impacting permeability (Otitoju et al. 2017, Kochan et al. 2009). Ethanol causing hollow fiber swelling and reducing permeability would explain the results for the ethanol rejection coefficient and permeate flow behavior. The hollow fiber swelling/reduction in permeability also appears to be a function of the ethanol

concentration, given the changes in the ethanol rejection coefficient as the retentate ethanol concentration decreases.

The ethanol concentration reducing the permeate flow rate only impacts the duration of the diafiltration step in the batch process. The total amount of buffer (six diavolumes) is required whether the permeate flow starts at a faster or slower rate. Additionally, the same equipment can be used whether the permeate flow is faster or slower.

3.3.2.2 Impact to Continuous Diafiltration

While not having a significant impact on the overall batch process, the ethanol related impact on permeate flow rate is interesting to consider in a continuous diafiltration system. To support continuous manufacturing, the batch process diafiltration would require reconfiguration. As stated in Section 1.4.1, the process could be reconfigured similar to Figures 1.3, 1.4, 1.5, and 1.6. The most likely candidate would be Figure 1.6, consisting of a series of single pass hollow fiber units with buffer replenishment/dilution in between each unit.

In an ILDF configuration such as Figure 1.6, the initial hollow fibers passes/stages of the system would be exposed to higher concentrations of ethanol and, based on the findings in the previous Section, would be less efficient/permeable than the later passes/stages. By having this diminished performance on the first few hollow fiber units, more passes would be needed to remove the first portion of ethanol as compared to later portions and more units overall would be necessary in order to support the equivalent buffer exchange/ethanol reduction in the ILDF system. This directly impacts

capital/running costs and complexity of the system. Addressing the impact of the ethanol on the permeate flow rate would be prudent.

Given this, additional upfront dilution of the initial liposome generation mixture should support increased permeability and efficiency within each ILDF pass/stage and reduce the overall amount of passes/stages needed in a continuous design.

3.3.3 Additional Considerations for a Continuous Liposomal Drug Product Process

Liposomal drug product manufacturing has various other aspects to consider when designing a process, continuous or otherwise. Due to the use of organic solvent (ethanol) as a raw material, materials of construction for the process equipment need to be considered. Ethanol presents a risk with respect to extractables/leachables when using of plastic or polymer derived single-use componentry. This risk will be exaggerated in a continuous design due to the expected use of larger amounts of organic solvent and longer process duration times. Stainless steel equipment or similar should be used whenever possible.

Another consideration is sterility assurance. Many aspects of the process are combined to build a robust sterility assurance package. The batch process referenced uses equipment that is designed to be cleaned-in-place (CIP), steamed-in-place (SIP), and a closed system design with sterilizing filters at all openings in the process boundaries. Also, the anticipated longer process durations will also increase risk in maintaining sterility assurance and integrity of process boundaries. Any new or modified equipment

that will support a continuous process will need to consider these requirements before implementation.

3.4 Conclusions

The batch process has an inherently continuous unit operation in the cross-flow liposome generation step. A continuous process could leverage the cross-flow liposome generation step with modification to the diafiltration steps. This could come in the form of ILDF or similar designs (Figures 1.3-1.6). Consideration would need to be given to the impact of ethanol concentration on permeate flow, materials of construction and sterility assurance. In the next chapter, the ethanol concentration dependency phenomenon will be further explored regarding its impact on the continuous ILDF arrangement.

Chapter 4

Continuous Diafiltration – ILDF

4.1 Introduction

In this chapter, continuous diafiltration of the previously described liposomal formulation will be explored, specifically in relation to the impact of the ethanol concentration on the diafiltration hollow fiber permeability. The results are compared to the commensurate batch process design. Per the previous chapter, the ethanol concentration in the retentate is suspected of having an inverse relationship on permeability of the hollow fiber cartridges. It is theorized that dilution of the ethanol in the initial liposome generation mixture will increase the hollow fiber permeability which will decrease the number of ILDF passes/stages needed to remove the ethanol, increasing the overall efficiency and benefits of the design.

One of the challenges with exploration of continuous diafiltration is scale. Full scale experimental runs, equivalent to the corresponding batch design can be expensive with respect to time, materials and resources. Conversely, small scale embodiments of continuous diafiltration may not be fully representative and introduce or mask other issues/variables. A case in point is the Pall Cadence™ In-line Diafiltration (ILDF) system (Pall Corp., Port Washington, NY, USA). The Cadence™ ILDF system essentially represents the continuous diafiltration concept as shown in Figure 1.6 but is more accurately represented in Figure 4.1. The Cadence™ ILDF system acts as a multistage single-pass tangential flow filtration (SPTFF) system with buffer supplied in between each stage, all contained within a single module or unit. Each stage concentrates the retentate through removal of the permeate, followed by a dilution with buffer prior to the

next stage. The buffer stream is fed through a manifold to the dilution points between each step and the permeate is conversely collection in a manifold into a single outlet stream.

While this embodiment of continuous diafiltration has advantages of convenient size and availability, there are disadvantages with respect to being representative of the potential full scale unit operation and the ability to explore of the impact of ethanol concentration on performance. First, the membrane material and pore size differ from the batch process hollow fiber membrane, which would presumably be used in a full scale continuous diafiltration system. The Pall Corporation Cadence™ In-line Diafiltration Module Omega (P/N DFOS030T120612) has a polyethersulfone (PES) membrane and a 30kDa pore size. The hollow fiber used in the batch process is the Cytiva model UFP-500-E-85MSM which is polysulfone (PSF) and a 50kDa pore size. It is unknown whether the different material will exhibit the same effect of reduced permeability with ethanol exposure or whether the reduced pore size would further interfere with permeate flow. Second, the manifold design of the system for the buffer feed and permeate outlet do not allow for assessment of the retentate and permeate for each pass or stage of the ILDF set up. Proper evaluation of a continuous diafiltration design will require assessment of the retentate and permeate between each pass/stage pre and post dilution with additional buffer solution.

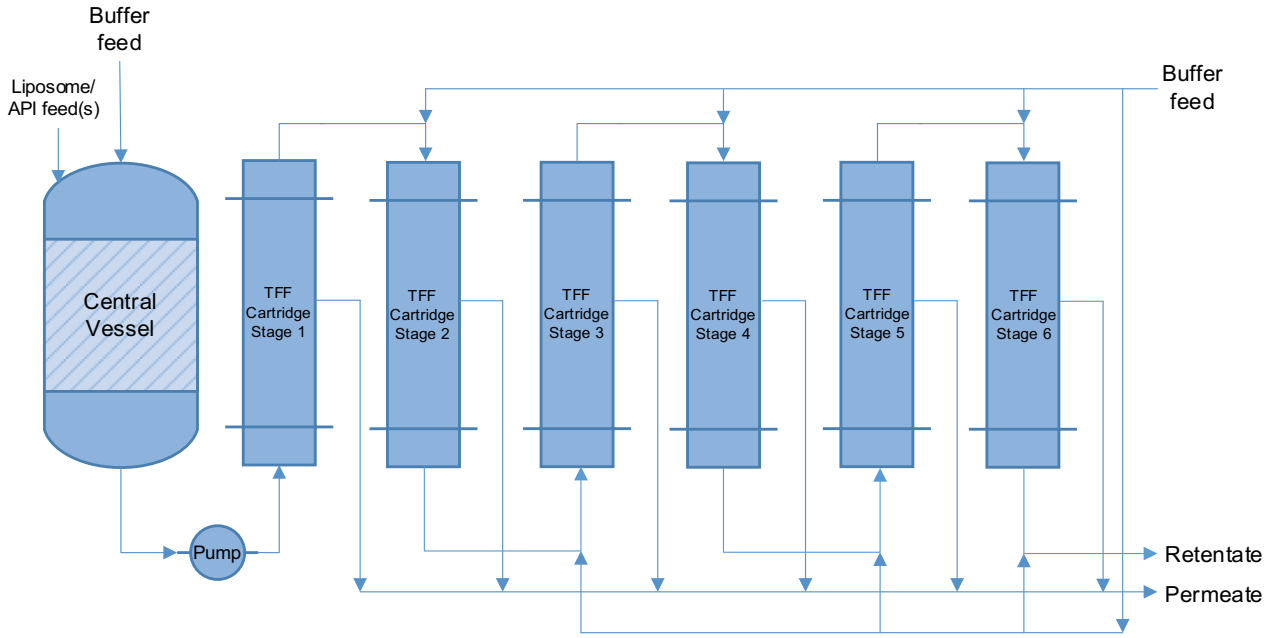

Figure 4.1 Process Flow Diagram of the Cadence™ ILDF system.

In order to allow for such an assessment, a pilot-scale system mimicking Figure 1.6 was explored (Figure 4.2), which is described in greater detail in **Section 4.2**. The set up in Figure 4.2 is designed to evaluate a simulated ILDF configuration by analysing each concentration pass and dilution step individually with the objective of determining the number of passes/stages and the buffer consumption needed to achieve target ethanol removal. Numerous runs were performed using the liposome formation step outflow with various amounts of dilution. This was meant to determine the impact of the ethanol concentration on the number of passes/stages required for ethanol removal (**Section 4.3.1**). The results include an assessment of the permeability performance and buffer consumption and comparison to the batch process (**Section 4.3.2**). Conclusions for the Chapter are contained in **Section 4.4**.

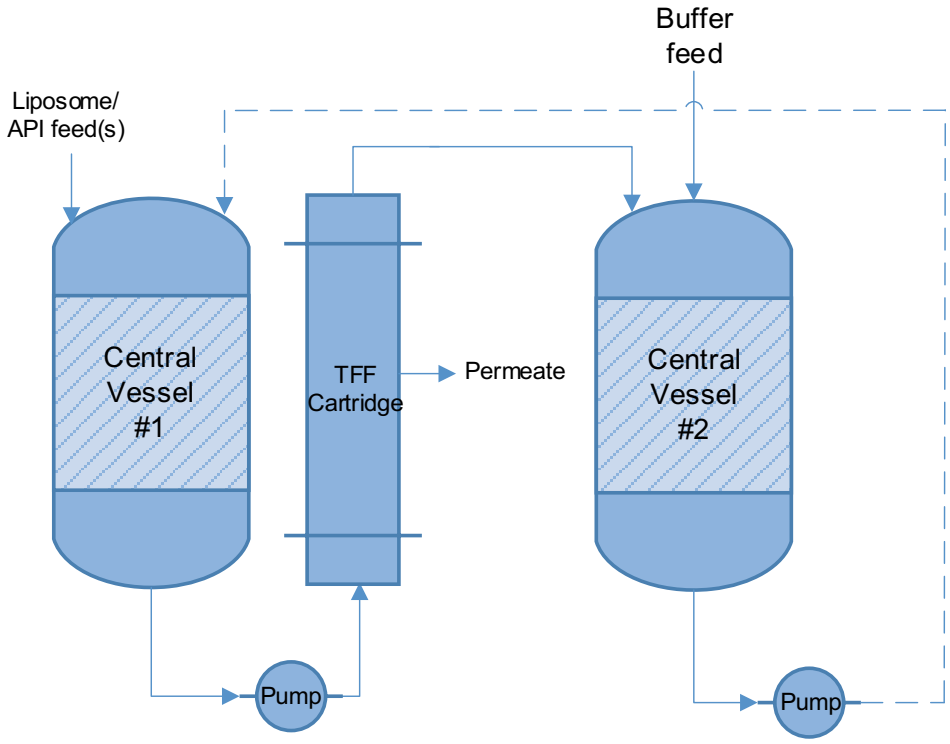

Figure 4.2 Simulated ILDF Set-up: Process Flow Diagram of the single-pass concentration/buffer exchange, which can be used to simulate ILDF with the ability to analysis retentate/permeate in between passes/stages. Solution vessels and liposome formation equipment not shown. The dashed line indicates the bulk is not returned to Central Vessel #1 until after analysis/dilution of its entirety.

4.2 Methods

4.2.1 Simulated ILDF System

The simulated ILDF system consists of the same or similar equipment used in the batch process and specifically the 50kDa MaxCell hollow fiber cartridge (Cytiva, Marlborough, MA, USA) model UFP-500-E-85MSM hollow fiber. The equipment was arranged in a manner supporting multiple single pass tangential flow filtration (SPTFF) with opportunity for sampling and dilution with buffer in between SPTFF passes (Figure 4.2). The hollow fiber cartridge was not replaced in between passes. A new hollow fiber was used for each run on the simulated ILDF system.

4.2.2 Feed Solutions/Liposome Mixture

The starting solutions and undiluted liposome mixture as described in Chapter 2 were used as a starting material. The buffer solution as described in Chapter 2 was used to pre-dilute the liposome mixture as described in the Results Section.

4.2.3 Analytical Methods

Analytical methods as described in Chapter 2 were used to determine the ethanol concentration.

4.3 Results and Discussion

4.3.1 Continuous Inline Diafiltration

4.3.1.1 Hollow Fiber Passes and Ethanol Rejection Coefficient

Continuous ILDF was explored using the arrangement shown in Figure 4.2 to mimic the arrangement in Figure 1.6, but with the ability to analyze the output of each pass/stage. The entirety of the bulk of the liposome formation mixture was fed through the hollow fiber as a single pass into the second vessel. The retentate and permeate were analyzed, buffer added to replace the permeate, then the entirety of the adjusted bulk returned to the first vessel in preparation for another pass. This was repeated until the target ethanol removal was achieved (<1% ethanol in the retentate). The intent was to simulate the arrangement in Figure 1.6 with the ability to assess the output of each pass/stage discretely.

The assessment of the continuous ILDF arrangement involved processing the post-liposome formation bulk mixture as described in Chapter 3 with various levels of pre-dilution from undiluted (36% EtOH) to significantly diluted (5% EtOH). The ethanol concentration was assessed after each pass/stage.

Figures 4.3 shows the results of the runs using various initial ethanol concentrations and the ethanol removal curves over the repeated SPTFF passes. As expected, ethanol concentration was reduced with each SPTFF pass and the amount of SPTFF passes needed to remove the ethanol decreased with the initial ethanol concentration. For example, an initial concentration of 24% required 36 passes for target removal while starting at 5% EtOH required 9 passes.

The behavior of the curves followed the pattern of the diafiltration model (Equation 3.2) with passes in place of diavolumes. Instead of

$$c = c_0 e^{-(1-\sigma)\frac{V_b}{V_s}} \quad \text{(Equation 3.2)}$$

where c_0= initial component concentration, c= final component concentration, V_b = the volume of the buffer added, V_s = the constant volume value, α = the component rejection coefficient and the ratio $\frac{V_b}{V_s}$ is the number of diavolumes (Foley 2016). A continuous ILDF equation can derived where c_0= initial component concentration (%), c = final component concentration (%), N_p = number of simulated ILDF passes and α = component rejection coefficient. The component being ethanol in this case.

$$c = c_0 e^{-(1-\alpha)N_p} \quad \text{(Equation 4.1)}$$

A given component would be expected to have a constant rejection coefficient (α), but as shown in the Figures 4.3, the ethanol rejection coefficient increased with decreasing initial ethanol concentration. This supports the notion from Chapter 3 of an ethanol concentration dependent rejection coefficient.

The ethanol rejection coefficient can be expressed as a function dependent on the initial ethanol concentration, $\alpha = f(c_0)$. This function can be derived from the results in Figures 4.3 as shown in Figure 4.4 and represented with Equation 4.2.

$$\alpha = f(c_0) = 0.059 \ln(c_0) + 0.99, \{0 < c_0 < 0.4\} \quad \text{(Equation 4.2)}$$

Equations 4.1 and 4.2 have a high degree of fit (R^2) and predict the outcome of the Figure 4.2 arrangement with respect to a continuous ILDF design.

Initial Concentration %EtOH	No. of Passes to <1%EtOH/Target Density	Fitted Equation	SPTFF "Rejection Coefficient" (α)	Coefficient of determination (R^2)
36%	53 (extrapolated)	$y = 0.35e^{-0.051x}$	0.949	0.995
24%	36	$y = 0.30e^{-0.088x}$	0.912	0.979
15%	24	$y = 0.17e^{-0.117x}$	0.883	0.994
7.5%	13	$y = 0.08e^{-0.166x}$	0.834	0.998
5%	9	$y = 0.05e^{-0.176x}$	0.824	0.999

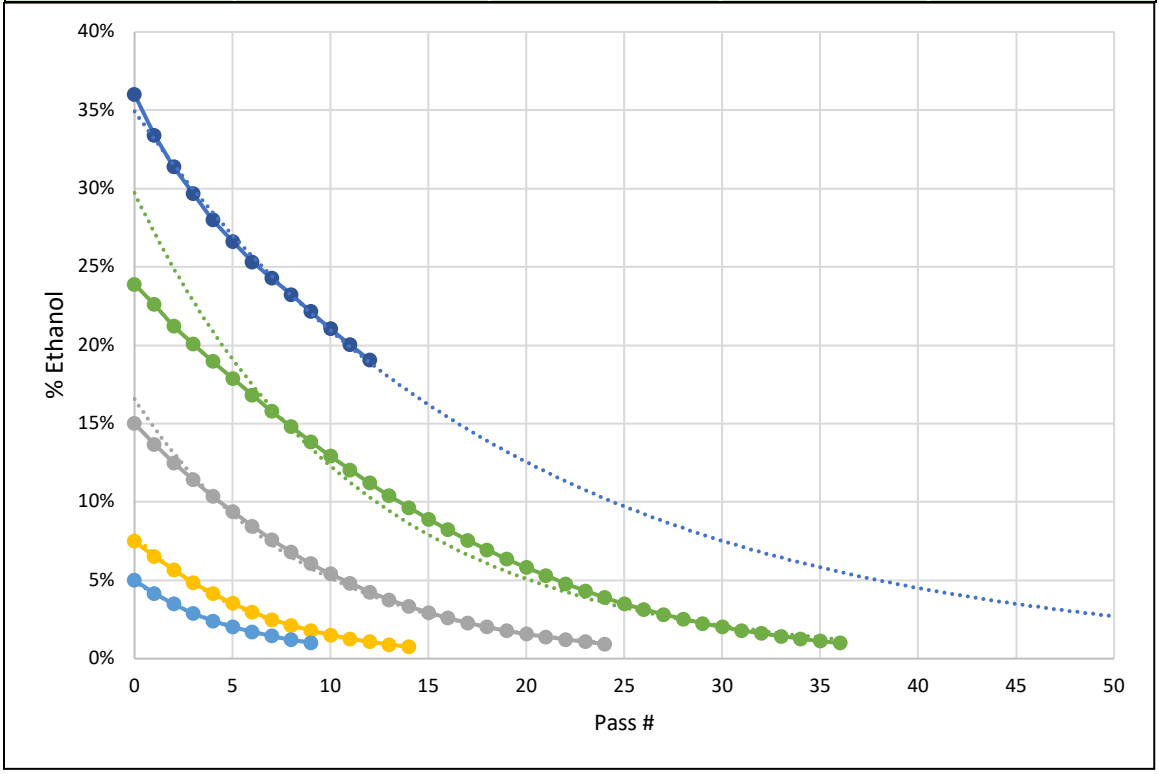

Figure 4.3 Ethanol removal curves and SPTFF total passes required to meet ethanol removal target for various starting ethanol concentrations. Decreasing initial ethanol concentration reduces the SPTFF passes required in a pattern similar to the diafiltration equation (Equation 3.2).

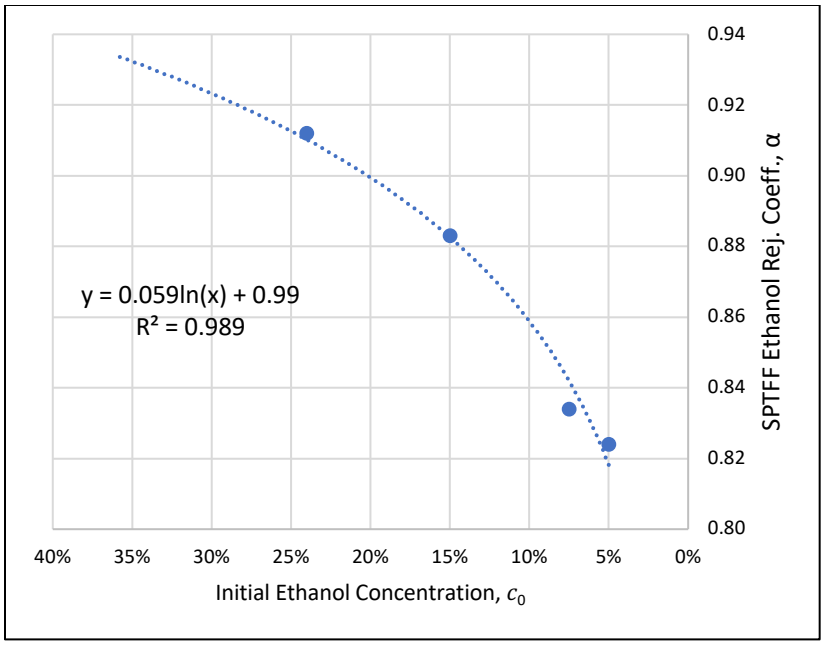

Figure 4.4 Calculated function for the ethanol concentration dependent rejection coefficient for the continuous TFF simulation in Figure 4.2 derived from the data in Figure 4.3. (Note the decreasing x-axis in order to maintain the left-to-right chronology of the data.)

4.3.1.2 Simulated ILDF Permeate

Before fully applying the above equations to continuous ILDF systems, aspects of the permeate stream should be considered. A further assessment of the Figure 4.2 arrangement with respect to a continuous ILDF design was the collection and assessment of permeate flow and permeate amount (kg per pass) during each of the runs using the various initial ethanol concentrations.

Figure 4.5 shows the amount of permeate collected with each pass for the various initial ethanol concentrations runs (not all are show for visual simplicity). As initial ethanol concentration decreased, the amount of permeate collected increased. Additionally, as the ethanol concentration decreased with each pass, the amount of permeate collected increased and plateaued, but did not reach the same level as the plateaus for other

initial concentrations runs. For example, 24% initial ethanol concentration started at 4.5kg/pass and plateaued at approximately 8kg/pass, 15% initial ethanol concentration started at 7kg/pass and plateaued at approximately 10kg/pass, and 7.5% initial ethanol concentration started at 10.5kg/pass and plateaued at approximately 12kg/pass.

Figure 4.6 shows the permeate flow rates versus the retentate ethanol concentration for each pass for various initial ethanol concentrations runs (not all are show for visual simplicity). Similar to the permeate collected in Figure 4.6, flow rates increased as ethanol concentrations decreased and peak flow rates increased with decreasing initial ethanol concentration. Specifically, 24% initial ethanol concentration started at 4kg/min and peaks at approximately 9kg/min permeate flow while starting with 7.5% ethanol gave 10.7kg/min and peaked with approximately 13kg/min.

It would be expected that a given ethanol concentration would yield a given permeate amount and flow rate, similar to the ethanol concentration/permeate flow rate results in Figure 3.4 (Permeate Flow vs. Ethanol concentration). Instead, when the initial ethanol concentrations of 24%, 15%, and 7.5% were reduced to 5%, for example, the flow rates were 7.5, 9.0 and 11.3kg/min, respectively.

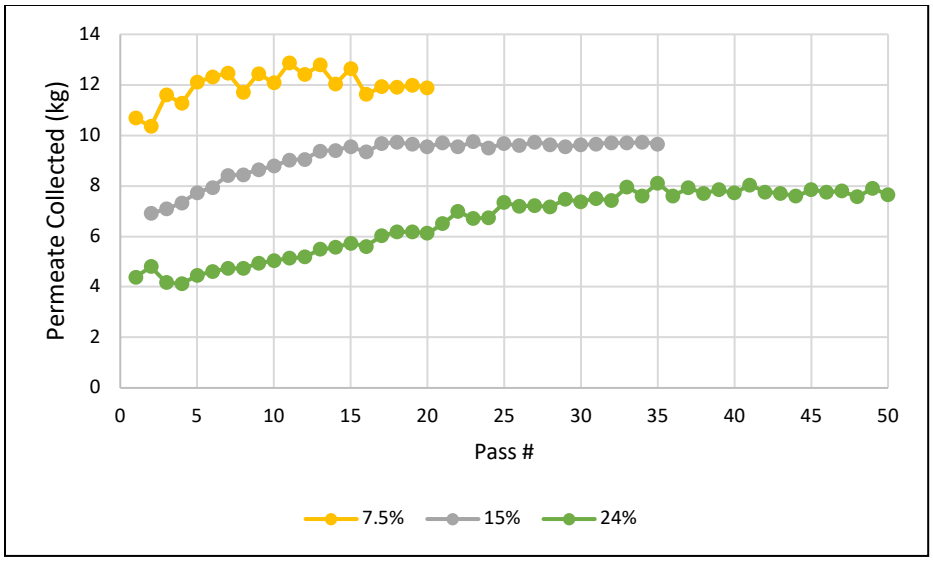

Figure 4.5 Permeate collected from each SPTFF pass for various starting ethanol concentrations runs for the continuous TFF simulation in Figure 4.2.

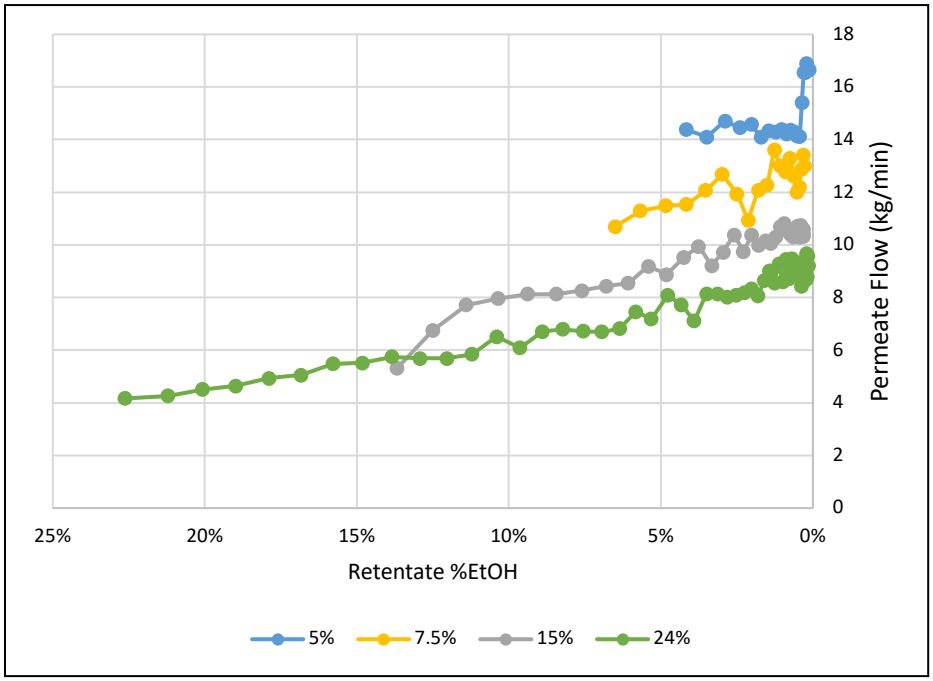

Figure 4.6 Permeate flow rate vs. the retentate ethanol concentration for each SPTFF pass for various starting ethanol concentrations runs for the continuous TFF simulation in Figure 4.2. (Note the decreasing x-axis in order to maintain the left-to-right chronology of the data.)

This indicates that hollow fiber permeability is not only impacted and dependent on ethanol concentration during the process but that the permeability is set by the initial ethanol concentration exposure and only able to recover a limited amount of performance. Stated another way, when the ethanol concentration levels between runs are equivalent in the retentate, it does not correlate to a specific permeability, but rather permeability is set by the initial ethanol concentration and will only improve a relative amount. This aligns with Figure 3.1 (Permeate flow rate vs. Diavolumes). Figure 3.1 captures the plateau effect, but not the effect of the initial ethanol concentration since there is only one initial concentration in that assessment.

Once the hollow fiber was exposed to the initial concentration in the Figure 4.2 set up, the permeability was set and overall performance limited. (Reminder: Each run used a new hollow fiber.) Therefore, the Figure 4.2 set up is not a fully valid representation of a continuous ILDF process. The Figure 4.2 set up used the same single hollow fiber to facilitate all passes/stages for each run, whereas a continuous ILDF process like Figure 1.6 would have separate new individual hollow fibers for each pass/stage. More appropriately, the permeate values for only the initial ethanol concentration passes, should be used and extrapolated; these values being accurate representations of the initial ethanol exposures of some of the independent hollow fibers in a continuous ILDF set up (Figure 1.6).

4.3.1.3 Modelled/Calculated ILDF Stage Requirements

Using the permeate removal data for the initial pass of the five initial ethanol concentrations examined, a continuous ILDF (Figure 1.6) was modelled and the number of passes/stages for the target ethanol removal calculated. Figure 4.7 shows the permeate removed for each of the measured initial ethanol concentration passes with an extrapolated curve/function. This function was used to model each independent pass/stage of a continuous ILDF process until the target ethanol removal was achieved (Figure 4.8). Each pass/stage was modelled using the following equations:

$$c_{p+1} = \frac{c_p R - c_p P}{R} \qquad \text{(Equation 4.3)}$$

$$P = -5.696 \ln(c_p) - 3.8359, \{0 < c_p < 0.4\} \qquad \text{(Equation 4.4)}$$

where c_p= ethanol concentration at the beginning of a pass (%), c_{p+1}= ethanol concentration at the end of a pass (%), R= mass of the retentate (kg), and P = permeate mass as a function of the incoming ethanol concentration (kg). Using Equations 4.3 and 4.4, starting ethanol concentrations were selected and the ethanol concentrations calculated after each subsequent pass/stage. The calculation continued until the target ethanol removal was achieved and the number of passes/stages determined (Figure 4.8).

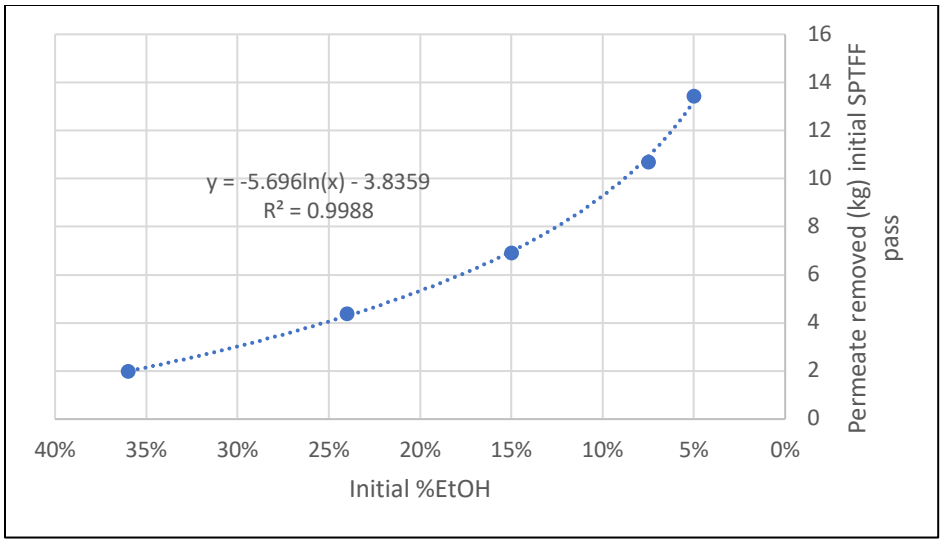

Figure 4.7 Permeate removed at initial ethanol concentration passes and the extrapolated function using the data shown in Figure 4.5. (Note the decreasing x-axis in order to maintain the left-to-right chronology of the data.)

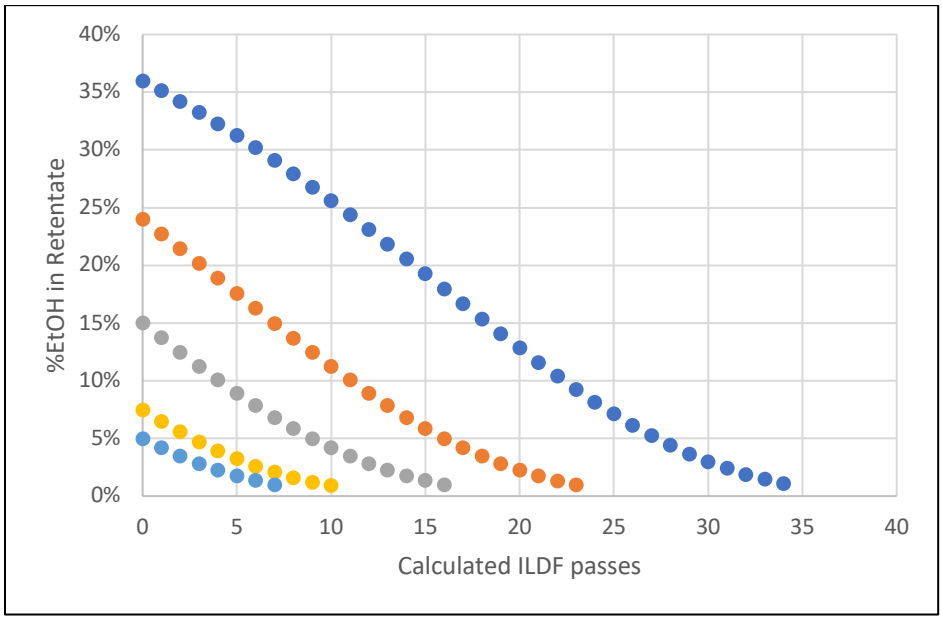

Figure 4.8 Calculated number of passes/stages to achieve target ethanol removal given the initial ethanol concentrations for each independent hollow fiber/pass/stage. Data generated using Equations 4.3 and 4.4.

Table 4.1 Comparison of the extrapolated and simulated amount of required ILDF passes/stages required to achieve target ethanol removal.

Initial %EtOH	Calculated ILDF passes	ILDF passes from Figure 4.3
36%	35	53
24%	23	36
15%	16	24
7.5%	10	13
5%	7	9

The number of passes needed when starting at different concentrations was calculated and are shown in Figure 4.8. Table 4.1 compares the calculated pass data to the pass/stage data determined with the Figure 4.2 set up in Figure 4.3. The modelled/calculated continuous ILDF passes required are significantly less than the Figure 4.3 results, but the difference becomes smaller with lower initial ethanol concentrations. This shows that the impact of carrying through the Figure 4.2 initial ethanol concentration limitation on permeability was mitigated by calculating the passes independently. Note that the continuous ILDF ethanol reduction curve does not follow the diafiltration equation, while the Figure 4.3 results did. This is most likely due to the reuse of the same hollow fibers for each run, which caused the permeability limitations similar to a CVDF batch process. The calculated ILDF model's use of new hollow fibers for each pass/stage leveled out the permeability/rejection coefficient effect into a linear reduction as the permeability of each pass/stage was set independently. Overall, this showed that a continuous ILDF process would minimize any impact of the ethanol rejection coefficient or permeability reduction as compared to the batch CVDF process.

4.3.2 Continuous ILDF Buffer Consumption

Another means of evaluating the continuous ILDF process versus the batch process involves comparing buffer consumption. Buffer consumption, in this case, included the buffer needed for the initial dilution of the starting liposome formation mixture as well as that needed to perform the diafiltration to the target ethanol concentration.

The continuous ILDF buffer consumption was calculated as part of the exercise shown in Figure 4.8. The experimental/calculated permeate was replaced by buffer for each ILDF pass/stage. The total permeate/buffer was summed for each initial ethanol concentration run/model. This total was added to the amount of buffer required for the dilution to the liposome mixture initial ethanol concentration, for a grand total of buffer consumed. These values are summarized in Table 4.2.

Table 4.2 Total buffer consumption for Continuous ILDF based on the calculated model using various initial ethanol concentrations.

	Initial ethanol concentration				
Continuous ILDF	36%	24%	15%	7.5%	5%
Diafiltration (kg)	310.8	261.4	223.9	178.1	139.2
Dilution (kg)	0.0	27.5	48.1	65.3	71.0
Total (kg)	310.8	288.9	272.0	243.4	210.3

For comparison, the total buffer consumption was calculated for the equivalent scale CVDF based batch process using the same initial ethanol concentrations and the diafiltration equation (Equation 3.2).

$$c = c_0 e^{-(1-\sigma)\frac{V_b}{V_s}} \qquad \text{(Equation 3.2)}$$

where c_0= initial component concentration, c= final component concentration, V_b = the volume of the buffer added, V_s = the constant volume value, α = the component rejection coefficient and the ratio $\frac{V_b}{V_s}$ is the number of diavolumes (Foley 2016).

Totals were calculated for two different conditions:

1) an ethanol rejection coefficient of zero (α=0)

2) an ethanol rejection coefficient of 0.5 (α=0.5)

The 0.5 rejection coefficient is representative of the real-world batch process example as described in Figure 3.5. Tables 4.3 and 4.4 show the total buffer requirements for the two comparison batch process conditions.

Table 4.3 Total buffer consumption for calculated for the batch process using an ethanol rejection coefficient of 0.5.

	Initial ethanol concentration				
α=0.5	36%	24%	15%	7.5%	5%
Diavolumes	7.2	6.4	5.4	4.0	3.2
Diafiltration (kg)	591.3	524.4	446.8	332.5	265.6
Dilution (kg)	0.0	27.5	48.1	65.3	71.0
Total (kg)	591.3	551.9	495.0	397.8	336.6

Table 4.4 Total buffer consumption for calculated for the batch process using an ethanol rejection coefficient of 0.

	Initial ethanol concentration				
α=0	36%	24%	15%	7.5%	5%
Diavolumes	3.6	3.2	2.7	2.0	1.6
Diafiltration (kg)	295.6	262.2	223.4	166.2	132.8
Dilution (kg)	0.0	27.5	48.1	65.3	71.0
Total (kg)	295.6	289.7	271.5	231.5	203.8

Figure 4.9 shows the buffer consumption (dilution and diafiltration) for batch process examples with rejection coefficients of 0 and 0.5 compared to the continuous ILDF model. Surprisingly, the continuous ILDF is in line with the zero rejection coefficient batch process. Previous evaluations of similar ILDF set ups in the realm of biologics have shown continuous designs to require more buffer than batch (Kavara, et al. 2020, Jabra, et al. 2019). The Figure 4.9 results again show how the use of independent passes/stages offsets the impact of ethanol on the hollow fiber permeability.

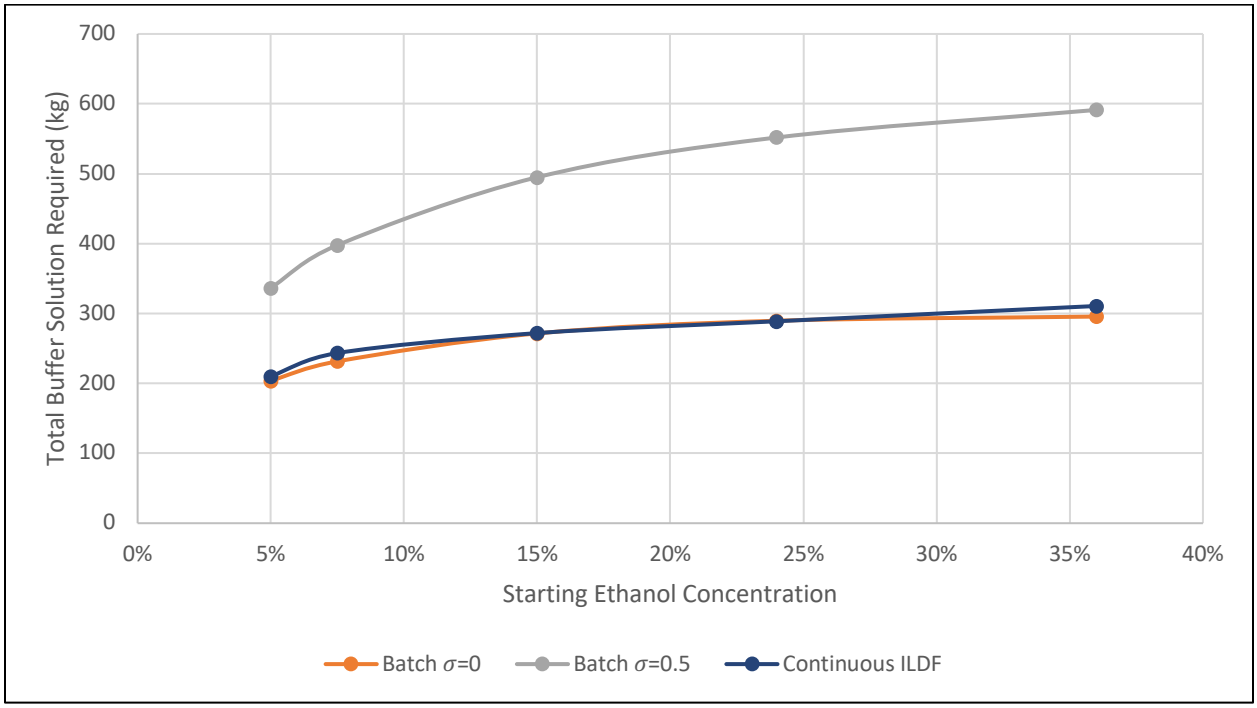

Figure 4.9 Buffer required (Dilution+Diafiltration) to achieve target ethanol removal when using various initial ethanol concentration for theoretical batch processes with rejection coefficient of 0 and 0.5 and a continuous ILDF process as calculated in Table 4.2.

4.3.3 Outlook & Perspectives

The assessment of the continuous ILDF set up for liposomal drug product formulation refinement was successful and provided a range of intriguing results. The notion of the permeate being impacted by the ethanol concentration in the batch process was reinforced by the results from the continuous ILDF experiment(s). The use of the Simulated ILDF Set up (Figure 4.2) showed that reducing of the initial ethanol concentration reduced the number of passes/stages necessary to achieve the target ethanol removal and that the reduction rate was dependent on ethanol concentration. This led to the derivation of an equation (Equation 4.1) for the simulated ILDF concentration/dilution arrangement (Figure 4.2) including the derivation of a function for ethanol concentration dependency of the rejection coefficient (Equation 4.2).

While these equations prove applicable and predictable under the simulated ILDF set up from Figure 4.2, these equations proved less applicable to a real-world set up such as that shown in Figure 1.6. The simulated ILDF method showed that the permeability of the hollow fiber was limited by the initial exposure to ethanol. The permeability then only improved a limited amount as the ethanol concentration decreased with each pass. By using the same hollow fiber to simulate each pass of a ILDF system, the efficiency of each subsequent pass was limited and not representative of a true ILDF arrangement. By using the data from the initial passes of the separate runs, a true ILDF data set was assessed and extrapolated.

The continuous ILDF model showed an almost linear reduction as compared to the exponential reduction in the simulated ILDF model (Figure 4.8 vs. Figure 4.3). This may be because the permeability of the hollow fibers in the continuous ILDF model were set

independently and had no impact on the subsequent hollow fibers as the ethanol concentration decreased. This appeared to smooth the impact of the ethanol dependent rejection coefficient phenomenon. Additionally, the linear reduction of the ethanol in the continuous ILDF model limited the overall buffer consumption to that of a batch process where the rejection coefficient is zero. This showed that continuous ILDF would be more efficient than a solvent-based batch TFF with respect to buffer required.

Based on these findings, the impact of ethanol on the continuous ILDF design is less driven by a traditional rejection coefficient concept and more on ethanol's effect on hollow fiber permeability. By reducing the initial exposure of each individual hollow fiber in a continuous ILDF system, the performance and overall efficiency of the system is improved. The most optimal design for a liposomal continuous ILDF process would involve significant upfront dilution (i.e. 5% initial ethanol concentration) in order to start the ILDF with minimal ethanol concentration. This would minimize the amount of ILDF stages needed and buffer required. This is similar to the dilution strategy recommended for albumin diafiltration though optimization is not specifically correlated to the impact of ethanol concentration on permeability (Jaffrin et al. 1994, Paulen et al. 2011). Additionally, these finding may prove applicable and beneficial to mRNA-LNP vaccines, which often look to minimize mRNA exposure to organic solvents as well as having massive production demands (Hou et al. 2021, Schoenmaker et al. 2021, Verma et al. 2023), which a continuous process design could help to meet.

4.4 Conclusions

Continuous manufacturing designs for liposomal drug products will help meet the demand for these formulations, which are critical for future drug delivery options. Understanding what impacts these continuous manufacturing designs has benefit to their optimization. What is viewed to be a minor phenomenon (i.e. the increasing permeate flow pattern) in a batch process design could have significant impact to a continuous design. The deduction of an ethanol concentration-dependent rejection coefficient in the batch process has led to a greater understanding of how ethanol concentration will impact a continuous TFF/ILDF process. Ethanol reduces and limits hollow fiber permeability with the impact increasing with concentration. It was determined that by diluting the post-liposome formation bulk and reducing the initial ethanol concentration that fewer passes/stages of ILDF (5% retentate ethanol concentration requiring 7 ILDF stages versus 35 stages needed for 36% ethanol) and less buffer the commensurate batch design would be needed to achieve target ethanol removal (At 5% initial ethanol concentration, 210kg of buffer for continuous versus 337kg for batch). In understanding this impact, continuous ILDF presents as a competitive alternative to the batch process that can be scaled at will.

Chapter 5

Economic Assessment of Batch vs. Continuous Design

5.1 Introduction

Given the established feasibility and operational advantages from the previous chapters, an economic assessment of a continuous liposomal process design is prudent. Current manufacturing processes for liposomal drug products are designed as batch processes with few options for commercial scale (Worsham, et al, 2018). In order to increase scale or output of these few viable processes, the batches must be produced more frequently, additional manufacturing lines must be installed, and/or larger equipment/capital investments for scaled up processing must be made. Depending on the combination of options employed, the impact to Cost-of-Goods (COG) can be detrimental. Additionally, there may be limitations to the options being leveraged. Facilities have space, capacity, and utilization limitations and equipment has limited scale, particularly for aseptic/pharmaceutical grade systems. A means of overcoming these limitations is the employment of the concept of continuous manufacturing. Continuous manufacturing has shown economic benefit in other applications such as solid oral dosage manufacture (Kleinebudde et al., 2017) and monoclonal antibody manufacture (Subramanian et al., 2015, Mahal et al., 2021; Mahajan et al., 2012; Pollock et al., 2013a, 2013b, 2017; Walther et al., 2015). Detailed economic assessments related to selection of batch or continuous technologies for oral solid dosage forms have been performed, which take into account numerous factors internal and external to the manufacturing process (Matsunami et al., 2018, Rossi, 2022).

Reviews or assessments of the economics of liposomal drug product manufacturing are limited as there are few commercial scale products or processes to assess (Justo et al., 2010; Trucillo et al., 2020). Additionally, processes and process costs can be very product specific as meeting the needs of the end drug product (solubility, particle size, stability, etc.) may be dictated by manufacturing technique. Therefore, limited optimization options from a financial perspective are available. For example, a supercritical fluid liposome formation technique and the associated economics (Trucillo et al., 2020) may not be applicable to a different drug product or process with other requirements/applications. Thus, any economic assessment performed on a liposomal drug product manufacturing process will be very case specific, but some recommendations, particularly with respect to batch versus continuous processing, may be more universally applicable.

In this chapter, the liposomal drug product manufacturing process, described in the previous chapters will be assessed and compared as the real-world batch design and with various implementations of continuous process design scenarios. Previously, this process has shown promise as a liposomal drug product process that can be converted from a batch to a continuous design to enable higher productivity in terms of amount of vials/doses produced per time (Worsham, et al, 2018). The assessment is intentionally kept to a basic premise to maintain confidentiality of the real-world commercial example. The assessment does not provide for variation of the individual components within the economic calculations or influence of external factors on the manufacturing process such as location related economic inputs. The focus was economic shifts specifically related to batch process design versus continuous design. The scenarios were assessed with respect to COG over a range of production demands, capital investment,

inherent risks and recommendations for optimization/implementation. **Section 5.2** describes the methods for the economic assessment including details of the process designs. **Section 5.3** provides the description of the specific scenarios used as case studies for the comparisons and evaluations. The results and discussion for each of the assessments of the scenarios are provided in **Section 5.4**. Which scenario should be selected and when is described in **Section 5.5** with final conclusions and recommendations contained in **Section 5.6**.

5.2 Methods/Assumptions

5.2.1 Modelling methodologies/approach

5.2.1.1 COG Model and Cost Data

Cost of goods (COG) values were calculated using an in-house model created in Microsoft Excel (version 2311). The calculation for COG involved annual direct (variable) and indirect (fixed) costs divided over the annual production demand. Annual production demand is calculated as total number of filled vials (1 vial = 1 dose). The direct costs include Reagents (chemical raw materials for production, cleaning, etc.), Consumables (e.g. filters, tubing, vials), and Labor (variable labor for production, in-process and release testing, waste disposal, utilities generation, etc.). The indirect costs include Depreciation of initial fixed capital investment (FCI) and ongoing annual capital investment (ACI) as well as Other (annual maintenance, calibrations, requalifications). The annual direct costs are dependent on the batch size/run time and number of batches required to fulfill the annual production demand. The annual indirect costs are

fixed with activities such as maintenance being dictated on schedule related to equipment usage. Table 5.1 summarizes the calculation.

Table 5.1 Summary of COG model

COG calculation		
Cost Category		Equation
Direct	Reagents	Per batch raw materials x Annual batches
	Consumables	Per batch consumables x Annual batches
	Labor	Tiered per dose cost x Batch size x Annual batches
Indirect	Depreciation	10% x (FCI + ACI) annually
	Other	Fixed annual costs to maintain production and compliance
COG		(Direct + Indirect)/annual production in vials

Direct and indirect costs for the batch process design scenario were based on a real-world example. These include real-world pricing data, resource planning, and facility utilization. For the scenarios involving the theoretical continuous process design, the batch process design scenario costs were extrapolated or manipulated as needed. For purpose of confidentiality, the COG values for each scenario are presented in relative monetary units (RMU/vial).

5.2.1.2 Capital Investment Assumptions

Capitalized spend includes all process and utility equipment, build out of an existing facility (not greenfield), and tech transfer activities to achieve a validated state ready for commercial production. The capital investment for the batch design is also based on a

real-world example. For purpose of confidentiality, the other design options are normalized to the batch design capital cost.

The continuous design capital expense includes the same aspects and uses the real-world batch design costs as applicable and extrapolated as necessary. As compared to the batch design, the continuous design would require changes such as additional hollow fiber housings for the inline diafiltration configuration and tankage modifications. Additional materials and components are also needed to produce engineering and validation batches at the high end of the run duration (240k vials).

The convertible model capital expense is portioned from the batch and continuous design capital expenses. Both process configurations need to be fully equipped and installed. Production runs would be needed for engineering and validation of both the batch and continuous designs.

5.2.1.3 Qualitative Risk Assessment Method

As a means of comparing the more intangible aspects of the process designs, a qualitative risk assessment was performed. Risk around aspects such as implementation, operation, and economics were relatively assessed at various demand levels for the batch and continuous process designs. The risk levels are shown qualitatively using a three-level scale (Low, Medium, High). The separate aspects were evenly weighted and an overall risk comparison was generated. The method for determination of the risk levels was subjective and is meant to illustrate the need to capture aspects of the process design selection beyond the economic factors of more or less costs.

5.2.2 Case Study Setup

5.2.2.1 Process Designs

The process designs consist of two independent options. A batch process option based on a real-world design and a continuous design option, using similarly scaled equipment to the batch design, but arranged to allow unit operations to occur simultaneously/continuously. A third option allows for either of these options to be selected independently within the same system.

5.2.2.1.1 Batch Process

The batch process for manufacturing liposomal drug product, as depicted in Figure 5.1A, involves mixing/infusing streams of lipid solution and API solution at the crossflow point in an approximate 1:2 ratio. The output of the crossflow point is fed into a central vessel concurrently with the buffer solution. Constant volume diafiltration is performed using 50kDa hollow fiber cartridges (Cytiva model UFP-500-E-85MSM, Marlborough, MA) until six diavolumes have been processed. The product is then concentrated through the hollow fiber membrane until the retentate achieves the target final product API concentration. The bulk is then aseptically filled into 10mL vials as the final drug product unit. Each vial is equal to one dose of the product. Note: the process is run aseptically once the starting solutions have passed through the sterilizing filters.

5.2.2.1.2 Continuous Process

The continuous process is similar to the batch process until the diafiltration step. As depicted in Figure 5.1B, the diafiltration consists of 50kDa hollow fiber (HF) cartridges (Cytiva model UFP-500-E-85MSM, Marlborough, MA) set up in series similar to an in-line diafiltration (ILDF) arrangement where buffer can be added between each HF stage/pass to replace the permeate. The specific amount of HFs/steps was based on the results of Chapter 4 and is significantly more than the batch design uses (Worsham et al., 2023). This continues until the drug product formulation targets, similar to the batch process formulation targets, are achieved. Similarly, the final drug product is aseptically filled into 10mL vials (one dose each). All these steps are performed simultaneously and in a continuous fashion until the target output of filled vials are produced.

5.2.2.1.3 Convertible Process

The Convertible process, Figure 5.1C, has both the batch design and the continuous design as an option and can be used in either configuration depending on production requirement tradeoffs. In this design the batch and continuous designs are isolated/separated at the three-way valve just before the hollow fibers. Selecting the flow in upward direction of the diagram provides the batch process option shown in Figure 5.1A. Selecting the flow through option for the valve provides the continuous process option shown in Figure 5.1B. Only one option can be chosen/used per run. They cannot be used simultaneously.

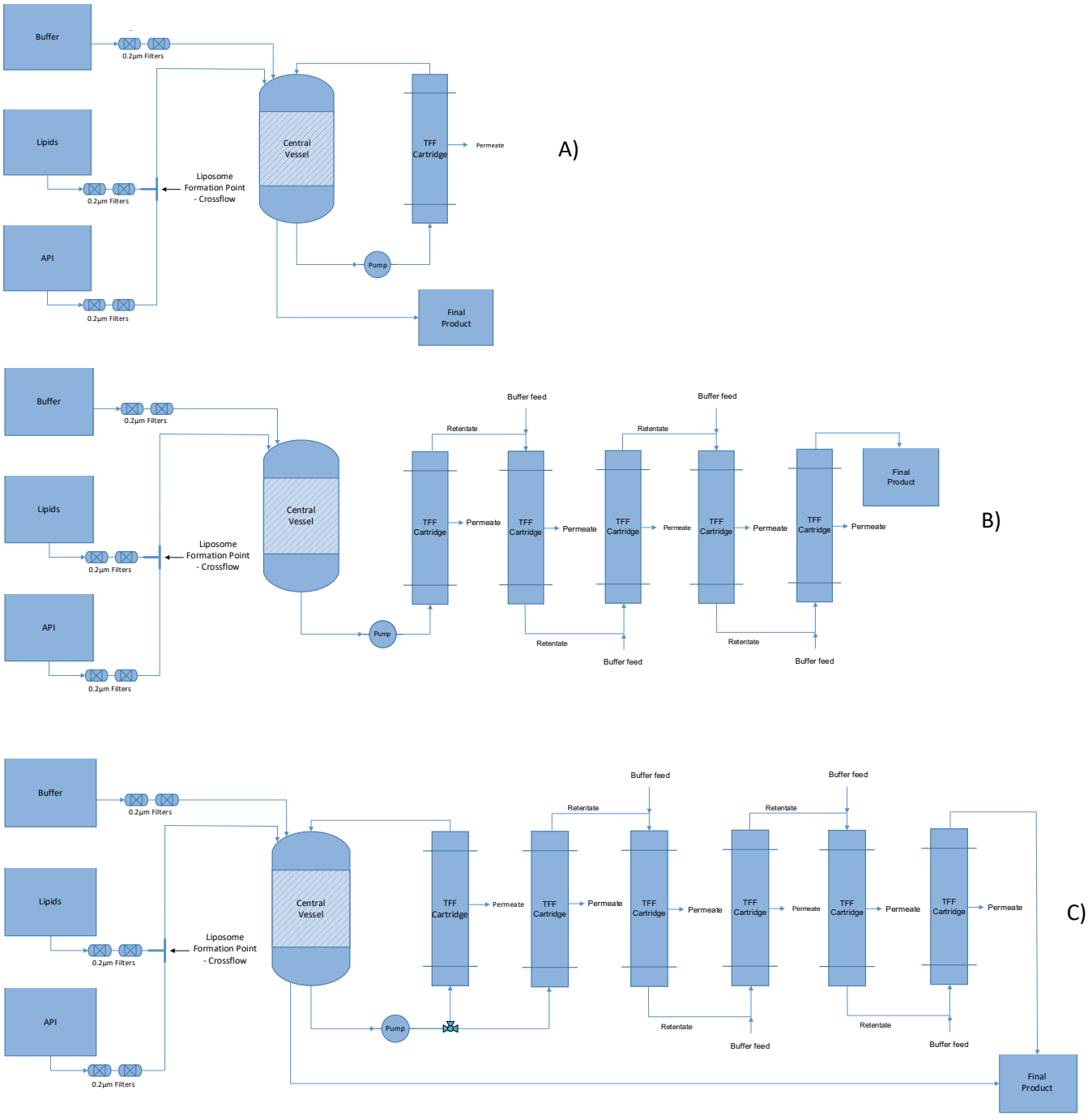

Figure 5.1 Liposomal Drug Product Manufacturing Process Flow Diagrams:
A) Batch Design, B) Continuous Design, C) Convertible Design where both batch and continuous flow path options exist.

5.2.3 Scenarios

Three different scenarios using the process designs were assessed over a range of annual production demands. The cleaning-in-place (CIP), steam-in-place (SIP), and production times are fixed. The amount of filled drug product vials or doses produced by the batch process is fixed at 40,000, consistent with the real-world example. The total batch time is 48 hours including prep, formulation, and filling (Figure 5.2). Due to the fixed nature of the batch process design, increasing annual production demands requires more batches be produced or additional production lines be added.

For the continuous process design, the CIP and SIP times are fixed, but the production time (formulation/filling) is adjustable depending on the desired output of vials.

Increasing annual production demands can be met by increasing batch size/run time and/or by increasing the number of batches/runs per year. Note: For the continuous design, the term “run” may be used in place of “batch” to avoid confusion with the batch design.

For the purposes of this examination, the total run time was limited to 48 hours, similar to the batch design (Figure 5.2). This limit was chosen in order to maintain some equivalency of validation requirements, particularly aseptic process validation, to the batch design. This produces a maximum output option of 240k vials per run. The continuous process design could, in theory, be run for much longer times, but additional risks, which are not specifically examined in this chapter should be considered. These risks include the ability to maintain limited bioburden in unfiltered starting solutions as well as significant loss of product if a single long production run has an issue.

	Batch	Continuous
Total Batch duration/time (hr)	48	48
Maximum Production rate (vials/hr)	1.7k	10k
Maximum batch/run size (vials)	40k	240k
Maximum Total Annual Capacity (vials)	10M	35M

Step	Net processing time – Batch
Prep (Assembly, CIP/SIP, etc.)	
Solution prep	
Liposome Formation	
Buffer exchange	
Filling	

Step	Net processing time – Continuous
Prep (Assembly, CIP/SIP, etc.)	
Solution prep	
Liposome Formation	
Buffer exchange	
Filling	

Figure 5.2 Batch and Continuous design details including assumptions standardizing batch durations, corresponding run sizes and capacities as well as a visualization of the serial and parallel unit operations for the batch and continuous designs, respectively.

The scenarios examined are as follows:

1. Batch – The standard batch process design was based on a real-world example (Figure 5.1A). The facility utilization was targeted for 70-80% with shifts being added when utilization was greater than 80%. This scenario has a maximum annual output of 10 million vials/doses (Figure. 5.2).

2. Continuous – The continuous process design (Figure 5.1B) targeted 70-80% facility utilization by adjusting batch size/run time and labor/production shifts. This scenario has a maximum annual output of 35 million vials/doses (Figure 5.2).

3. Convertible – A convertible process design (Figure 5.1C) where a batch or continuous design can be employed depending on what is most advantageous for the demand level. Either can be selected to fulfill the demand, but they are not used simultaneously. This scenario takes into consideration facility utilization. The process option (batch or continuous with a specific size/run duration) was selected based on the optimal COG while targeting 70-80% facility utilization.

5.3 Results and Discussion

The three scenarios employing batch and continuous process designs were compared using the in-house COG model that captured direct and indirect costs. General trends as well as aspects of the COG composition, capital investment, and risk are assessed.

5.3.1 COG vs. Demand

The COG assessment sought to compare and determine whether the various design options would prove advantageous over a range of demand levels. Figure 5.3 shows the COG for the three scenarios: Batch, Continuous, and Convertible, over a range of demand (1M to 15M annual vials). The assessment showed that a Batch scenario is favorable until the demand surpasses a certain threshold (approx. 5M annual vials), beyond which the continuous design driven scenarios (Continuous/Convertible) become favorable. Additionally, at even greater demand levels (10M+ annual vials), the

continuous driven options are highly favorable due to the higher capacity without the need of additional production lines.

The range of demand was selected because the batch design has an upper end limitation of 10M annual vials based on a 24/7 80% facility utilization operation while the continuous design has a theoretical limitation of 35M annual vials at a 24/7 80% facility utilization operation. The 1-15M range best illustrates the differences between the scenario/design options. As expected, the COGs decrease as annual demand increases, but each scenario presents some specific behaviors.

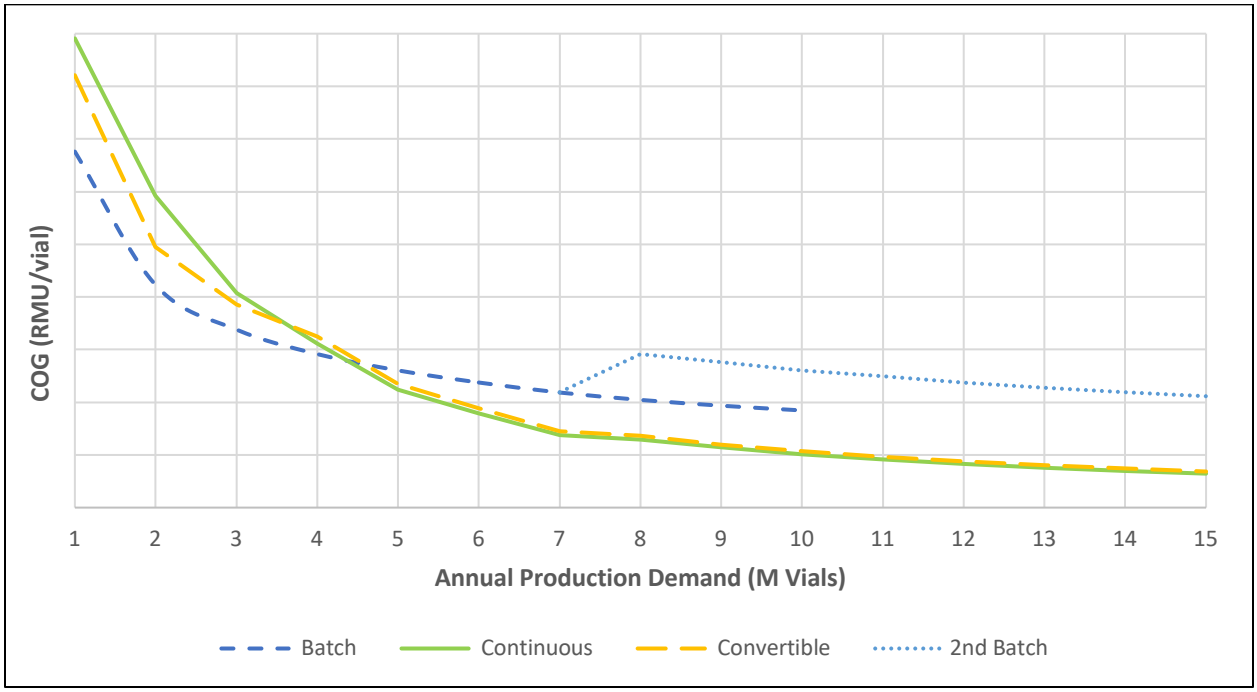

Figure 5.3 COG (RMU/vial) for the Batch, Continuous and Convertible scenarios, over a range of demand (1M to 15M annual vials). RMU = relative monetary units.

The Batch scenario is the lowest cost option through 4M vials and the highest starting at 5M. The batch process is also limited in overall capacity as it reached 80% utilization of

a 24/7 operation at 10M annual vials. Increasing beyond this would require additional production lines or facilities. Figure 5.3 shows the addition of a second batch operation once the 70% capacity is reached for the first batch operation.

The Continuous scenario has the highest COG (16% higher than Batch at 1M vials) until beyond 4M annual vials. The COG is higher compared to the Batch design at lower production volumes due to the increased Consumable and Depreciation costs per vial for the continuous design (Section 5.3.2). As demand increases, these costs are divided over more vials, decreasing the COG.

By slowly increasing batch size/run time, the continuous process meets demand and maintains 70-80% facility utilization using one shift. At 9M annual vials, a second shift is required. The Continuous process could theoretically achieve 35M annual vials at 80% facility utilization and a 24/7 operation.

The Convertible scenario uses the Batch process option through 3M annual vials and the Continuous process option for greater. The Convertible scenario used in batch mode has a 11% higher COG than the purely Batch scenario at 1M annual vials. Once the continuous option is employed, the COG closely follows the Continuous scenario with a slight increase (1%). As will be discussed below, these increases are due to the increased capital investment and depreciation for building and installing both processing options.

5.3.2 COG composition with Demand

The COG composition assessment sought to evaluate the different inputs to the COG calculation and determine which had the most influence on the various design options over the range of demand. Figure 5.4 contains the breakdown of the COG data for each of the three scenarios across the annual demand. The breakdown consists of Consumables, Reagents, Labor, Depreciation, and Other, which includes the annual overhead costs and services. The assessment found that Reagents and Labor inputs were generally fixed regardless of scenario or demand. The Consumables, Depreciation and Other categories diminished as they were spread over the larger demand levels. This further incentivizes the investment into a continuous design driven option when demand is high.

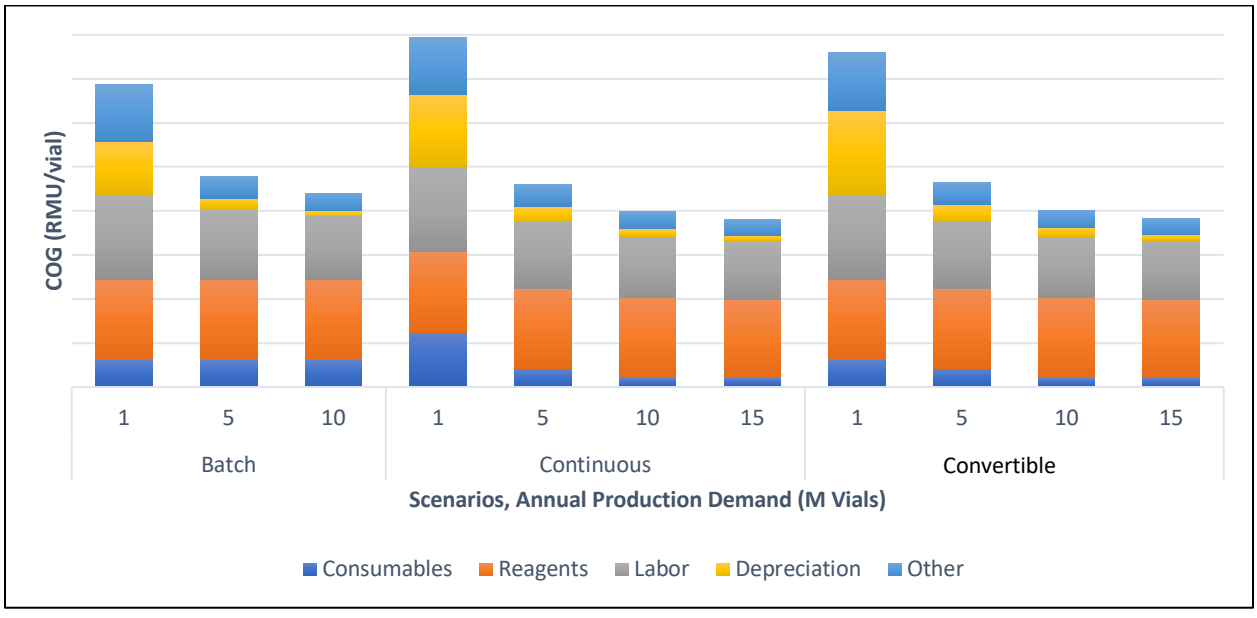

Figure 5.4 COG breakdown comparison for the Batch, Continuous, and Convertible scenarios, over a range of demand (1M to 15M annual vials). RMU = relative monetary units.

The composition of the Batch scenario COG is typical for a batch process. The COG per vial values attributed to Consumables and Reagents are constant regardless of annual demand. Each vial contains the same amounts of materials and uses the same portion of consumables, regardless of demand, since the batch size is fixed. The other components of the COG are spread across the annual demand and thus decrease per vial as demand increases. Labor decreases at a slower rate as there is some increase in total labor as the workload increases.

The Continuous scenario has some fluid elements. The fixed components (Depreciation, Other) decrease as annual demand increases. Note the increased Depreciation component as compared to the Batch, due to the increased Capital costs (Section 5.3.3). Labor costs decrease as a portion of COG and decrease faster than the Batch scenario as batch size/run time increases. The Reagents COG component only decreases slightly as demand and run size increase. Prep Reagent costs per vial will decrease with increased batch size, but the majority of the Reagents consumption is proportional to the batch size and will be constant per vial. The Consumables costs start higher, as compared to the Batch scenario, due to the increased requirement for components such as hollow fiber filters, but quickly decrease as those costs are spread over more vials in the increasing batch size. This cost levels out once maximum batch size/run duration is achieved.

The Convertible scenario is meant to follow the optimal COG for either process design option at the given demand levels. This equates to the batch option in the first 3M annual vials and the continuous option thereafter. The COG values are increased as compared to the individual design scenarios because of the increased Depreciation.

This is due to the capital investment needed to build out and implement both options. This causes a 5-11% increase in overall COG when comparing the Convertible batch option to the purely Batch design, but becomes less of a factor as the demand increases. There is only a 1% increase in the overall COG when comparing the Convertible continuous option to the purely Continuous design.

Each of the categories (Consumable, Reagents, Labor, Depreciation, Other) that constitute the COG contain within them many discrete items and services. For example, Consumables includes all sterilizing filters, hollow fiber filters, vials, stoppers, caps, etc. Pricing/cost fluctuations for any of these individual items could have impact on COG given the magnitude of the increase, but in and of themselves, they constitute a very small percentage of the total COG. Therefore, the COG values are not particularly sensitive to changes in the individual items or services unless the change is unusually significant.

One aspect of the design that should be noted is the quantity of hollow fibers assumed. The batch design consists of a fixed number of hollow fibers and depending on the scale of the process only one is required. The quantity of hollow fibers for the continuous design is dictated by the formulation of the bulk as it enters the ILDF train. Dilution of the bulk will allow the system to run more efficiently and minimize the amount of hollow fibers required (Chapter 4; Worsham et al, 2023). The COG calculation assumes that the system has been optimized and the hollow fiber requirement has been minimized. Operating at a less optimal condition could significantly increase the amount of hollow fibers required, which would have a tangible impact on COG (multiple dollars per vial depending on the system efficiency, demand, etc.).

Another aspect that could have tangible impact on the Convertible scenario, specifically, is the timing of the validation of the different manufacturing options. The Batch option presents as advantageous at low demand with respect to the Convertible scenario COG while the Continuous option is advantageous at higher demand. The crossover could be leveraged in that the Batch option could be implemented and validated first and the Continuous option implemented and validated later when demand is expected to be an advantage for that option. This could defer the continuous design capital costs until a later point, which would reduce the Depreciation component of the COG during the low demand Batch option period. Deferring this cost could reduce the COG by more than 7% during that period. Additionally, once the Continuous option is fully implemented and validated that additional Depreciation cost would be divided over a larger annual demand and be more easily absorbed.

5.3.3 Capital Investment

The Capital Investment assessment sought to compare the aspects of the capital investment for each process scenario (Figure 5.5). The Convertible scenario required the highest investment as it contains aspects of both the other scenarios.

The capital investment for each scenario consists of process and utility equipment, facility build out and tech transfer activities to achieve a validated process. The facility build out involves the retrofit of a space within an existing facility and not a greenfield situation. Another factor to note is that it is assumed that the facility has existing services and systems for operational aspects like quality systems, warehousing, shipping/receiving, etc. This is reflected in the Lang factor (Total Capital Costs ÷ Capital

Equipment Cost = Lang Factor), which is 3-4 for each of the scenarios as opposed to higher factors (4-6) seen in more extensive builds (Wain, 2014). The capital investment described is focused on product specific requirements for process equipment, utilities, and facility modification. The values in Figure 5.5 are normalized to the Batch scenario, which is based on a real-world example.

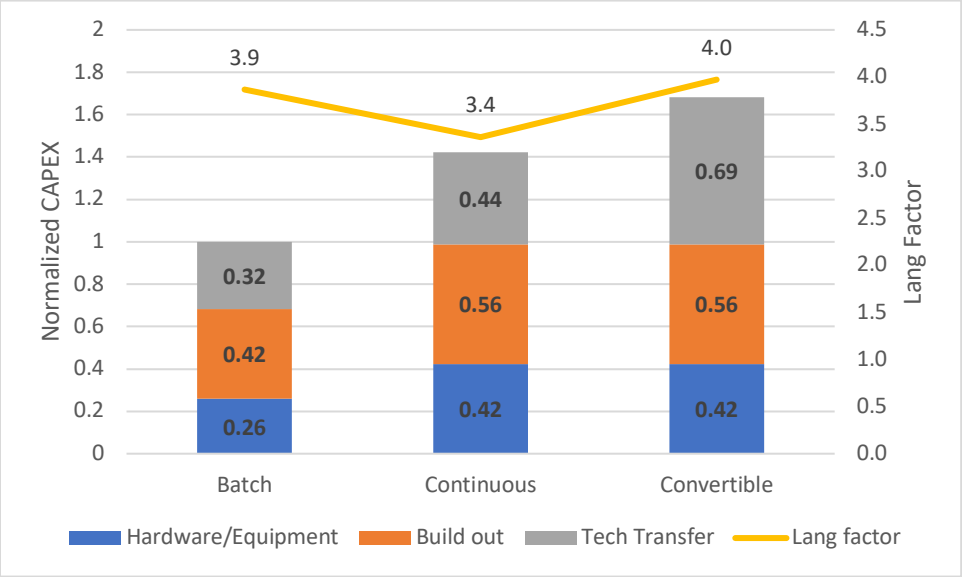

Figure 5.5 Normalized Capital Expenditure and Lang factor for each process scenario.

The investment for the Continuous scenario is increased (42%) as compared to the Batch scenario. The necessary equipment costs increased approximately 60% as compared to the Batch design due to tankage modification and the additional hollow fiber housings and piping to support the ILDF configuration. Overall build out costs are 25% higher for the additional integration of the configuration. Tech transfer costs increased approximately 35% due to the additional materials and components needed to produce batches at the high end of the run duration.

The Convertible scenario is 68% more as compared to the Batch scenario. The Convertible scenario capital expense is portioned from the batch and continuous design capital expenses. Equipment costs and build out are similar to the Continuous scenario as the Batch configuration can be encompassed with the Continuous. The tech transfer costs are significantly higher (more than 2x) as production runs will be needed for both the batch and continuous designs.

5.3.4 Qualitative Aspects

Another means of comparing the scenarios is a qualitative risk assessment examining less tangible aspects of each option. The intent of this assessment was to consider aspects that may influence selection of scenarios/options beyond the numerical comparisons of economic calculations. These aspects include difficulty or risk around implementation, on-going operation and the general economic landscape. The risk for these aspects also depends on the demand to be fulfilled. The assessment of these risk across the annual demand is summarized in Figure 5.6 and described below. The assessment shows that the threshold for the favorability of a continuous design driven option is greater than the approximate 5M vial demand mark previously shown in the COG calculation. Note the Convertible option is not explicitly assessed as it contains both the Batch and Continuous option.

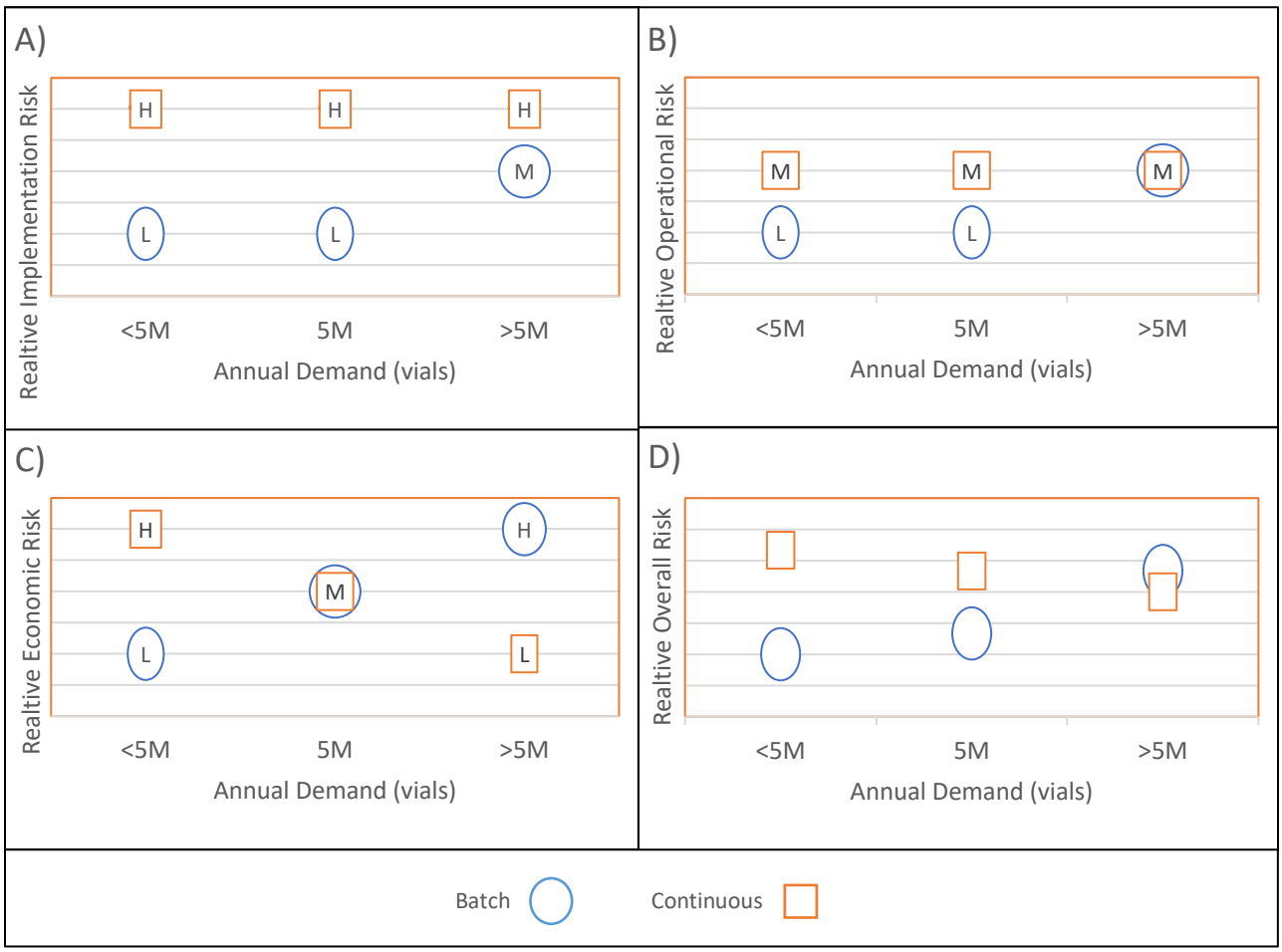

Figure 5.6 Qualitative Risk Assessment of Batch and Continuous scenarios in terms of A) Implementation risk, B) Operational risk, C) Economic risk and D) Overall risk. 5M annual vials was used as a division point based on the crossover of benefit in the COG calculation.

5.3.4.1 Implementation Risk

The Batch scenario involves a standard configuration for unit operations such as constant volume diafiltration (CVDF) and presents lower risk relative to the Continuous scenario. The implementation risk for the Batch scenario does increase as the demand increases as the system has a limited capacity (10M annual vials) and will require another production line. The second implementation increases the risk.

The Continuous design presents a higher risk as it is a less well established configuration (ILDF) and difficult to evaluate at full scale. The design also requires a high level of automation, process analytics, and balancing of process parameters such as flow rates, pressures, etc. at the different stages of the ILDF set up. This risk is present regardless of annual demand.

5.3.4.2 Operational Risk

The operational risk for the Batch scenario should be considered low at low annual demand. The low demand presents a consistent manageable cadence (batches per week) to meet the requirement. At higher demands, the operational risk increases. The need to produce near maximum capacity or operate a second production line increases the probability of an issue.

The complexity of the continuous system relative to the batch system (increased amount of connections/risk to aseptic processing, maintenance of balanced flows, etc.) presents a higher relative risk, but again a level profile across the annual demand. The operational risk from requiring more batches/longer processing times (risk of aseptic processing, greater exposure to lost product with failure) does not present until the demand significantly increases and is matched by multiple Batch scenario production lines running simultaneously.

5.3.4.3 Economic Risk

The qualitative perspective on the economics reflects the previous COG calculations as well as the need for additional production lines in the Batch scenario. The Batch scenario has the lower COG at lower demand as the fixed costs per vial are lower for this design. The COG becomes higher than the Continuous option once demand increases past 5M annual vials and would increase significantly (approximately 2x) beyond 10M, in both COG and Capital investment, when a second production line is needed. This presents as low risk at low demand and high risk at high demand.

The Continuous scenario has higher COG at low demand due to the higher direct and indirect costs being spread over low production levels, but the COG are substantially lower at higher demands, particularly given the designs total capacity of 35M annual vials, which would require multiple batch operations (3.5x) to produce an equivalent amount. This presents as the opposite economic risk of the Batch scenario; high risk at low demand and low risk at high demand and approximately equivalent at the break point.

5.3.4.4 Overall Risk

Summating these risk factors into an overall perspective, the Batch scenario presents as the lower risk for low annual demand while the Continuous scenario presents as the lower risk at the high annual demand. The summation plot for the overall risk shows a crossover point that is further to the right than the selected demand midpoint of 5M annual vials, which is based solely on COG. This suggests that when selecting which of

the scenarios should be employed, the COG calculation crossover (5M annual vials) should not be the sole selection factor and that the crossover point for the Continuous scenario to be fully advantageous is a greater demand level than what COG would suggest alone.

5.3.5 Scenario Selection

Given the information above, it becomes clear that a good understanding of the production forecast over the product lifecycle is imperative when selecting which scenario is best for the application. Additionally, understanding the timing within the production forecast can lead to further means of optimizing the selection.

With knowledge of the production forecast, the selection of a scenario can be simplified into three outcomes:

1. If annual forecast projections over the product lifecycle are low (i.e. peak of 5M or less), a batch design is the optimal choice with respect to COG, Capital investment, and risk and a continuous option need not be pursued.
2. If annual forecast projections start high (i.e. minimum annual demand of 5M) and remain high over the product lifecycle, a continuous design is the optimal choice with respect to COG, Capital investment and risk and should be pursued from the beginning.
3. If annual forecast projections are varied (i.e. above and below 5M) due to clinical trial demands or other product lifecycle factors, the Convertible option should be explored. For example, the batch process design may be

most suitable for clinical phase and early commercial production, but unable to optimally meet maximum demand during the product lifecycle. When the batch process becomes suboptimal due to increased demand, the continuous process pathway could be employed.

Additionally, understanding the timing within the production forecast can lead to further means of optimizing the COG. If the Convertible option is projected to be needed, but the high demand necessitating the continuous pathway is not forecast until the later years of the product lifecycle, the investment, installation, validation, etc. of this pathway could be postponed. The process could start as the batch design (Figure 5.1A) and later be modified to the convertible design (Figure 5.1C), given the space within the facility is reserved. This would have the benefit of deferring the increased capital investment into the continuous pathway until the product demand is at a point where it can be better absorbed into the COG. This would yield the low COG of the Batch scenario in the earlier years of the product lifecycle and the even lower COG of the Continuous scenario in the later years. This strategy does have the risk of competing for production time when the continuous pathway needs to be implemented.

5.4 Conclusions/Recommendations

Liposomal drug product manufacturing is very product specific and therefore, the economics can be unique and have limited applicability. In spite of this, some general lessons can be learned. The standard strategy for increasing manufacturing capacity involves scaling up or adding production lines. This often comes with limited or no

benefit to COG. With the incorporation of continuous manufacturing, capacity can be increased with additional benefit to COG. The case examined here shows that a continuous manufacturing design provides an economic advantage over the batch design and adding production lines when production demand surpasses a particular threshold (>5M annual vials). Given the inflection in economic advantage and qualitative shift in risk, when selecting batch or continuous designs, the best option would be to allow and provide for a convertible process design that can be strategically implemented to leverage the advantages of both designs.

Chapter 6

Conclusions & Future Work

6.1 Overall Conclusions

The primary aim of this thesis was to determine whether a basic design and process requirements can be determined for a continuous liposomal drug product manufacturing process and whether economic benefit could be realized. The results of the preceding chapters show that there are feasible options for arrangement of a continuous liposomal drug product process. These options would include the inherently continuous cross-flow mixing step for liposome generation coupled with an inline diafiltration (ILDF) arrangement.

Process conditions were examined to support an ILDF arrangement. It was determined that the ethanol necessary in the liposome formation negatively impacts the permeability of the hollow fiber during the diafiltration step used for formulation refinement. This negative impact can be mitigated by pre-dilution of the liposome formation mixture with buffer which improves permeability and greatly reduces the amount of stages needed in the ILDF arrangement (5% retentate ethanol concentration requiring 7 ILDF stages versus 35 stages needed for 36% ethanol). Additionally, the ILDF arrangement was found to require less buffer than the commensurate batch design due to the ethanol permeability impact improving with each stage (At 5% initial ethanol concentration, 210kg of buffer for continuous versus 337kg for batch).

The economic assessment showed that the additional investment and validation required for a continuous process design was offset by a favorable COG once the production demand exceeded a certain threshold. When considering the demand driven

COG advantage and the risk factors in the continuous design, the recommendation was to build a flexible system that allowed for conversion from batch to continuous processing to optimize economic benefits.

These chapters clearly demonstrate the advantages of continuous processing with optimized processing conditions and strategic implementation.

Chapter 2 detailed the batch process design including raw materials, process conditions, and testing methods. The chapter also includes the experimental set up for a system used to simulate an ILDF arrangement. Additionally, the methods used to support an economic comparison of the batch and continuous options are listed. This includes Cost of Goods, capital investment, and a qualitative risk assessment. These methods generated the data to support the evaluation, examination and comparison of batch and continuous processing of the liposomal drug product that led to Chapters 3, 4 and 5.

Chapter 3 presented and evaluated of the batch process for the liposomal drug product, which serves as the baseline and touchstone for a continuous process design. The batch process was reviewed and examined for conversion to a continuous process design. The batch process has various points which are favorable for conversion to continuous processing including the critical step of liposome formation. Of note, the formulation refinement step of diafiltration requires conversion to a design similar to in-line diafiltration (ILDF). Upon deeper examination of the batch process constant volume

diafiltration, the ethanol required in the liposome formation step, which is meant to be removed by diafiltration, has a negative impact on the permeability of the hollow fiber membranes used in diafiltration. This is expected to cause the ILDF configuration to be onerous if not properly mitigated.

Overall, the system is viewed as favorable for conversion to a continuous design given further examination of the impact of ethanol on the ILDF configuration.

Chapter 4 evaluated the impact of ethanol concentration on the performance of an ILDF design to a continuous liposome process. The assessment determined that upfront dilution of the retentate is necessary to reduce the impact of ethanol on permeability of the hollow fibers. This minimizes the overall number of passes/stages in the ILDF. 5% retentate ethanol concentration required 7 ILDF stages versus 35 stages needed for 36% ethanol. Also, by diluting, the overall buffer requirements for a continuous process are less than the batch process. At 5% initial ethanol concentration, 210kg of buffer was needed for the continuous process versus 337kg needed for the batch process. Additionally, further experiments should be careful to consider the ethanol impact when simulating ILDF.

Chapter 5 compared the economics of batch and continuous designs. The comparison concluded that the continuous process design becomes advantageous when demand surpassed a certain threshold, in this case, approximately 5M annual vials. This threshold is dependent on the assumptions built into the COG and Capital investment

calculation and the risk tolerance of the implementation. The recommended strategy was to allow for a flexible design that can produce in both batch and continuous configurations and selected either option based on the optimized real-world circumstance.

6.2 Future Work

The objective of this body of work was to evaluate the batch process for conversion to a continuous design, determine feasibility of the design, recommend conditions for process and implementation and calculate any benefit thereof. The preceding chapters have completed these objectives and demonstrated that continuous processing of liposomal drug products is feasibility and advantageous under certain conditions. Additional aspects to expand on the evaluation and development of this design/process are discussed below.

6.2.1 Fully arranged ILDF

The continuous process design and inputs were primarily evaluated using a simulated ILDF system (Figure 4.2) and then extrapolated to determine recommended process conditions. The next steps in this evaluation would be to test a full-scale/fully arranged mockup of the recommended conditions. This would involve an ILDF system, similar to Figure 5.1B, with approximately seven to ten hollow fiber cartridge stages being fed by a sufficient quantity of diluted liposome generation mixture. The validity of the assumed

number of stages could then be tested and the benefits to both process and economics more accurately calculated.

This full-scale mockup would also provide an opportunity to explore the needs and application of PAT to the system. Each stage of the ILDF system will need an array of pumps and flow meters to not only control and track the flow rates and mass balances of each stage, but measure the retentate and permeate densities and calculate the ethanol concentrations and removal rate. Ideally, these controls and calculations could be automated as well. More elaborate PAT such as online HPLC could also be explored for direct measurement of the concentrations. This would allow for the evaluation of the control and consistency of such a system over a long duration production run.

6.2.2 Small-scale ILDF

Another alternate means of testing this on a smaller scale would be continued development of the Cadence ™ ILDF system as a scaled down surrogate for the full-scale system. As mentioned in Chapter 4, the Cadence ™ comes with the disadvantages of the buffer inlet and permeate outlet being manifolds (Figure 4.1), preventing the ability to evaluate each stage. Additionally, the membrane material and pore size differ from the hollow fiber used In the batch process, making comparison difficult. Customization of the Cadence™ could be performed in order to allow for interstage sampling and measurement to overcome the evaluation challenges. Crossover studies could be performed to determine the impact of the differences in the material/pore size. If these

gaps can be closed, this presents a more convenient means of evaluating the full-scale ILDF concepts on liposomal drug products.

6.2.3 Novel liposome generation/buffer exchange in single step

Another completely unique concept for continuous processing of liposomal drug products is the use of a counter-current extractor such as the Podbielniak Contactor or POD®. The Podbielniak Contactor is a liquid-liquid centrifugal extractor/separator previously used to manufacture antibiotics as well as other biologics and food product applications. This piece of equipment could take the place of both the liposome generation step and buffer exchange (diafiltration) in a singular unit operation. The system supports a liquid-liquid interaction (liposome generation) as well as a density driven separation of liquids (ethanol removal/buffer exchange) (Figure 6.1). Preliminary work was performed with a lab scale POD system. Liposomes were generated and buffer exchange was determined to be feasible, but the equipment, which involves a liquid-fed system revolving at thousands of RPM, was decided to be too difficult and dangerous for further experimentation. Additionally, other concerns around the future requirements are cleaning, seal integrity and sterility made continuation of the work not prudent at this time.

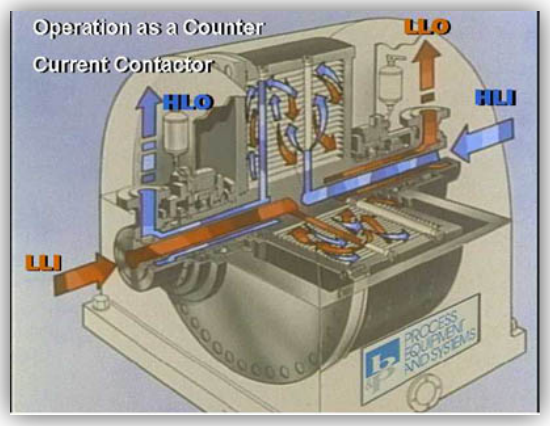

Figure 6.1 Representation of the Podbielniak Contactor with a cross section removed

6.3 Patents and Publications

The research completed within this thesis has yielded the two published papers and a third in submission/review. Additionally, a US patent for the method of continuous manufacture of liposomal drug products was approved with other regional patents in review.

Patents:

1. Worsham R. Methods for continuous manufacture of liposomal drug products. US11571386B2. 2023

Papers:

1. Worsham, R., Thomas, V., Farid, S., Potential of Continuous Manufacturing for Liposomal Drug Products, *Biotechnology Journal*, 14(2), 2018
2. Worsham, R., Thomas, V., Farid, S., Impact of ethanol on continuous inline diafiltration of liposomal drug products, *Biotechnology Journal*, 18(11), 2023
3. Worsham R., Thomas V., Farid S. Economics of Continuous Manufacturing of Liposomal Drug Products. *Pending*

Chapter 7

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