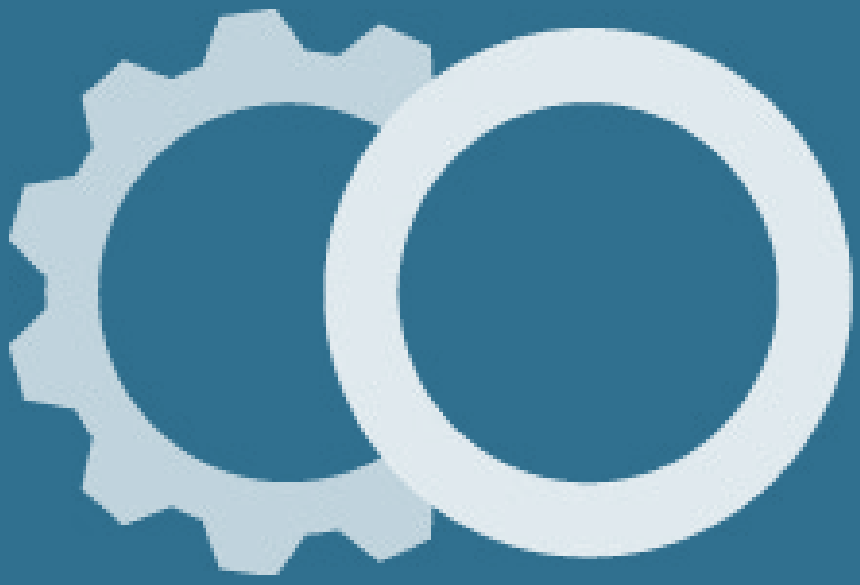
ALTHEA

THE POWER TO MAKE®

IS YOUR COMPLEX FORMULATION PROCESS SET UP FOR SUCCESS?

As the biopharmaceutical industry continues to advance its methods of treating disease, novel drug delivery systems are being developed to target specific areas in a patient as opposed to administering treatment systemically. For example, in the case of antibody drug conjugates (ADCs), the cytotoxic cancer drug is conjugated to the antibody so that it can be received by the tumor only and not the rest of the body. This reduces the harmful side effects to the patient while also increasing the drug's efficacy. The formulations for these types of drug delivery systems are complex and therefore present challenges regarding the stability, efficacy, potency, and even safety of drug products during clinical and commercial phases. Often, the implementation of specialized equipment and/or processes is necessary to ensure successful formulation.



This article discusses six major types of complex formulations as well as the important equipment and processes necessary to develop GMP-compliant processes. Any company pursuing a drug delivery system that involves a complex formulation must be prepared for the requirements around this type of drug development in order to achieve successful scale-up and manufacturing.

COMMON COMPLEX FORMULATIONS IMPLEMENTED TODAY

A formulation is considered complex if the process requires additional steps beyond simple mixing and filtration. Below are some of the most widely used complex formulations in the pharmaceutical industry today. Each formulation is unique in the manufacturing challenges it presents, but some solutions may be shared.

1. SUSPENSIONS

A suspension is a heterogeneous mixture that contains solid particles sufficiently large for sedimentation. There are three main types of suspensions:

▶ *OIL/WATER EMULSIONS*

An emulsion is a mixture of two or more liquids that are normally immiscible (non-mixable or unblendable). The terms colloid and emulsion are sometimes used interchangeably. In an emulsion, one liquid is dispersed into the other. Two liquids can form different types of emulsions. As an example, oil and water can form an oil-in-water emulsion, where the oil is suspended in water. A water-in-oil emulsion can also be formed, where water is dispersed into the oil. Multiple other emulsions are also possible, including a "water-in-oil-in-water" emulsion and an "oil-in-water-in-oil" emulsion.

Because emulsions are liquids, they do not exhibit a static internal structure. The droplets dispersed in the liquid matrix (called the dispersion medium) are usually assumed to be statistically distributed. Microemulsions are used to deliver vaccines. Typical emulsions used in these formulations are nanoemulsions. The oil is emulsified with detergents using a high shear mixer to stabilize the emulsion, so when the oils encounter lipids in the cell membrane or envelope of bacteria or viruses, they force the lipids to merge with themselves. Some examples of drugs that are formulated as oil/water emulsions are Diprivan® (propofol), an injectable anesthetic, and Restasis® (cyclosporine), an ophthalmic drop for dry-eye syndrome. Other applications of nanoemulsions include treatment of reticuloendothelial system infections, enzyme replacement therapy in the liver, and treatment of cancer.

There are three processes used in oil/water emulsions:

High shear mixing — disperses or transports one-phase ingredients into a continuous phase with which it would normally be immiscible. There are two types of high shear mixing equipment: overhead, which is used for batches that are 20 liters or less, or inline, which is used for batches over 20 liters.

Homogenization — intensive blending of mutually related substances or groups to form a constant of different insoluble phases to obtain a suspension or emulsion. The goal of homogenization is to decrease particle size and increase stability.

Extrusion — a process used to create objects of a fixed, cross-sectional profile. A material is pushed or drawn through a die of the desired cross-section. For pharmaceutical products, extrusion through nanoporous, polymeric filters is used to produce suspensions of liposomes or transfersomes of a particular size and a narrow size distribution. For example, the anti-cancer drug Doxorubicin in liposome delivery system is formulated by extrusion. Reproducibility, the ability to achieve a uniform particle size down to submicron dimensions, is one of the main advantages. This results most often in achieving desired product quality in a single pass.

High shear mixing and homogenization are typically

performed in IS0 7 cleanrooms and are considered nonsterile operations. If a sterile formulation operation is required, it must go through aseptic process validation. The industry standard for a sterilizing grade filter membrane is 0.22 micrometers or 220 nanometers (nm). Suspended particles larger than 220 nm cannot be sterile filtered, and thus require aseptic formulation techniques. Anything sterile also has to be validated and tested.

▶ *LIPOSOMES*

A liposome is an artificially prepared vesicle composed of a lipid bilayer. They can be used as a vehicle for administration of nutrients and pharmaceutical drugs. Liposomes are difficult to make and require solvents to dissolve the lipid. The reason drugs are encapsulated in lipids is the basic principle, "like dissolves like." Lungs, for example, have lipid all around the bronchioles, which allow them to expand and contract during inspiration/expiration. Liposomal delivery systems offer key advantages because they are more easily absorbed into a cell and allow sustained release of API from liposomal delivery systems. It is possible that some cancer treatments will be distributed using this method as a way to deliver drugs directly to the areas where cancer cells are located. You can then heat these in situ and "break" the lipid to release the drug. It is also used for higher uptake of toxic drugs to target sites, therefore reducing the danger of a systemic exposure to toxic drugs just to see their effects on patients.

The following processes can be used in liposome production:

Encapsulation — There are three ways to encapsulate a liposome. They are:

1. the infusion of two or more solution streams
2. sonication, which is the act of applying sound energy to agitate particles in a sample for various purposes
3. high shear mixing.

Tangential Flow Filtration (TFF) — TFF is a type of filtration in which the feed is passed through a membrane or bed. The solids are retained by the filter and the filtrate is released "tangentially" from the recirculated bulk. In TFF, the fluid is pumped across the surface of the membrane. An applied transmembrane pressure serves to force a portion of the fluid through the membrane to the filtrate side. This allows for single-use TFF, which eliminates the need for cleaning validation as well as offers the ability to achieve a tight concentration specification.

Validation — An aseptic process validation (APV) is necessary to show that the process can be performed without compromising the sterile integrity of the product, thus making it safe for human use. Various QC assays are also done to verify that the product is good for use. For example, a process simulation is performed to demonstrate that the sterility of the formulated bulk is not being compromised at any time. This is achieved by using nutritive liquid media instead of API and excipients.

▶ *NANOPARTICLES*

Nanoparticles are loosely defined as substances with a product dimension of less than 100 nm, but the category, particularly in the area of pharmaceuticals, includes substances that are as large as 500 nm. For a point of comparison, a human hair is approximately 80,000 nm wide. Nanoparticles have been used in drug delivery for many years. For example, Abraxane® utilizes nanoparticle delivery of the anticancer agent Paclitaxel (Taxol). Drug developers may require lipids to be manufactured and sized down to nanoparticles in order to enable better drug absorption by the body. This is typically done by microfluidization, which is essentially homogenization using microfluidics.

There are several considerations that need to be made when it comes to nanoparticles, such as:

- The formulation of the nanoparticle may require the blending of aqueous and oil phases and then homogenizing to a particle size specification.
- If new equipment is introduced for formulation, Installation,

Operational, and Performance Qualification (IQ/OQ/PQ), cleaning, and sterilization protocols may be required. This ensures the equipment is functioning properly, and all process parameters are in a qualified state of control.

- If mean particle size is greater than 220 nm, it is likely aseptic processing will be required.
- The nature and severity of any waste must be considered. If it is determined that waste poses a hazard, it must be collected in Department of Transportation (DOT)-approved containers and handled only by qualified operators. Specific protocols must be followed to ensure proper disposal.

2. DRUG CONJUGATES

Drug conjugates consist of a drug covalently bound to another molecule. Some examples include drugs conjugated to proteins or polymers. One of the most promising types of conjugates is ADCs (as mentioned above). ADCs consist of monoclonal antibodies attached to a biologically active drug via a chemical linker. As mentioned above, by linking a cytotoxic drug with an antibody, the amount of cytotoxic material absorbed by the rest of the body is reduced by specifically targeting receptors on the cancer cells. ADCs are challenging to produce and are predominantly used in cancer treatments.

With ADCs, it is important to assess the toxicity of the conjugate and the linking agents. Many times, solvents must be added and subtracted from the mixture for the bonding to occur. Because of the potency of these compounds, high containment is required to protect both the environment and the technicians. An example of the process used to conjugate a linker to an antibody using aseptic processing is described later in this paper.

3. CRYSTALLIZED PROTEINS

When a solution in which a protein is being dissolved becomes supersaturated, the proteins form into crystals. The individual protein molecules created under these conditions pack in a repeating array, which are held together by noncovalent interactions. There are two main challenges to developing a crystal suspension formulation. The first is the challenge of finding a robust crystallization condition that will produce crystals in less than 24 hours, which is sufficiently short for GMP manufacturing. The second challenge is the development of a drug product formulation that is suitable for injection while maintaining stability in the crystal structure. Crystallized proteins can address viscosity issues, potentially provide sustained release, and improve syringeability and injectability that are often associated with soluble protein formulations.

4. MONOCLONAL ANTIBODIES (mAbs)

Drug development pipelines are filled with mAbs, and a number of these products have become some of the best-selling drugs in the world. Typically, high doses of mAbs are needed to achieve the desired clinical benefit; however, the viscosity of solutions with a high concentration of mAbs present challenges during both manufacturing and administration.

In addition, as with other protein-based biopharmaceuticals, mAb-based therapeutics are susceptible to degradation. This presents stability challenges throughout the supply

chain as well as during long-term storage and drug product administration. Prior to formulation, degradation pathways and environmental risks to the molecule should be identified. To reduce molecule degradation during formulation, buffer, pH, and excipients should be optimized.

ASEPTIC VS NONASEPTIC FORMULATIONS

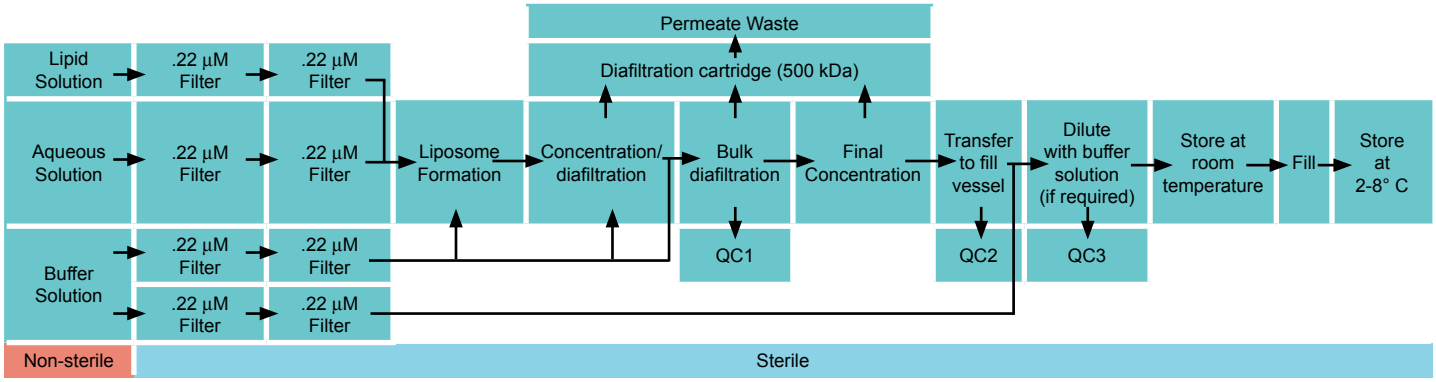
Aseptic processing is the handling of drug components, container closure systems, and excipients so as to prevent microbial contamination. The process used for a formulation is either aseptic or non-aseptic, which is dependent on whether or not the final product is sterile filterable. This, in turn, determines what class of cleanroom is needed. Formulation cleanrooms often contain equipment, such as laminar airflow hoods, isolators, sterilizing filters, autoclaves, and depyrogenation tunnels.

Formulations involving the use of organic solvents are an example of when aseptic processing would be required. Organic solvents are used in certain formulations to dissolve lipids or other hydrophobic formulation components that are insoluble in aqueous solutions. Some examples include ethanol, dimethyl sulfoxide (DMSO), and methanol. These solvents pose serious risks due to their flammability and require a very strategic engineer design, as the flame that is introduced into a suite has to be protected from explosion. A system must be able to detect the risk of ignition and shut down before that risk results in a danger to the staff as well as the product.

The example below illustrates the execution of a process for an aseptic liposome in a nonaseptic space, using a fully contained, fully enclosed system that is sterilized and validated.

The process begins with three separate solutions: a flammable ethanol lipid solution, an antibiotic solution in water, and a salt solution, which is used diafiltration. The three solutions are filtered separately into a sterile vessel (a stainless steel tank that was steamed and sterilized) using gamma-irradiated, single-use filtration. They are each filtered separately into the tank. Everything downstream of that solution occurs in an aseptic process where a concentrate is diafiltered. Because the liposome is more than a few hundred nanometers, the product is transferred out of the skid using aseptic connector technology in a sterile manner so that further filtration is not required. The process is a synthesis of stainless steel clean-in-place and sterilize-in-place coupled with the use of single-use bags, tubing, and filters, which are all gamma-irradiated, preassembled, and then steamed onto the skid.

Another example of an aseptic formulation under nonaseptic conditions is above. This shows both an antibody conjugation and ultrafiltration diafiltration (UF/DF). It is a TFF performed



SAMPLING	PROCESS	CLASSIFICATION
Incoming Inspection/ Release	Receipt & Release of Raw Materials	Unclassified ISO 8/Grade D Warehouse 2-8° C Storage
	Preparation of Components (Vial and Component Washing, Depyrogenation, Steam Sterilization)	ISO 8/Grade D via pass-through sterlilizers to ISO 5/6, Grade A/B
Samples from each formulation vessel	Process Solution Formulation & Mixing	ISO 7 Grade C
Pre-filtration Bioburden	Sterile Filtration	ISO 7 Grade C
Post Use Filter Integrity Testing Concentration via HPCL (QC-1, QC-2, and QC-3)	Bulk Production (Consisting of Infusion, Diafiltration, & Concentration)	ISO 7 Grade C to ISO 6 Grade B
	Product Transfer	ISO 6 Grade B
In-process samples collected after the fill. Finished product sampling conducted during inspection.	Aseptic Connection to Filler Aseptic Filling Operation Fill, Stopper, Seal	ISO 5 Grade A
	100% Visual Inspection/AQL	Inspection Areas (Unclassified)
	Transfer Product to Cold Storage	Warehouse 2-8° C Storage (Unclassified)

under nonaseptic conditions because it can be filtered after formulation, whereas liposomal formulations that cannot be filtered after are completed under aseptic conditions.

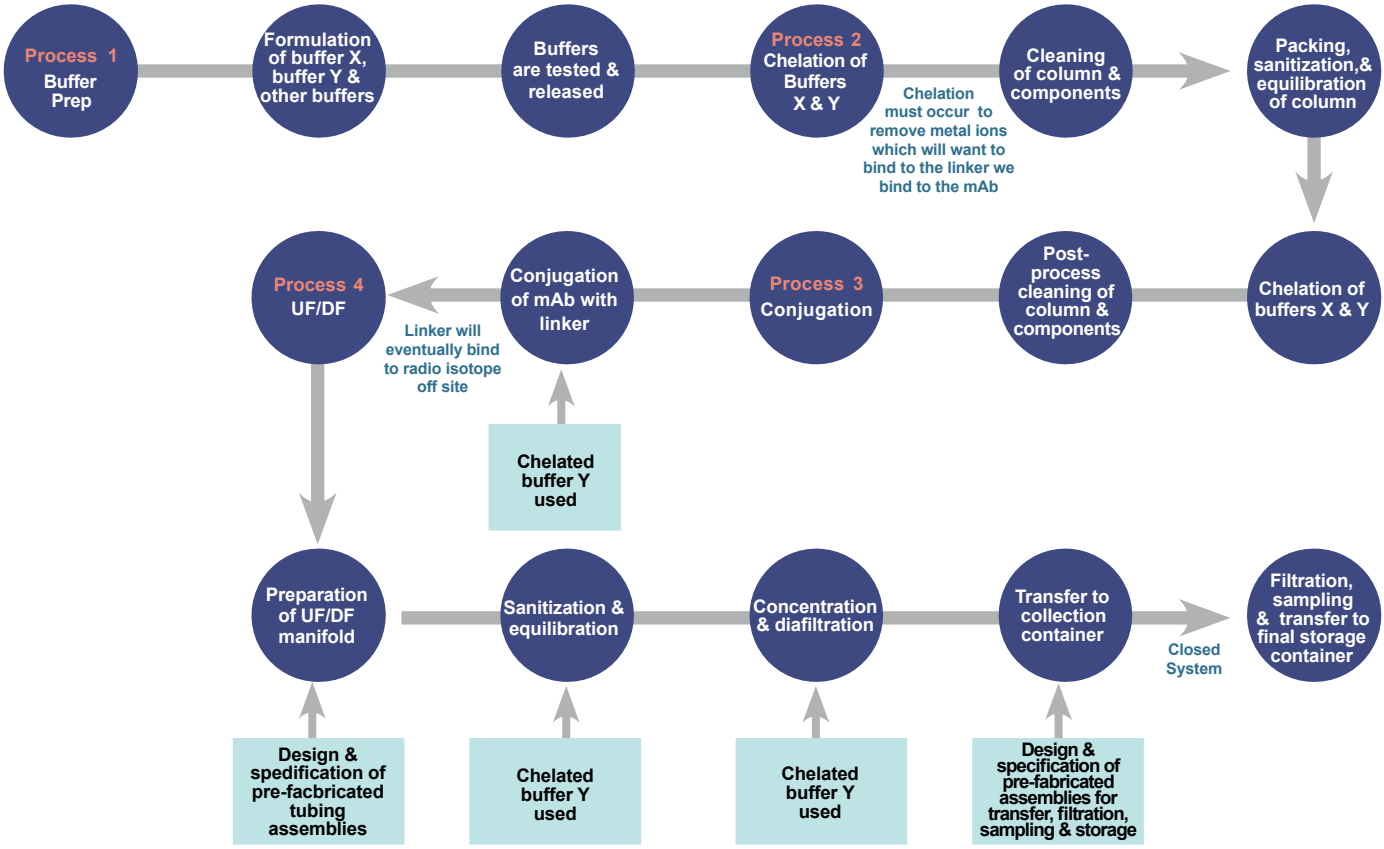
Generally, when a complex formulation is done in an aseptic environment, it goes straight to filling once the formulation has been completed. However, additional steps must be completed for formulations done in nonaseptic environments, which are illustrated in the example on the next page. In this case, buffers X and Y are being prepared for part of a larger process. There are a variety of steps using various excipients that were made previously so the buffers could be formulated later.

The buffers are chelated to remove metal ions because the metal ions will compete with the linker that is going to be conjugated onto the mAb later. Once the buffers have been chelated over the first several days of the process, the conjugation step is implemented on the third day and makes use of chelated Buffer X. After the conjugation is complete, the ultrafiltration and diafiltration (UF/DF) step is next, which requires another of the previously chelated buffers.

That conjugated antibody is then concentrated and diafiltered using prefabricated tubing assemblies, a TFF cassette holder and appropriate TFF cassette filter membrane. Chelated Buffer Y is then used to do the diafiltration portion followed by the transfer of material through use of additional prefabricated tubing assemblies. Finally, this closed-system transfer and final dilution is followed by another transfer/filtration and then aseptic sampling utilizing aseptic disconnectors. Final filtration can be completed later. In the meantime, it is stored in a preassembled storage vessel at a very specific temperature.

SUMMARY

Because of the complexity and sensitivity of complex formulations, not all companies have the resources necessary to complete these sophisticated processes under the appropriate conditions for cGMP manufacturing. Doing so successfully requires a considerable investment in equipment as well as skilled labor. A company must also be ready to present its processes to regulators for clinical trials and commercial filing.



Experienced CMOs that offer a one-stop shop formulation plus fill finish manufacturing deliver completely seamless handoffs from one phase to the next all at one single location. By doing this, supply chain risks as well as communication and process errors associated with transfers among multiple vendors can be minimized or even eliminated. CMOs that provide both single location services, paired with experience and a track record of success with complex formulations, can help overcome many of the current challenges in this field. As one of these CMOs, Althea continues to explore innovative drug formulation processes in order to advance the field of drug development.

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