

Michael De La Cruz, Senior Scientist and Tom Fletcher, Director of Process Development

Department of Research and Development, Manufacturing Sciences and Technologies, FUJIFILM Irvine Scientific, 1830 E. Warner Ave., Santa Ana, CA 92705

## CURRENT CHALLENGES IN MEDIA PREPARATION

### Mixing and Handling Problems/Needs

- "Difficult...mixing protocols...powder mixing involving much art and experience, along with much variability."
- "I would like to avoid the long mixing times required to mix the components."
- "The manipulation of powders for packaging is not easy and powders are volatile."

### Consistency Problems

- "Variation in powders is normal. We see differences [inconsistencies] between the media we purchase for GMP manufacturing and non-GMP research... grinding is likely done at different scale, likely using different mixing equipment."
- "We see inconsistencies...the 'same' media from different manufacturers' facilities, in manufacturer's and our own mixing."
- "Variability [with our powdered media]. Sometimes we redo a process and the results are different."

### Lack of Industry Information and Support

- "Media companies are not adding value. All they do is fill our orders."
- "Never any investigation or discussion of the powders we ask them to make."
- "They never suggest how we could be doing things better."

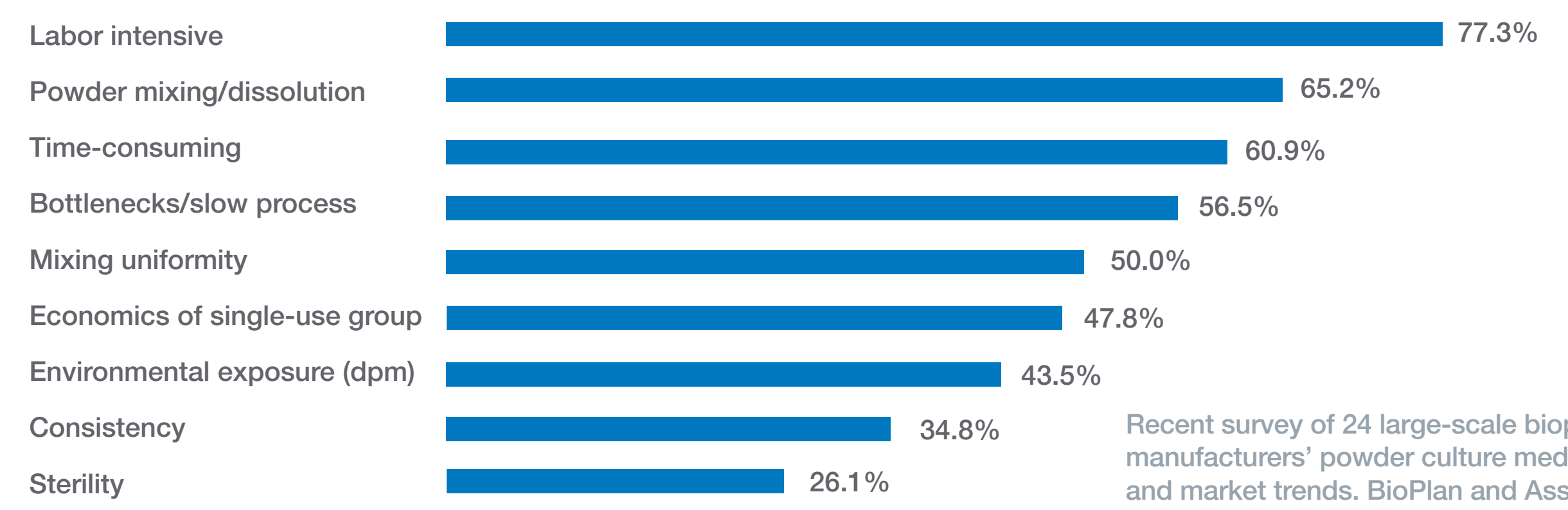
### Need for Closed Systems

- "Regulators want more closed processing or cleaner air in media prep."
- "If all powder media components were mixed together without exposure to the environment, these would be preferred and a big advantage."

**Where can we add value to your process?**  
We offer new opportunities to collaborate with you.

## THE NEED FOR BETTER LARGE-SCALE MEDIA PREPARATION METHODS

### How can the methods be improved?



Recent survey of 24 large-scale biopharmaceutical manufacturers' powder culture media packaging, preparation, and market trends. BioPlan and Associates, Inc. (2014).

## SOLVING THE MEDIA PREPARATION PUZZLE

### Process Challenges in Biologics Manufacturing

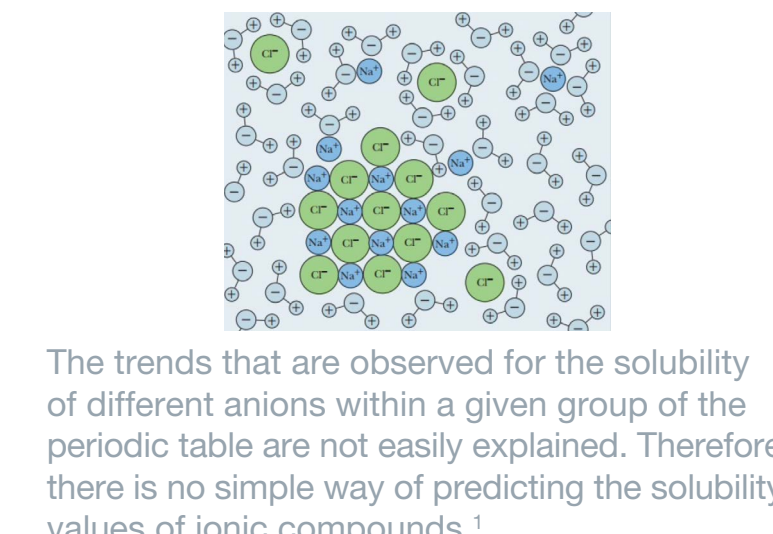
- Issues with media powder hydration:
- Solubility
  - Interactions
  - Complexation
  - Reduction/Oxidation
- Liquid supplementation
- Error due to human intervention from:
- Handling and weighing
  - Avoidable adjustments
  - Volume [quantum satis, or q.s.]
  - pH
  - Osmolarity

### Applied Design Principles

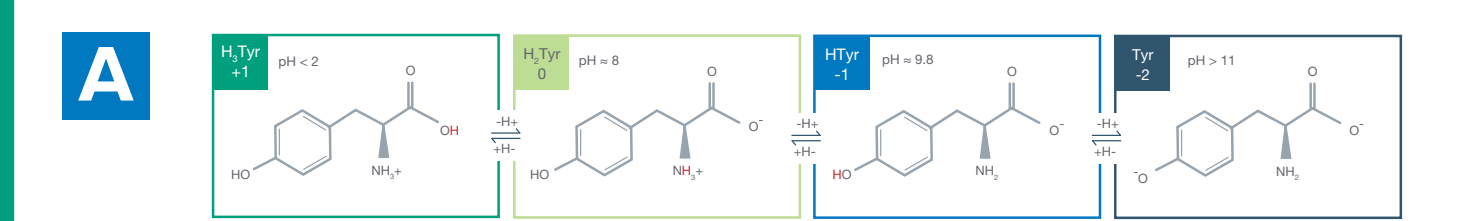
- Simplicity
- Risk reduction
- Efficiency
- Risk mitigation
- Cost reduction
- Maintained product quality attributes
- Maintained productivity

### Dissolution of a Salt is Conceptually Understood as a Sequence of the Two Processes

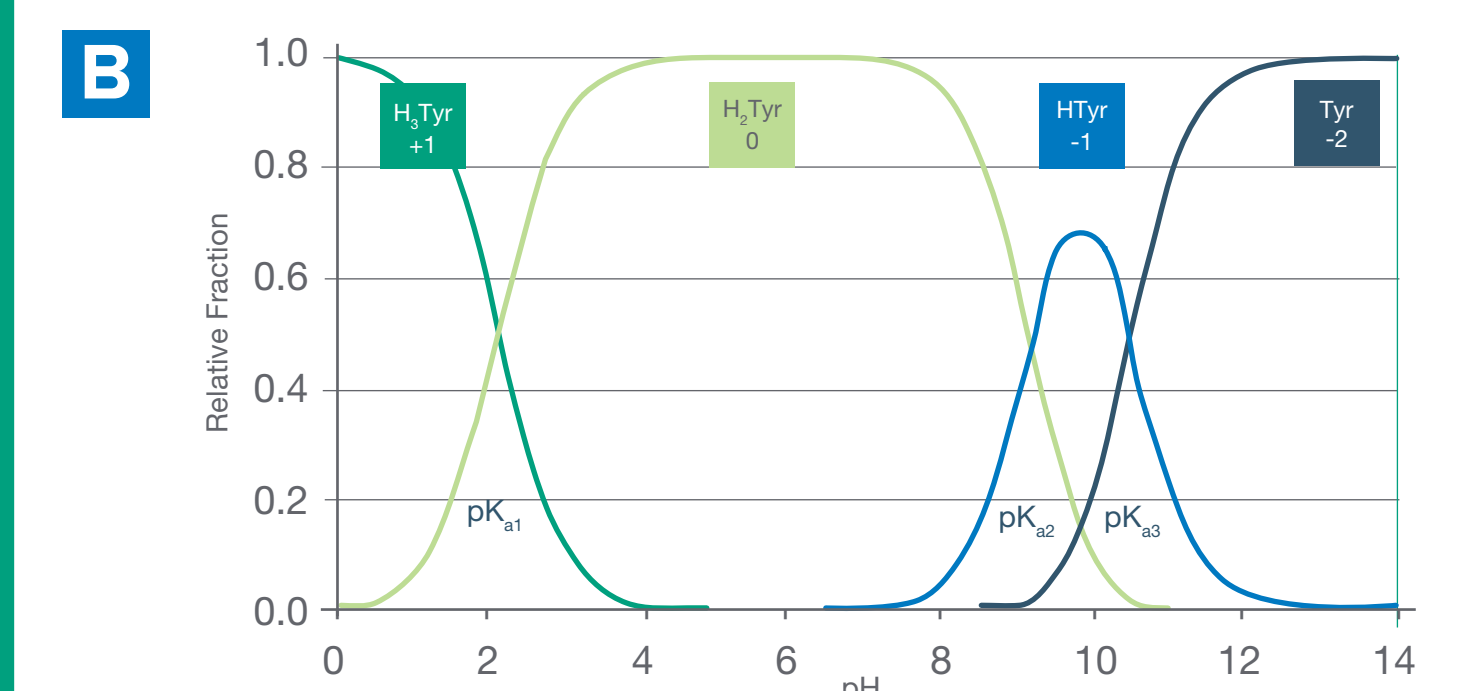
The breakup of the ionic lattice of the solid (Lattice energy).  
The attachment of water molecules to the released ions (Solvation or Hydration energy).  
Solubility of solid compounds depends on a competition between these two engines, including entropy effects related to changes in the solvent structures (the degree of disorder or randomness in the system).



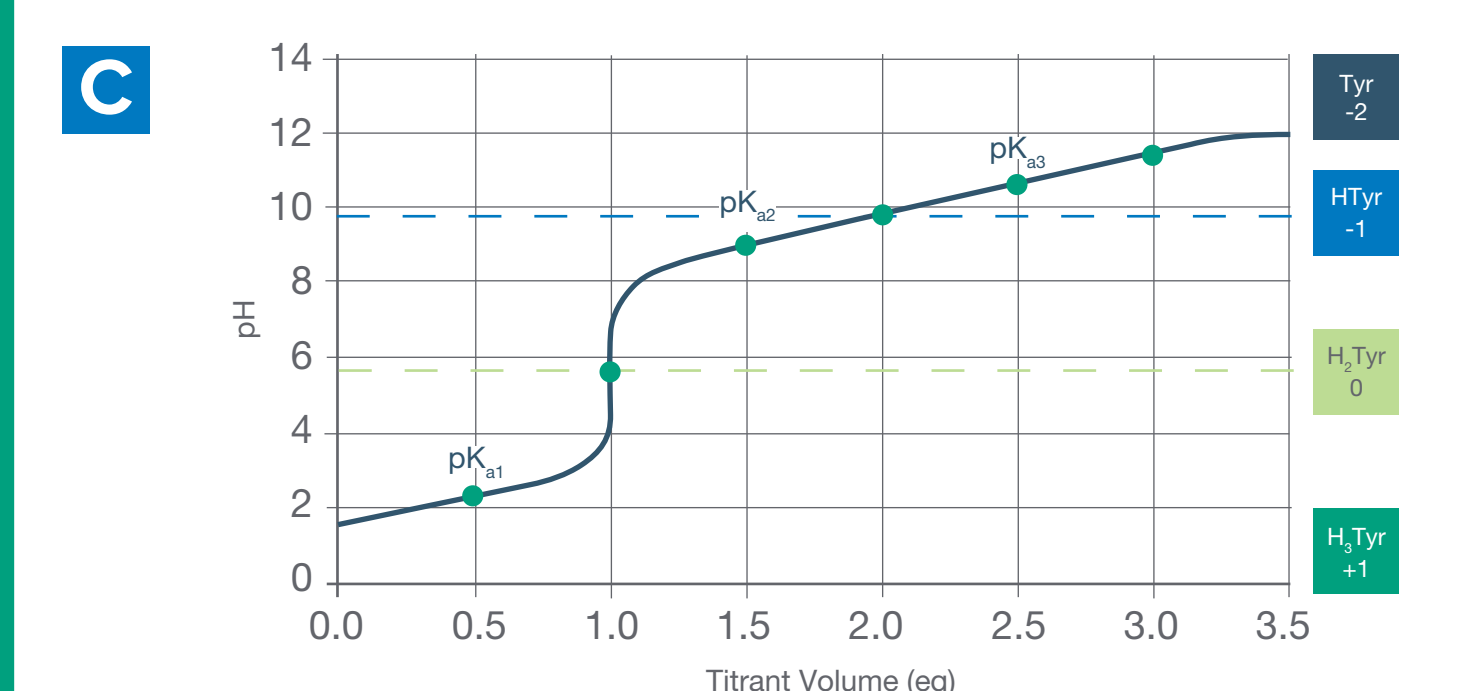
### Low Solubility of Tyrosine at Neutral pH is Partly Due to the Low Acid Dissociation Constant of its Phenol Side Chain Group



The relative concentration of the monoprotonated tyrosinate base species, HTyr, does not reach unity.



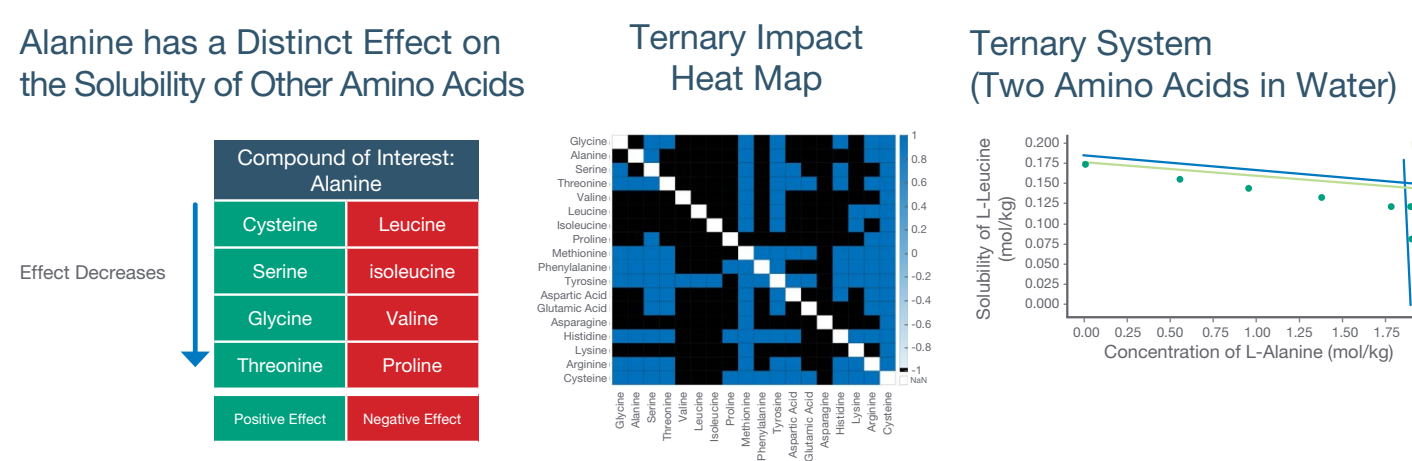
The tyrosine solution is effectively buffered by H2Tyr, HTyr, and Tyr at the second equivalence point.



Tyrosine is triprotic, dibasic, the only aromatic amino acid with an ionizable side chain, and one of the three amino acids containing a hydroxyl group.<sup>2</sup>

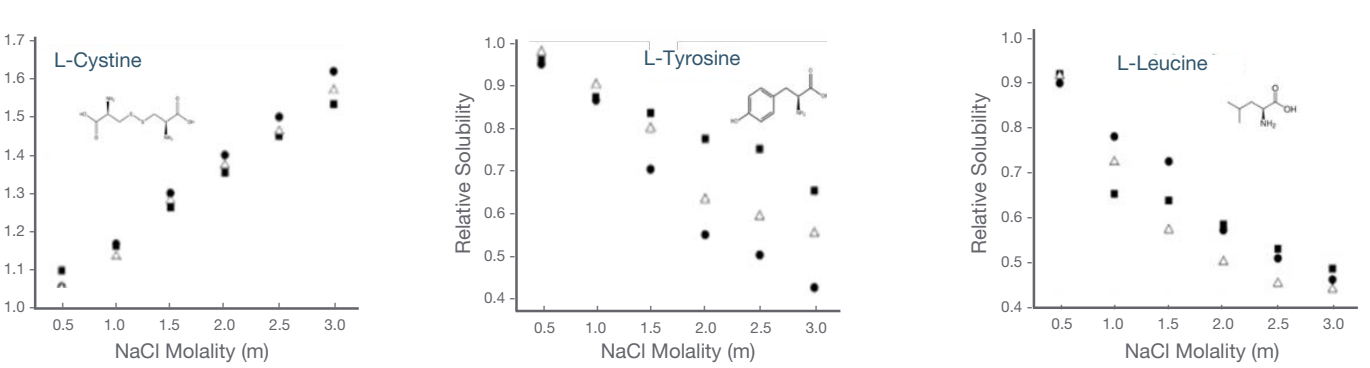
### Interactions of Medium Components Can Lead to Contrasting Effects on Their Solubility

The solubility limit of one amino acid as a function of another AA's concentration was experimentally measured.  
Solubility limit was ensured by observing precipitate in sample.<sup>3</sup>



### Sodium Chloride Concentration Has Disparate Effects on the Solubility of Each Amino Acid

Results showed a salting-out effect for L-Tyrosine and L-Leucine while a salting-in effect was observed for L-Cysteine in different experimental conditions.<sup>4</sup>



### Manufacturing Science

Development of innovative media handling and simple preparation methods that are easy for end-users.

### Common Media Components

Amino Acids	Inorganic Salts
Glycine	Calcium Chloride
L-Alanine	Cupric Sulfate
L-Arginine	Ferric Nitrate/ Sulfate
L-Aspartic Acid	Magnesium Chloride/ Sulphate
L-Cysteine	Potassium Chloride
L-Cystine	Sodium Bicarbonate
L-Glutamic Acid	Sodium Chloride
L-Glutamine	Sodium Phosphate, mono/dibasic
L-Histidine	Zinc Sulfate
L-Isoleucine	
L-Leucine	
L-Lysine	
L-Methionine	
L-Phenylalanine	
L-Proline	
L-Serine	
L-Threonine	
L-Tryptophan	
L-Tyrosine	
L-Valine	
Others	
D-Glucose	Linoleic Acid
Hypoxanthine Na	Lipoic Acid
Thymidine	Putrescine-2HCl
	Sodium Pyruvate

### Holistic Approach

The component parts are best understood in context and in relation to one another and to the whole medium.

### Reductionist Approach

A complex system is the sum of its parts. Gain a basic understanding of every medium component and its interactions.

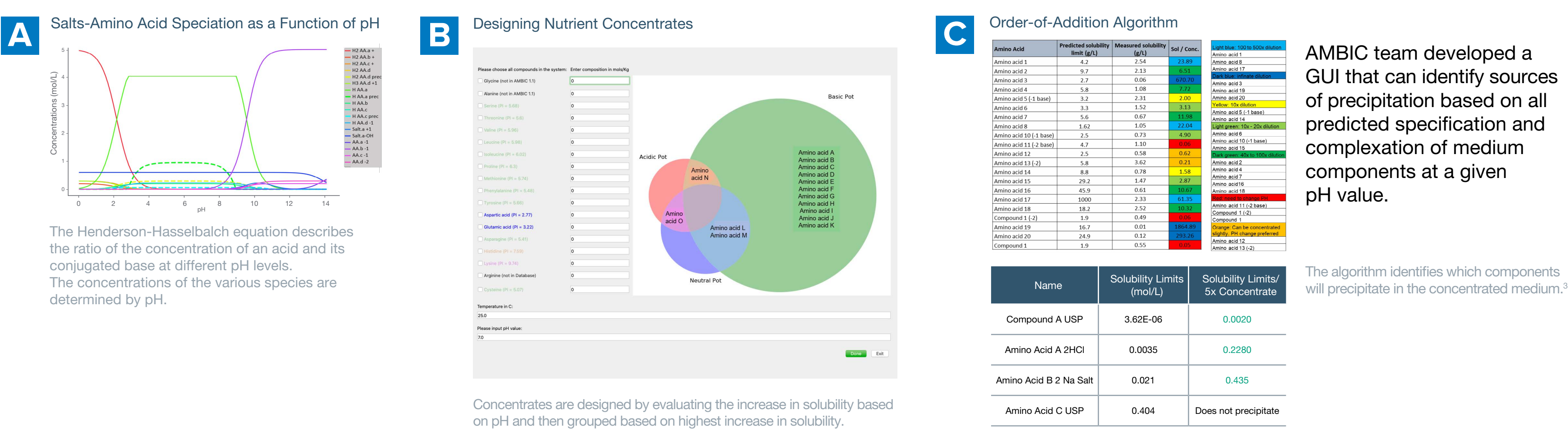
### Innovative Technology

Evaluation and development of new technologies that help improve customer experiences, provide risk reduction, or cost advantages.

### Examples of Novel Media Components that Improve Efficiency and Reduce Risk

- Dipeptides
- Iron Chelates
- Acetylated amino acids
- Glycyl-L-Tyrosine Dihydrate

### In Silico Modeling Provides a Quick and Cost-Efficient Way to Identify Potential Sources of Precipitation



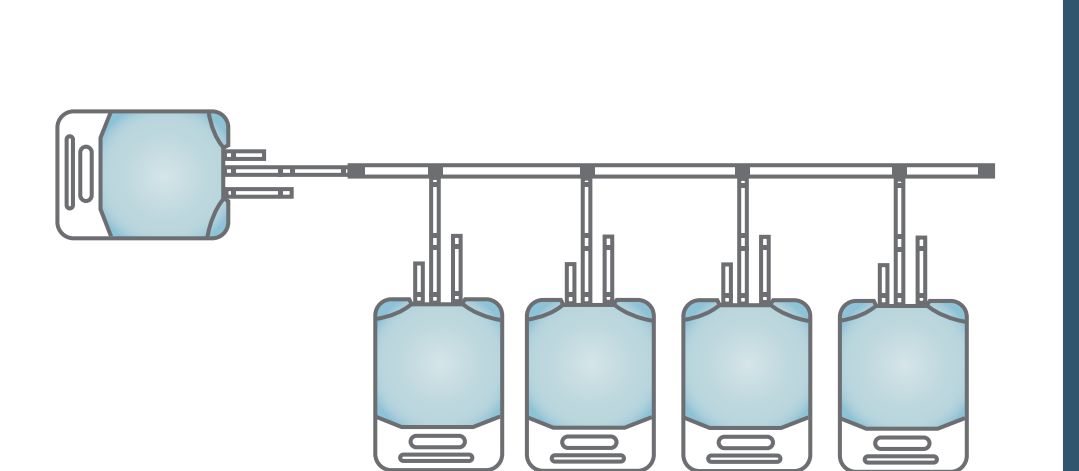
### Automated Solutions Being Developed at FUJIFILM Irvine Scientific

Fully automated media preparation is now possible for most cell culture media.

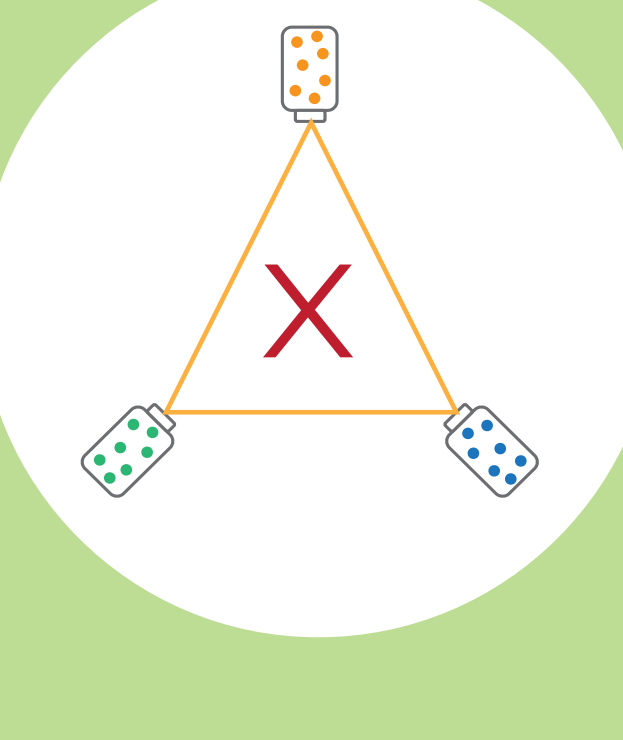


### Comprehensive Packaging Solutions

Both off-the-shelf and custom plastic bags with manifolds of weldable tubing.



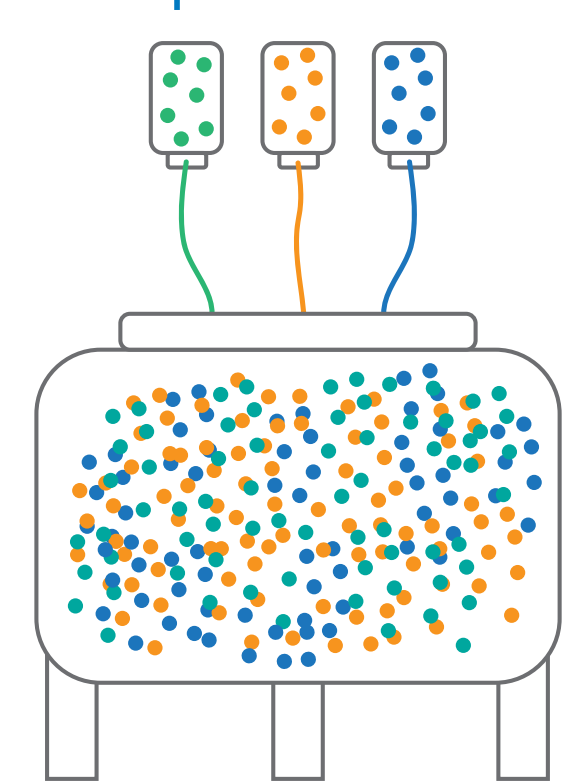
User reports pH related solubility issues for a powdered supplement



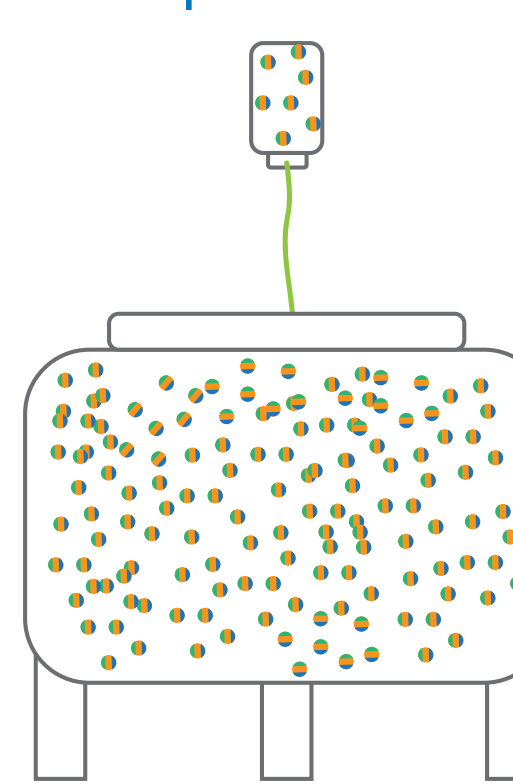
Past media services offerings could not fully address solubility issues encountered by a user

FUJIFILM Irvine Scientific provided user with panel of media with verified performance for screening

### Complex/Insoluble



### Simple/Soluble



MSAT resolves the solubility issue by simplifying the media preparation process, reducing risk, and increasing efficiency

Step 1

Step 2

Step 3

Step 4

User identifies the optimal mixture and orders the supplement powder. Soon after support is requested to resolve a solubility issue.

MSAT medium designing/ engineering activities occur

Issue is resolved with a user-verified, user-friendly solution for cell culture media solubility and performance