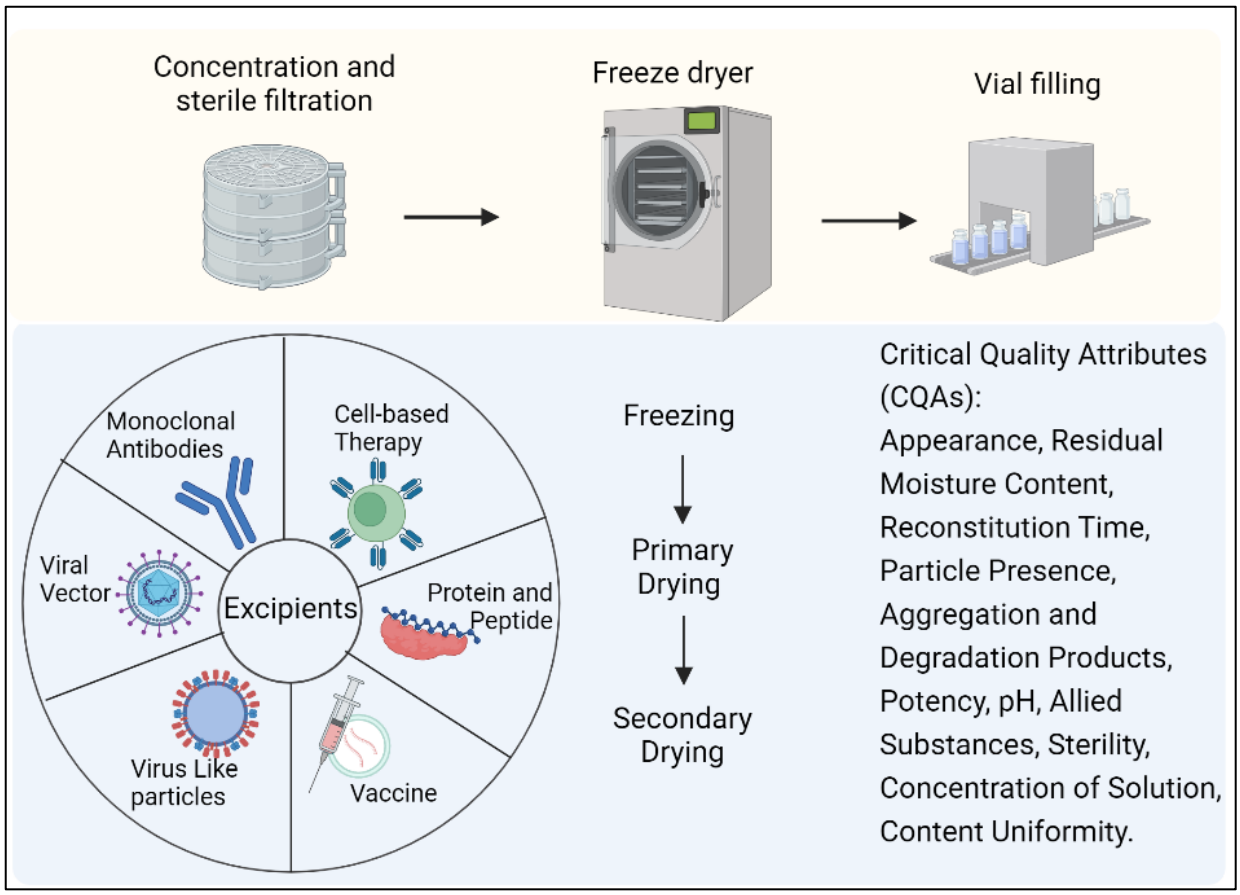

Graphical Abstract. Showing the formulation of biologics, Freeze-Drying process, fill and finsh product. (Created with BioRender.com).

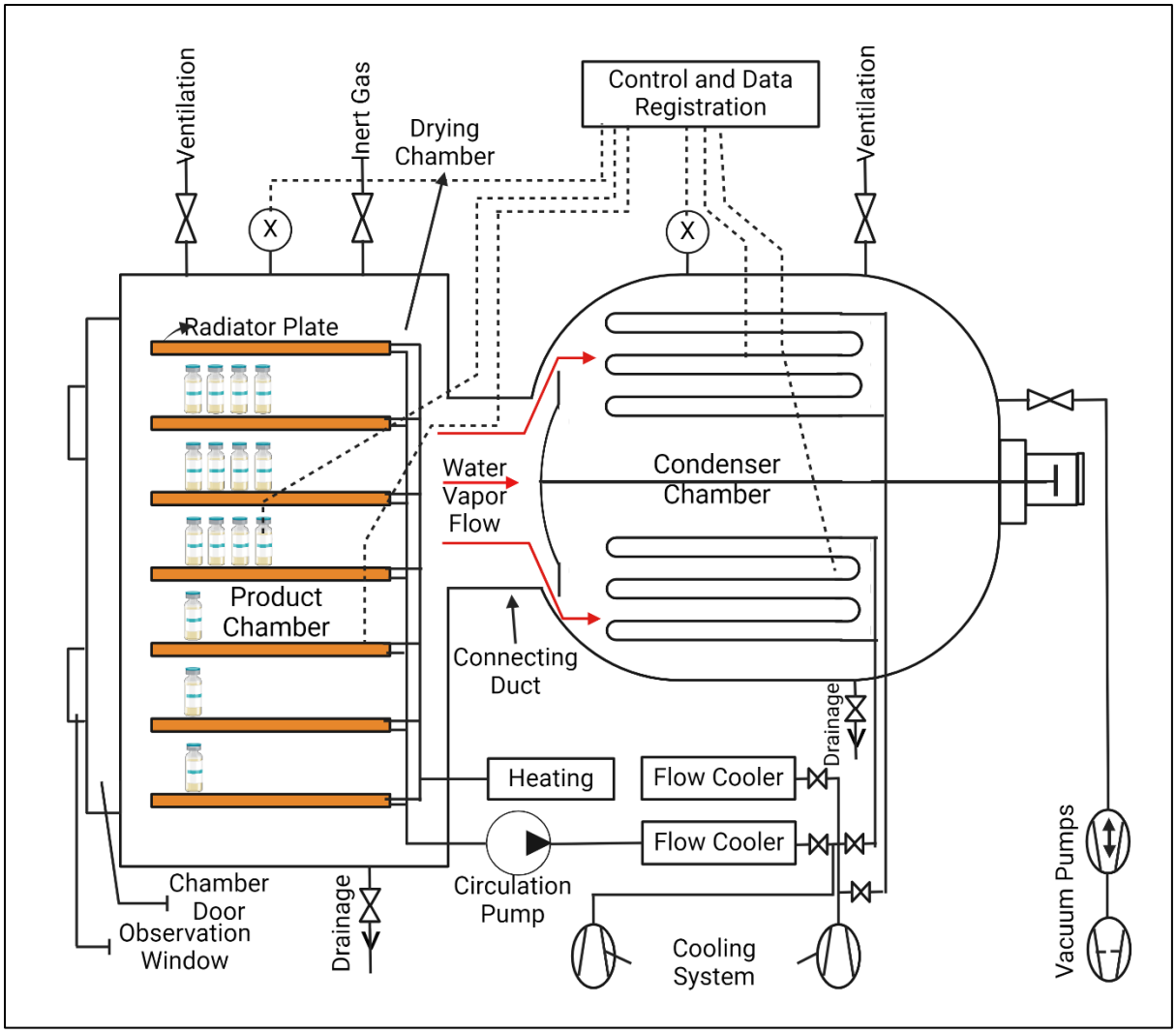

Figure 1. Schematic Overview of the Basic Design of a Lyophilizer. (Created with BioRender.com).

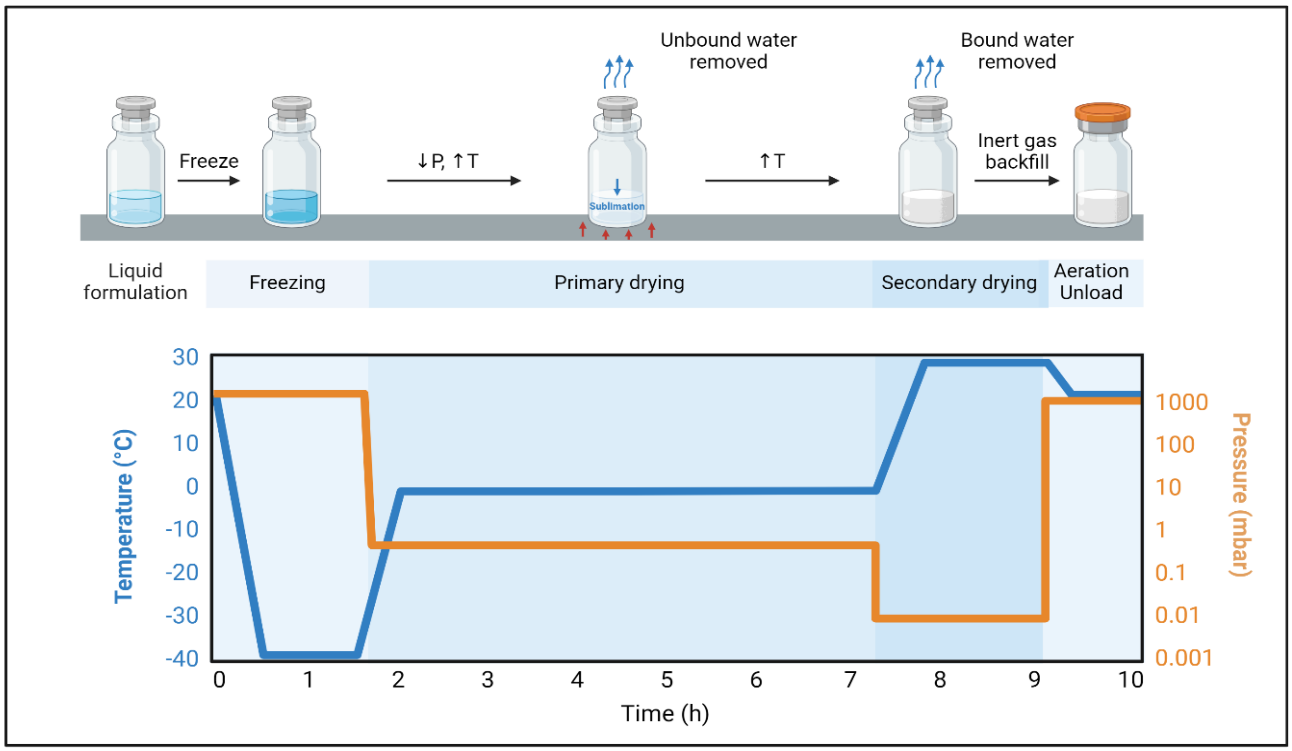

Figure 2: Lyophilization Process Overview, illustrating Freezing, Primary Drying, and Secondary Drying Stages (Created with BioRender.com).

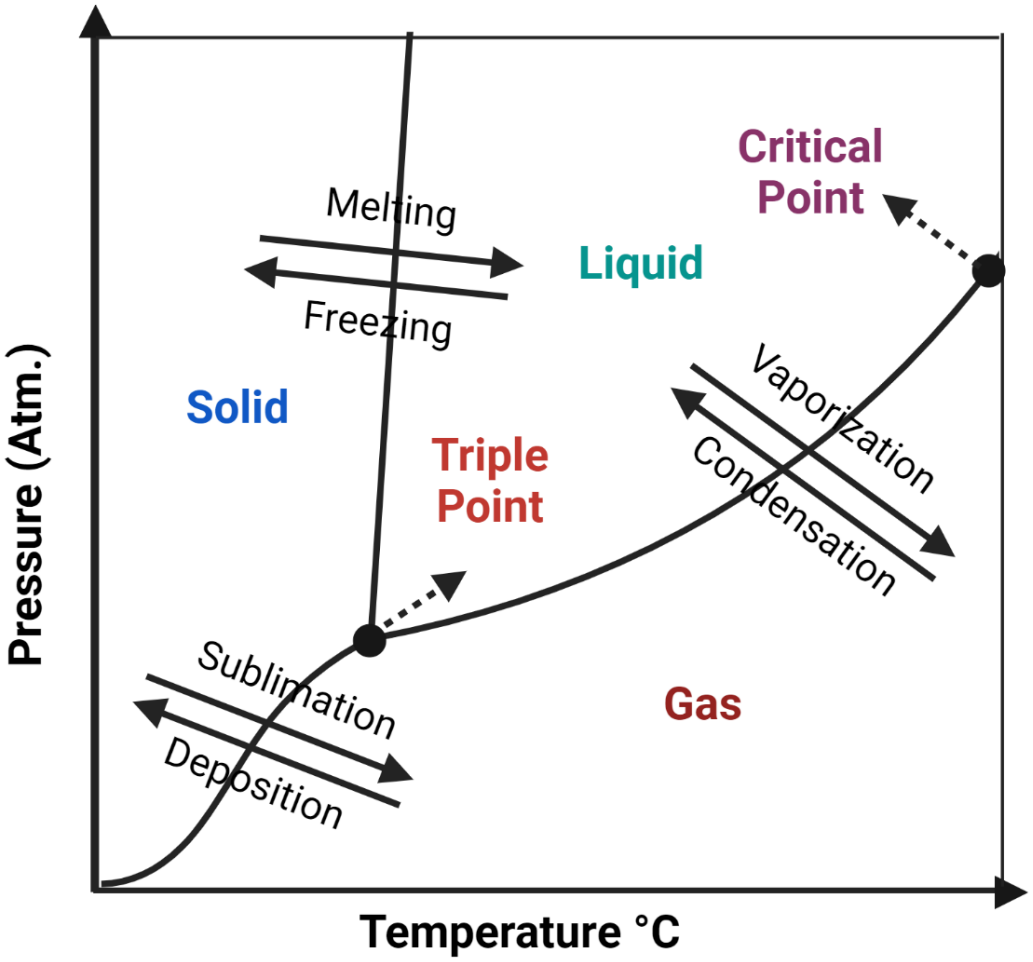

Figure 3: Principles of Lyophilization, Depicting Substance States and Triple Point Conditions. (Created with BioRender.com).

Table 1. Comparative Analysis of Primary Stabilization Technologies for Biologics

Technology	Key Principle	Advantages	Disadvantages	Ideal Use Cases	Ref
Liquid Formulation	Stabilization in aqueous solution.	Simple, low-cost, immediate administration, patient-friendly.	Limited shelf-life, requires cold chain, prone to chemical degradation (hydrolysis, deamidation) and microbial growth.	Stable mAbs, some vaccines with robust adjuvants, short-term use in-hospital products.	[9]
Spray-Drying	Rapid solvent evaporation via atomization into hot gas.	Continuous process, fast, more energy-efficient than lyophilization, good for particle engineering.	High shear and thermal stress can denature proteins, less suitable for very large or complex structures (e.g., viral vectors, LNPs).	Peptides, some enzymes, inhaled biologics, where a dry powder is required but extreme sensitivity is not a concern.	[10]
Lyophilization (Freeze-Drying)	Sublimation of ice under vacuum.	Excellent long-term stability, enables ambient temperature storage, preserves structure of highly sensitive molecules (proteins, mRNA, viral vectors), widely accepted by regulators.	Batch process, time-consuming, high energy cost, requires reconstitution, risk of freezing and drying stresses (aggregation, collapse).	High-value, sensitive therapeutics: mAbs, mRNA vaccines, gene therapies, plasma products, live microbes (probiotics/LBPs).	[11]

Table 2. Key Excipients in Lyophilized Biologic Formulations: Mechanisms and Applications

Excipient Category	Examples	Primary Mechanism of Action	Critical Considerations & Empirical Effects	Ref
Lyoprotectants (Sugars)	Sucrose, Trehalose	Water replacement hypothesis; form hydrogen bonds with proteins, vitrification to form a stable amorphous glassy matrix.	Amorphous state is crucial for stability. Tend to increase collapse temperature (T_c). High concentrations can increase viscosity and prolong primary drying. Trehalose has higher T_g than sucrose.	[40] [41] [42]
Bulking Agents	Mannitol, Glycine	Crystallize upon freezing, providing mechanical strength and elegant cake structure.	Crystalline form provides no protein stabilization. Must be fully crystallized (via annealing) to prevent crystallization during storage. Mannitol crystallization can cause pH shifts.	[43]
Surfactants	Polysorbate 20, Polysorbate 80	Minimize surface-induced aggregation at interfaces (air-liquid, ice-liquid) during freezing and drying.	Can undergo oxidative degradation, generating reactive species. Optimal concentration is a balance between protection and potential destabilization.	[50] [52]
Buffers	Histidine, Succinate, Citrate	Maintain pH stability during process-induced concentration shifts.	Must resist crystallization (e.g., Histidine is preferred over phosphate). Buffer capacity and pKa at various temperatures are critical.	[46] [47] [48]

Table 3. Application of PAT in Lyophilization Process

Stage	**Measurement Type**	**Method Used**	**Control Parameter**	**Ref.**
Freezing	Temperature	Thermocouples, Resistance Thermometers	Shelf Temperature, Product Temperature	[99]
	Ice Formation	Differential Scanning Calorimetry (DSC)	Freezing Rate, Nucleation Temperature	[100]
Primary Drying	Pressure	Capacitance Manometers, Pirani Gauges	Chamber Pressure, Shelf Temperature	[101]
	Residual Moisture	Near-Infrared Spectroscopy (NIR), Tunable Diode Laser Absorption Spectroscopy (TDLAS)	Product Temperature, Drying Rate	[16]
	Sublimation Rate	Manometric Temperature Measurement (MTM)	Chamber Pressure, Shelf Temperature	[102]
Secondary Drying	Residual Solvent	Gas Chromatography (GC), Mass Spectrometry (MS)	Product Temperature, Drying Time	[103]
	Final Moisture Content	Karl Fischer Titration, NIR Spectroscopy	Product Temperature, Drying Time	[104]
Post-Lyophilization	Product Stability	Dynamic Vapor Sorption (DVS), X-Ray Powder Diffraction (XRPD)	Storage Conditions, Residual	[105]

			Moisture Content	
	Reconstitution Time	Manual or Automated Reconstitution Tests	Moisture Content, Product Quality	[106]