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The Critical Role of pH in Biopharmaceutical Formulation and Processing

pH is a defining variable in biopharmaceutical development, directly impacting molecular stability, purification efficiency, and final product quality.

Even small deviations in pH can lead to aggregation, reduced yield, or loss of activity, making pH both a critical quality attribute (CQA) and a tightly controlled process parameter (CPP) across biologic workflows, with measurement and control aligned to pharmacopeial standards and compendial expectations such as USP <791> and EP 2.2.3.[1,2]

The importance of pH spans multiple therapeutic modalities, including mRNA-lipid nanoparticles (LNPs), monoclonal antibodies (mAbs), gene therapies, and injectable formulations, and extends across the full development lifecycle from early formulation through to downstream purification and final fill-finish.

To achieve consistent control through the development process, researchers need to optimize and maintain pH using defined, reproducible procedures. The following workflows highlight the best practices for ensuring product safety, efficacy, and stability.

ABOUT THE AUTHORS

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Andrew Witschi is Chief Technology Officer at Hudson Lab Automation, where he leads product development and automation strategy across the company's laboratory automation portfolio. He developed the Rapid pH technology after identifying an unmet need for automated pH measurement in bioprocessing workflows.

pH: A Critical Quality Attribute and Critical Process Parameter

pH directly affects molecular structure, stability, degradation pathways, solubility, and bioavailability. In product terms, it is often a CQA that defines product quality, safety, and efficacy across biologic formulations.[3–5] Because pH must be monitored consistently throughout development and manufacturing, pharmacopeial standards such as USP <791> and EP 2.2.3 define standardized methods for pH measurement to support accuracy, comparability, and regulatory confidence.

In process terms, the strategies used to achieve and maintain target pH, such as buffer optimization and composition, titration strategy, temperature, and other process conditions, are CPPs that impact product CQAs[3]. Together, these controls support reproducible pH measurement and robust process development under pharmacopeial expectations.

Thus, pH must be tightly specified and monitored across manufacturing stages. In biopharmaceutical systems, even minor pH deviations can lead to significant changes in product quality. For example, pH influences ionization states, which in turn affect the stability, intermolecular interactions, aggregation, and degradation kinetics of proteins[6–8] (Table 1).

Buffer systems are therefore designed not only to maintain pH but also to provide sufficient buffering capacity under dynamic processing conditions, including mixing, dilution, and purification. These considerations are central to robust and reproducible biologics development workflows and scale-up operations.

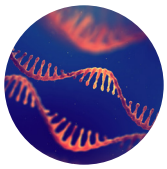
Rapid pH Plus supports plate-based pH measurement workflows, enabling consistent setup, execution, and data capture across experiments. By standardizing measurement and reducing operator variability, it supports more reliable comparison of conditions and faster optimization of pH-dependent processes.

Table 1. pH-Sensitive Transitions in Proteins

Protein	pH	Stability Profile
Bovine Serum Albumin	<3.0	Loss of stability and unfolding[8]
Human IgG4-based antibody (IgG4-N1)	≤3.3	Unfolding driven by intra-molecular repulsions[9]
Human IgG mAbs (IgG1, IgG2, IgG4)	5.0-5.5	Stable, with minimal degradation or aggregation[10]
Granulocyte-colony stimulating factor (G-CSF)	6.9	Rapid aggregation and loss of monomeric forms[11]

Complex Biologics Formulations Requiring pH Optimization

A common challenge amongst different therapeutic modalities is the need to evaluate and control pH conditions consistently across multiple samples, formulations, and process steps. This requires scalable, reproducible measurement workflows that can integrate with a range of complex biologics formulation processes, from mRNA systems to injectable products.



mRNA and LNPs

In mRNA-LNP systems, pH plays a foundational role in both formulation and delivery. During nanoparticle formation, acidic conditions in the range of pH 4-5 are required to protonate ionizable lipids, enabling electrostatic complexation with negatively charged RNA and efficient encapsulation.[12,13]

This pH-dependent ionization behavior is also critical for intracellular delivery. Ionizable lipids are designed to remain neutral at physiological pH, minimizing toxicity, but become protonated in the acidic endosomal environment, facilitating endosomal escape and cytoplasmic release of mRNA.[12,14]

Process parameters such as N/P ratio, buffer composition, and mixing conditions are tightly coupled with pH and collectively determine key CQAs, including particle size, polydispersity index (PDI), and encapsulation efficiency.[13,15]

Following formulation, pH is adjusted back to near physiological levels (approximately 7.4) to ensure stability and compatibility for administration.[12]

mAbs

For protein therapeutics, pH is a primary determinant of structural stability and aggregation propensity. Each antibody exhibits a characteristic isoelectric point (pI), and deviations from optimal pH ranges (i.e., 5.0-5.5) can increase the risk of aggregation, denaturation, or chemical degradation.[16]

When considering degradation, pH influences key degradation pathways such as deamidation, oxidation, and hydrolysis, all of which can compromise efficacy and increase immunogenicity.[17] Maintaining pH within a narrow stability window is therefore essential.

Buffer systems for mAbs must also balance competing requirements, including minimizing aggregation, maintaining solubility, and ensuring compatibility with excipients and delivery devices.[18] These challenges make pH control a central consideration in both formulation and long-term stability studies.

Complex Biologics Formulations Requiring pH Optimization

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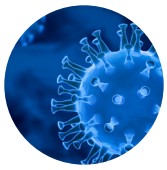

Gene Therapy and Viral Vectors

In gene therapy systems, including viral vectors such as adeno-associated virus (AAV), pH is critical for:

- Maintaining capsid integrity
- Preventing aggregation
- Preserving biological activity

Viral particles are highly sensitive to environmental conditions, and even modest pH shifts can lead to structural instability or loss of infectivity.[4] For instance, stress under low pH (i.e., pH 3.0) may trigger AAV8 aggregation and fragmentation.[19]

pH also influences interactions between viral particles and excipients, as well as the efficiency of purification processes such as chromatography and filtration.[20,21]

During the downstream cell lysis step, pH is adjusted to improve lysis efficiency, optimize endonuclease activity, and maximize AAV recovery while maintaining capsid stability. Different AAV production platforms use different lysis pH ranges, including pH 8.0-8.3 for transient transfection systems, pH 6.8-7.4 for infection-based systems, and pH 8.0-8.4 for stable producer cell lines.[22]

Maintaining optimal pH conditions is therefore essential across both upstream and downstream operations to ensure product consistency and potency.

Injectable Formulations

For injectable drug products, pH must be carefully controlled to ensure both product stability and patient tolerability. Certain APIs, such as exenatide, may undergo rapid degradation under elevated pH conditions.[23] High pH conditions (i.e., pH 10) may also induce irritation and discomfort at the injection site.[24]

Formulation strategies often involve buffer systems that maintain pH within a physiologically acceptable range while also stabilizing the drug substance. These systems must account for factors such as ionic strength, excipient compatibility, and storage conditions.[25]

The Rapid pH platform supports single- or 4-probe capacity, with optional Power Wash, enabling scalable performance across diverse biologics development workflows.

Purification and pH Control

pH remains a critical variable during downstream purification, where it is used strategically to separate, stabilize, and concentrate biopharmaceutical products. Techniques such as ion exchange chromatography rely on precise pH control to modulate charge interactions between the target molecule and the stationary phase.[18]

In LNP and RNA-based systems, downstream processing includes buffer exchange and purification steps to remove solvents (e.g., ethanol), unencapsulated RNA, and process impurities. These steps typically involve transitioning from acidic formulation conditions to neutral pH for stability and storage.[26]

Chromatographic separations, including ion exchange and size exclusion methods, are highly sensitive to pH, which governs binding affinity, resolution, and recovery. Optimized pH conditions are therefore essential for achieving high purity and yield in bioprocessing workflows.[27]

In addition, pH influences aggregation, precipitation, and degradation during purification, making it a key parameter for process robustness and scalability.[18]

Integrated buffer strategies are often required to maintain consistent pH across unit operations and minimize process variability. These unified, coordinated systems can manage buffers across multiple processing steps, ensuring pH is maintained within target ranges despite transitions between harvest, chromatography (polishing), ultrafiltration, and final formulation stages (Table 2).

Table 2. pH Ranges in mAbs Purification.[a]

Stage	Technique	pH Range
Capture	Protein A Affinity	6.0-8.0, with low-pH elution at 2.5-4
Polishing	Anion Exchange	8.0-8.2
Polishing	Cation Exchange	6.0-8.0 (elution)
Polishing	Membrane Chromatography	≥7.0

a Data reported from Liu et al., 201028

Reproducible pH Measurement Across Biologics Workflows

Accurate pH measurement is essential for ensuring process consistency, product quality, and regulatory compliance. Compendial methods such as USP <791> and EP 2.2.3 define how pH should be measured to support accuracy, reproducibility, and comparability of data across development and manufacturing workflows.

Variability in calibration, probe condition, temperature, or sample handling can introduce measurement error, leading to incorrect process decisions and inconsistent results.[29] Standardized measurement procedures, including calibration, probe handling, temperature control, and consistent sample preparation, are therefore critical to maintaining data integrity in regulated environments.

Unlock New Possibilities With Rapid pH Plus

pH is a unifying parameter across biopharmaceutical modalities, influencing everything from molecular stability and delivery efficiency to purification and final product performance. Its role as both a CQA and a CPP underscores its importance in modern drug product development workflows.

By integrating robust buffer systems, optimizing process conditions, and maintaining precise analytical control, manufacturers can ensure consistent product quality across diverse therapeutic platforms. As biologics formulations continue to evolve, from mAbs to mRNA and gene therapies, the strategic management of pH will remain central to successful biopharmaceutical innovation.

As pH workflows become increasingly complex and data-intensive, reproducible measurement systems are critical for maintaining consistency across experiments and development stages.

Rapid pH Plus enables plate-based pH measurement across complex biologics workflows, allowing teams to screen more conditions, compare results across experiments, and generate traceable data at scale.

With Rapid pH Plus, researchers can achieve more efficient buffer optimization, improved reproducibility, and faster development timelines across formulation and downstream processing workflows.

Contact Hudson Lab Automation to learn more about how Rapid pH Plus can accelerate buffer and formulation pH optimization workflows for your laboratory.

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