

Potential of Continuous Manufacturing for Liposomal Drug Products

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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 (e.g., improved economics, more consistent quality) that come with continuous processing. If relevant processing techniques and principles are selected, the opportunity arises to develop continuous manufacturing designs for additional pharmaceutical products including liposomal drug 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 paper reviews the current state of continuous manufacturing technology applicable to liposomal drug product manufacturing and an assessment of the challenges and potential of this application.

1. Introduction

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 recently 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.).^[1,2] 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.^[1,2] 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.^[3–14] 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.^[3,13,14] TFF systems support continuous filtration by clearing the mem-

brane surface with tangential fluid flow while ATF uses a cyclical backflush. Single pass tangential flow filtration (SPTFF)^[2] 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.^[15–19] 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 benefit 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. 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 explored. The consensus is that PAT around critical process measurements is a requirement for continuous processing as this replaces the testing at intermediate stages in a batch process, but often specifics of implementation are left to the end user.^[20,21] 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.^[17,22–24]

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Given this conclusion, 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 continuous pharmaceutical manufacturing and some unique aspects that require further exploration. Frequently, liposomal products are reformulations of compendial APIs meant to alleviate adverse clinical side effects and/or provide a more targeted delivery as compared to systemic dosages.^[25,26] 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, which will be examined further here.

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 h a day, 7 days a week, and 50 weeks per year.^[27] Others state that the term should also include restrictions around intermediate surge vessels or processing breaks between API and drug product.^[28] 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.^[29]

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 liposome formulations and how implementing continuous manufacturing can be achieved and provide benefit to the liposomal drug products.

3. Liposomal Manufacturing

Liposomes were first discovered in the early-1960s. Since that time, a number of strategies have been demonstrated for their manufacture.^[30,31] 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). 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.).^[30,31] Manufacture 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).



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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.^[32–34] For uniform product generation, these suspensions required post-formation mechanical size manipulations strategies.^[35,36] The combination of these methods, although effective and well-understood, have been 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.^[37]

The most successful examples of scaled methods for liposome manufacture to date have followed the principles of alcohol injection (Figure 1A) or crossflow techniques (Figure 1B), 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.^[38–42] A change in the local solubility of the lipids during this process ultimately leads to the spontaneous formation of liposomes that

Table 1. Liposome formation methods.

Method	Mechanism	Suitability for continuous manufacturing
Bangham ^[32–34]	Rehydration of thin lipid film	Not practical means of continuous dehydration/rehydration steps
Sonication method ^[65]	Sonication of an aqueous lipid suspension	Requires small scale batch operation for sonication to be practical
Reverse phase evaporation ^[45,66]	Aqueous phase added to organic phase and evaporated to form liposomes	Overly complex to regulate continuous solvent evaporation, sterile boundary hard to establish
Detergent depletion ^[67,68]	Liposomes formed through detergent lipid interaction	Slow process with difficult to establish sterile boundary, detergent use general disadvantageous
Microfluidic channel ^[69]	Intersection of lipid/API solutions in micro-channels	Very small scale, not a practical manufacturing process with existing technology
High pressure homogenization ^[35,36]	Liposome formation through high pressure mixing	Very high pressures required, difficult to sterilize equipment
Heating method ^[30,70,71]	Heating of a lipid aqueous/glycerol solution to form liposomes	Hydration step and high temperatures make continuous production impractical
Supercritical fluid methods ^[45–50]	Use of supercritical fluids as solvent for lipids instead of organic solvents	High pressures required for feed vessels make resupply/continuous operation impractical
Dense Gas methods ^[45,50,51]	Use of dense gas as solvent for lipids instead of organic solvents	High pressures required for feed vessels make resupply/continuous operation impractical
Ethanol/ether injection ^[38,46,72,73]	Precipitation of liposome from organic phase into aqueous	Simple process with inherently continuous liposome formation step, very suitable
Crossflow method ^[37,39–42]	In-line Precipitation of liposome from organic phase into aqueous	Simple process with inherently continuous liposome formation step, very suitable

encapsulate a small volume of the aqueous solution. Depending on the chemical nature of the API, it can be encapsulated in the aqueous core or embedded in the lipid bilayer. 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.^[39,43,44]

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). 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 adapt to a continuous design.^[45–51] 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 for comments on suitability for continuous manufacturing for each method.) With continuous formulation of the feed solutions, the liposome formation

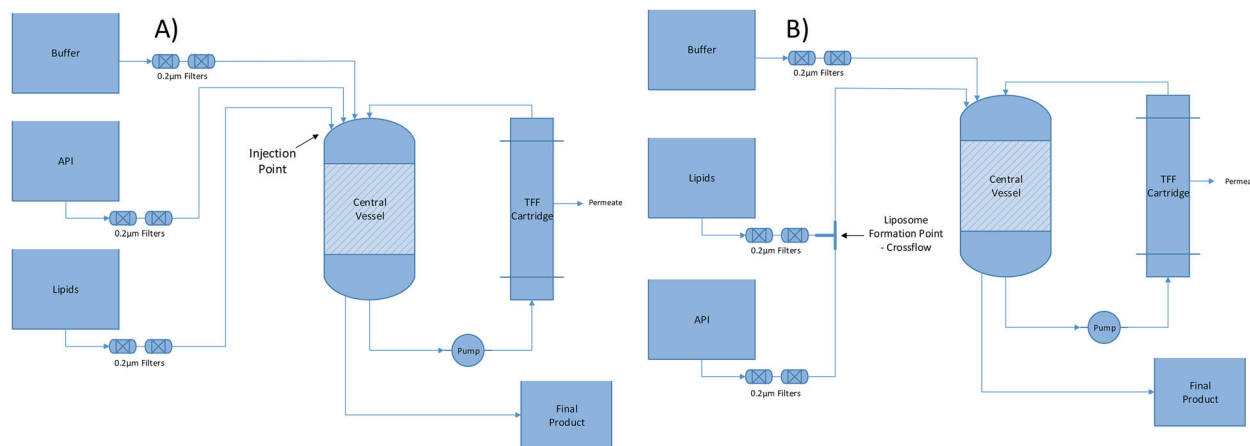


Figure 1. Liposomal Drug Product Manufacturing Process Flow Diagrams – Batch Design. A) Ethanol/Ether Injection Method: Lipid/Solvent solution is directly fed into the central vessel. B) Crossflow Method: Solvent/anti-solvent mix in-line at an intersection point. A,B) Formulations are refined in multi-step buffer exchange diafiltration and concentration steps.

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.

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.

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.^[52] 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), facilitating the buffer exchange. Once buffer exchange is complete, the product is concentrated to the desired strength by ceasing buffer addition (Figure 1). 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.^[52] 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 2A). 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 (Figure 2B,C). Additionally, more compact and elegant designs for continuous buffer exchange, such as the Cadence[®] In-line Diafiltration Module (ILDF), are becoming available and should be explored.^[53] An ILDF design concluding with SPTFF would eliminate the need for multiple vessels to support continuous buffer exchange (Figure 2D).

SPTFF is an additional unknown for liposomal formulations, but data has been generated for use of SPTFF for concentration of cell culture harvest^[15] or for protein concentration.^[16] 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 reinforces 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 HPLC.^[20,21]

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 to 4 min is a more likely candidate.^[54] Other in-line measurements, such as particle size, may be applicable, given they can be correlated to concentration.

4.2. Materials of Construction

Many of the benefits of continuously manufacturing biologics are leveraged from the incorporation of single-use systems and componentry.^[14,22,23,55] 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.^[56,57] Additionally, if single-use components are pre-sterilized through gamma-irradiation, there can be issues with free-radical generation and incorporation into 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.^[58] 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.

4.3. Sterility Assurance

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 0.2 μm (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).^[59,60] With aseptic processing comes the need to

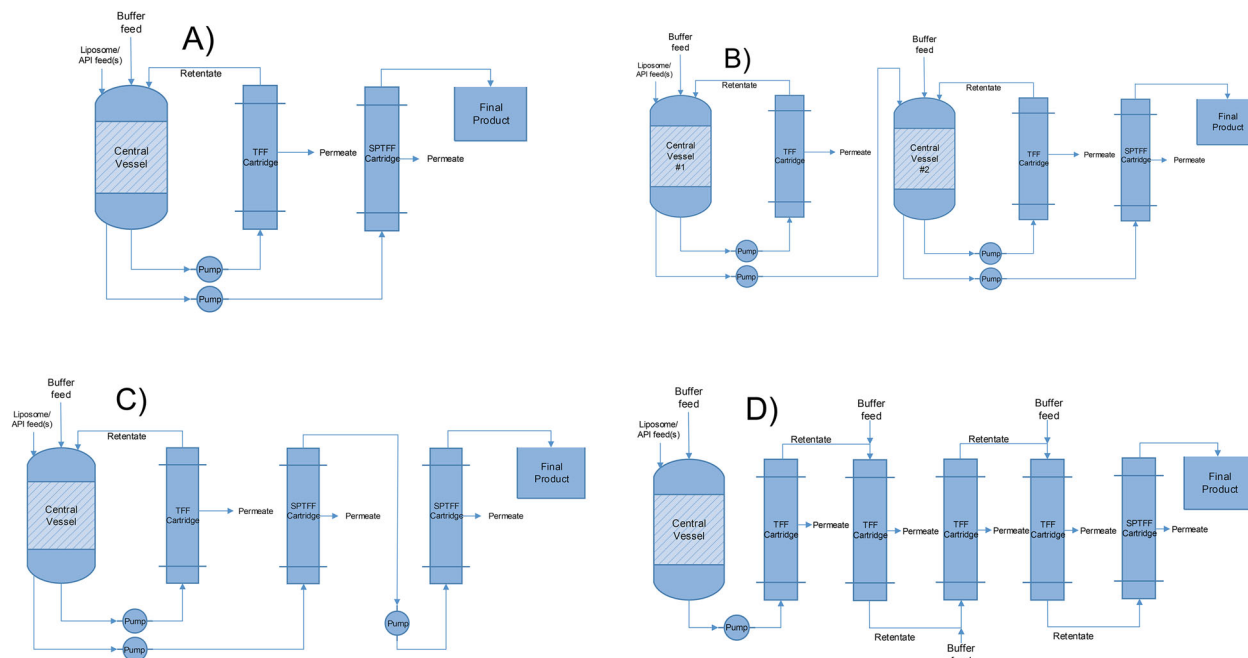


Figure 2. Proposed novel process designs for continuous liposome drug product manufacturing. A) Single tank buffer exchange TFF and single stage concurrent concentrating SPTFF, B) Continuous multistage (multi-vessel) buffer exchange TFF and single stage concurrent concentrating SPTFF, C) Single tank buffer exchange TFF and multistage concurrent concentrating SPTFF, D) Multistage buffer exchange (ILDF) with concurrent concentrating SPTFF.

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.^[61] Additionally, extended use of flexible tubing in pumping systems can lead to spalling and breaches as well.^[62]

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 an 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.^[63] 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.^[64] There is no PAT for microbiology on the horizon, so 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

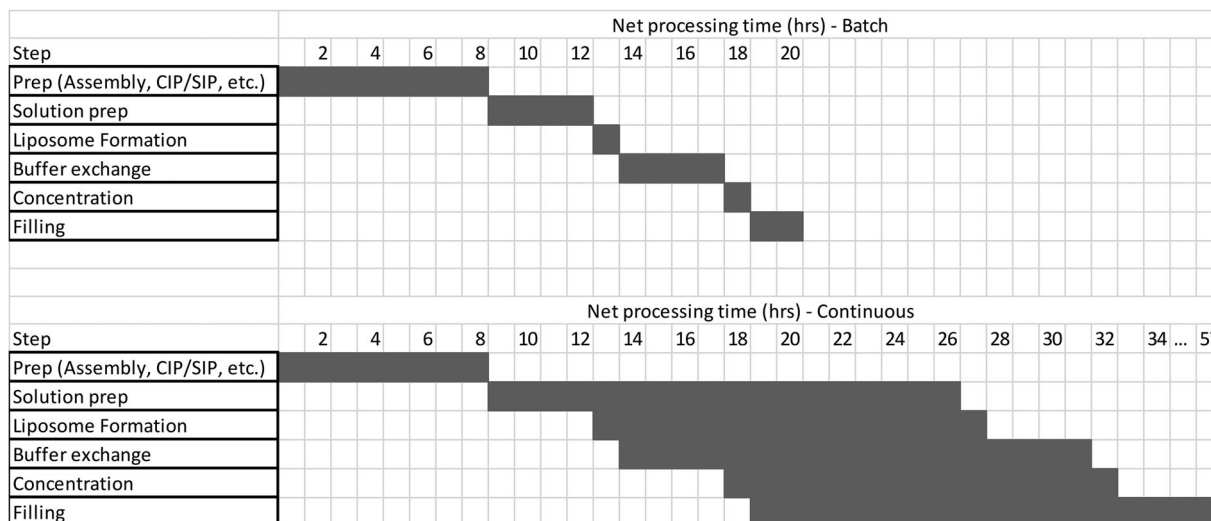


Figure 3. Batch vs. Continuous processing steps/times for a liposomal drug product.

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.

5. Assessment of Benefits

Based on the above assumption of a feasible continuous operation for the manufacture of liposomal formulations, a basic case study can be performed. For the purposes of the case study, the following options shall be compared: 1) a batch process design producing 2500 filled units from a 1 h liposome formation step with supporting batch process steps and 2) a continuous process design allowing for a 24 h liposome formation step with concurrent continuous unit operations. The batch process is based on a real-world example used for early phase clinical production (i.e., a 25 L bulk formulation with 10 mL fills). It is assumed that the batch and continuous designs are using similar scale equipment with similar processing rates (i.e., a filling speed of 1250 units h⁻¹). A summary of the unit operations and processing times is in **Figure 3**.

The batch process is able to produce 2500 filled units in 20 h of total processing time including preparation (assembly, CIP/SIP, etc.), or 125 units h⁻¹. The continuous process with a 24 h liposome formation step would produce 60 000 filled units in 57 h of total process time or 1053 units h⁻¹. This translates to a 8.4-fold increase in output for the same overhead costs based on units per hour and a 24-fold output increase for the same process preparation costs and single-use componentry costs (sterilizing filters, TFF cartridges). Additionally, the savings and output rate could be further increased with increased filling speeds. This ignores the additional capital expenses needed to achieve one of the continuous designs previously mentioned.

Another way to compare the processes would be their ability to fulfill a given production forecast. For a forecast of 1 million units per year, the continuous design would require the 57-h process to be run less than 1.5 times per month. For the batch design, the 20-h process would have to be run more than once

per day, necessitating multiple lines running at a higher rate to fulfill the forecast.

By converting the early phase clinical scale production line to a continuous operation, not only are cost savings and higher throughput achieved, but the need for scaling up the process is alleviated, which eliminates the need for supporting process development work and large-scale capital equipment purchases.

6. Conclusions

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. Given a process is designed with relevant unit operations and consideration to materials of construction and sterility assurance, liposomal drug products can reap the economic and quality benefits of continuous manufacturing.

Abbreviations

ADME, adsorption distribution metabolism and elimination; API, active pharmaceutical ingredient; ATF, alternating tangential flow filtration; CIP, clean-in-place; COG, cost of goods; HPLC, High performance liquid chromatography; ILDF, In-line diafiltration; PAT, process analytical technology; SIP, steam-in-place; SPTFF, single-pass tangential flow filtration; TFF, tangential flow filtration; VHP, vaporized hydrogen peroxide.

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Conflict of Interest

The authors declare no financial or commercial conflict of interest.

Keywords

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- [1] P. Kleinebudde, J. Khinast, J. Rantanen, (Eds.), *Continuous Manufacturing of Pharmaceuticals*, Wiley-VCH, Hoboken 2017.
- [2] G. Subramanian, *Continuous Process in Pharmaceutical Manufacturing*, Wiley-VCH, Weinheim 2015.
- [3] J. Pollock, S. Ho, S. Farid, *Biotechnol. Prog.* 2013, 110, 206.
- [4] V. Warikoo, R. Godawat, K. Brower, S. Jain, D. Cummings, *Biotechnol. Bioprocess.* 2012, 109, 3018.
- [5] E. Mahajan, A. George, B. Wolk, *J. Chromatogr. Sep. Tech.* 2012, 1227, 154.
- [6] R. Godawat, K. Brower, S. Jain, K. Konstantinov, F. Riske, V. Warikoo, *Biotechnol. J.* 2012, 7, 1496.
- [7] M. Bisschops, M. Brower, *Pharm. Bioprocess.* 2013, 1, 361.
- [8] J. Pollock, G. Bolton, J. Coffman, S. Ho, D. Bracewell, S. Farid, *J. Chromatogr. A.* 2013, 5, 1284, 17–27.
- [9] R. Orozco, S. Godfrey, J. Coffman, L. Amarikwa, S. Parker, *Biotechnol. Prog.* 2017, 33, 954.
- [10] S. Parker, L. Amarikwa, K. Vehar, R. Orozco, S. Godfrey, *Biotechnol. Bioeng.* 2018, 115, 606.
- [11] J. Pollock, J. Coffman, S. Ho, S. Farid, *Biotechnol. Prog.* 2017, 33, 854.
- [12] J. Walther, R. Godawat, C. Hwang, Y. Abe, A. Sinclair, *J. Biotechnol.* 2015, 10, 3.
- [13] L. Castilho, in *Continuous Process in Pharmaceutical Manufacturing*, (Ed: G. Subramanian) Wiley-VCH, Weinheim 2015, pp. 115–153.
- [14] W. Whitford, in *Continuous Process in Pharmaceutical Manufacturing*, (Ed: G. Subramanian) Wiley-VCH, Weinheim 2015, pp. 183–226.
- [15] A. Arunkumar, N. Singh, M. Peck, Z. Li, *J. Memb. Sci.* 2017, 524, 20.
- [16] C. Casey, T. Gallos, E. Ayturk, S. Pearl, *J. Memb. Sci.* 2011, 384, 82.
- [17] M. Brower, Y. Hou, D. Pollard, in *Continuous Process in Pharmaceutical Manufacturing*, (Ed: G. Subramanian) Wiley-VCH, Weinheim 2015, pp. 255–296.
- [18] A. Jungbauer, *Trends Biotechnol.* 2013, 8, 479.
- [19] J. Dizon-Maspat, J. Bourret, A. D'Agostini, F. Li, *Biotechnol. Bioeng.* 2012, 4, 962.
- [20] C. Callener, *Pharm. Technol.* 2017, 41, 22.
- [21] A. Rathore, H. Winkle, *Nat. Biotechnol.* 2009, 27, 26.
- [22] M. Bisschops, in *Continuous Process in Pharmaceutical Manufacturing*, (Ed: G. Subramanian) Wiley-VCH, Weinheim 2015, pp. 35–52.
- [23] J. Novais, N. Titchener-Hooker, M. Hoare, *Biotechnol. Bioeng.* 2001, 75, 143.
- [24] S. Farid, B. Thompson, A. Davidson, *mAbs* 2014, 6, 1357.
- [25] N. Maurer, D. Fenske, P. Cullis, *Expert Opin. Biol. Ther.* 2001, 6, 923.
- [26] T. Lian, R. Ho, *Expert J. Pharm. Sci.* 2001, 6, 667.
- [27] C. Badman, B. Trout, *J. Pharm. Sci.* 2015, 3, 779.
- [28] R. Hernandez, *Genet. Eng. Biotechnol. N.* 2017, 37, 16.
- [29] Stanton, D., Pfizer: Global regulatory divergence restricted continuous manufacturing ambition. *In-Pharma Technologist.com*. 29-Jun-2017.
- [30] MR. Mozafari, *Cell Mol. Biol. Lett.* 2005, 10, 711.
- [31] B. Maherani, E. Arab-Tehrany, M. R. Mozafari, C. Gaiani, M. Linder, *Curr. Nanosci.* 2011, 7, 436.
- [32] A. Bangham, M. Standish, G. Weissmann, *J. Mol. Biol.* 1965, 13, 253.
- [33] A. Bangham, M. Standish, J. Watkins, *J. Mol. Biol.* 1965, 13, 238.
- [34] D. Deamer, A. Bangham, *Biochim. Biophys. Acta.* 1976, 443, 629.
- [35] R. Barnadas-Rodriguez, M. Sabes, *Int. J. Pharma.* 2001, 213, 175.
- [36] D. Carugo, E. Bottaro, J. Owen, E. Stride, C. Nastruzzi, *Sci. Rep.* 2016, 6, <https://doi.org/10.1038/srep25876>
- [37] A. Wagner, M. Platzgummer, G. Kreismayr, H. Quendler, G. Stiegler, B. Ferko, G. Vecera, K. Vorauer-Uhl, H. Katinger, *J. Liposome Res.* 2006, 16, 311.
- [38] C. Jaafar-Maalej, R. Diab, V. Andrieu, A. Elaissari, H. Fessi, *J. Liposome Res.* 2010, 20, 228.
- [39] A. Wagner, K. Vorauer-Uhl, H. Katinger, *Eur. J. Pharm. Biopharm.* 2002, 54, 213.
- [40] A. Wagner, K. Vorauer-Uhl, G. Kreismayr, H. Katinger, *J. Liposome Res.* 2002, 12, 259.
- [41] A. Wagner, K. Vorauer-Uhl, *J. Drug Deliv.* 2011, 2011, <https://doi.org/10.1155/2011/591325>
- [42] A. Wagner, K. Vorauer-Uhl, G. Kreismayr, H. Katinger, *J. Liposome Res.* 2002, 12, 271.
- [43] Kim, S., Kim, T., Murdande, S., *Sustained-release liposomal anesthetic compositions*. US Patent 8,182,835 B2, 2012.
- [44] Li, Z., Boni, L., Miller, B., Malinin, V., Li, X., *High delivery rates for lipid based drug formulations and methods of treatment thereof*. US Patent 7, 879, 351 B2, 2011.
- [45] L. Meure, N. Foster, F. Dehghani, *AAPS Pharma. Sci. Tech.* 2008, 9, 798.
- [46] I. Santo, R. Campardelli, E. Albuquerque, S. Vieira De Melo, E. Reverchon, G. Della Porta, *J. Pharm. Sci.* 2015, 104, 3842.
- [47] I. Santo, R. Campardelli, E. Albuquerque, S. de Melo, G. Della Porta, E. Reverchon, *Chem. Eng. J.* 2014, 249, 153.
- [48] R. Campardelli, I. Santo, E. Albuquerque, S. de Melo, G. Della Porta, E. Reverchon, *J. Supercrit. Fluids.* 2016, 107, 163.
- [49] L. Frederiksen, K. Anton, P. Hoogevest, H. Keller, H. Leuenberger, *J. Pharm. Sci.* 1997, 86, 921.
- [50] K. Otake, T. Imura, H. Sakai, M. Abe, *Langmuir* 2001, 17, 3898.
- [51] Anton, K., van Hoogevest, P., Frederiksen, L., Preparation of a liposome dispersion containing an active agent by compression-decompression. EP616801, 1994.
- [52] A. Jungbauer, *Trends Biotechnol.* 2013, 31, 479.
- [53] X. Gjoka, R. Gantier, M. Schofield, *BioPharm Int.* 2017, 30, 26.
- [54] V. Kumar, V. Joshi, *LC GC N. Am.* 2013, 31, 948.
- [55] N. Hammerschmidt, A. Tscheliessnig, R. Sommer, B. Helk, A. Jungbauer, *Biotechnol. J.* 2014, 9, 766.
- [56] M. Ferrante, *Pharm. Process.* 2017. <https://www.pharmpro.com/article/2017/06/continuous-processing-and-standardization-impact-advances-single-use-technology>
- [57] R. Hernandez, *BioPharm Int.* 2017, 30, 6.
- [58] E. Schnitzer, I. Pinchuk, D. Lichtenberg, *Eur. Biophys. J.* 2007, 36, 499.
- [59] N. Jan Zuidam, E. van Winden, R. de Vreeh, D. Crommelin, in *Liposomes: A Practical Approach*, (Eds: V. Torchilin, V. Wessig), Oxford University Press, New York 2003, pp. 149–165.
- [60] M. Toh, G. Chiu, *Asian J. Pharm. Sci.* 2013, 8, 88.
- [61] C. Stock, *Contract Pharm.* 2014. https://www.contractpharma.com/issues/2014-01-01/view_features/risk-assessment-for-single-use-disposable-projects
- [62] S. Bahal, J. Romansky, *Pharm. Dev. Technol.* 2002, 7, 317.
- [63] L. Yu, *FDA Voice* 2016. <https://blogs.fda.gov/fdavoices/index.php/2016/04/continuous-manufacturing-has-a-strong-impact-on-drug-quality/> (Posted on April 12, 2016)
- [64] Guidance for Industry, Sterile Drug Products, Produced by Aseptic Processing – Current Good Manufacturing Practice. FDA. Sep 2004.

- [65] S. Perrett, M. Golding, W. Williams, *J. Pharm. Pharmacol.* **1991**, *43*, 154.
- [66] F. Szoka Jr, D. Papahadjopoulos, *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 4194.
- [67] J. Brunner, P. Skrabal, H. Hauser, *Biochim. Biophys. Acta* **1976**, *455*, 322.
- [68] J. Lasch, V. Wessig, M. Brandl, in *Liposomes: A Practical Approach*, (Eds: V. Torchilin, V. Wessig), Oxford University Press, New York **2003**, pp. 3–29.
- [69] A. Jahn, W. Vreeland, D. DeVoe, L. Locascio, M. Gaitan, *Langmuir* **2007**, *23*, 6289.
- [70] S. Mortazavi, M. Mohammadabadi, K. Khosravi-Darani, M. Mozafari, *J. Biotechnol.* **2007**, *129*, 604.
- [71] M. Mozafari, C. Reed, C. Rostron, *Technol. Health Care.* **2002**, *10*, 342.
- [72] S. Batzri, E. Korn, *Biochim. Biophys. Acta* **1973**, *298*, 1015.
- [73] D. Deamer, A. Bangham, *Biochim. Biophys. Acta-Biomembr.* **1976**, *443*, 629.