Mitigating Risk Through Simplified and Balanced Cell Culture Media

Michael De La Cruz* and Tom Fletcher
Department of Research and Development, FUJIFILM Irvine Scientific, 1830 E. Warner Ave., Santa Ana, CA 92705
*michael.de.lacruz@fujifilm.com

Introduction

Three Manufacturing Science and Technology (MSAT) Service Case Studies

Company A
- Simplify hydration method of production medium for continuous process

Company B
- Investigate potential sources of precipitate in concentrated feed medium

Company C
- Investigate if factors in preparation method cause the aberrant process performance

MSAT Service goals are:

- Develop user-friendly cell culture media and other process solutions for late-stage drug substance manufacturing, and cell and gene therapy process development. (focus shifts from speed to increasing efficiency and reducing risk)
- Improve media process workflows by:
  - Simplifying preparation methods
  - Reducing errors associated with avoidable adjustments, handling, and weighing
- Reduce cost by improving the reliability of the media preparation unit operation increasing the overall efficiency in the facility

Manufacturers' comments specific powder-media associated problems to be avoided:

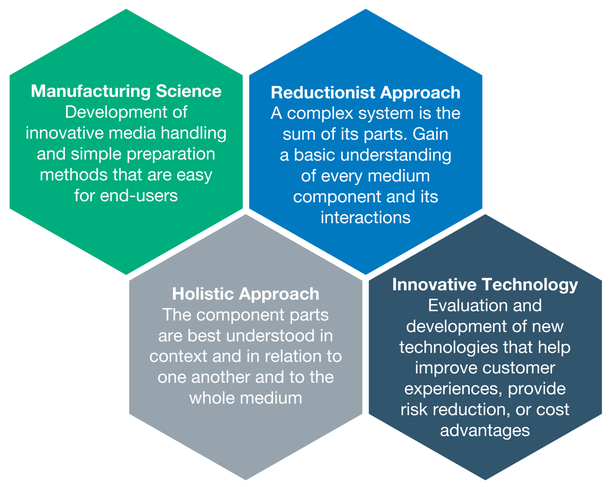
Mixing and Handling Problems/Needs
- *I would like to avoid the **long mixing times** required to mix the components.*

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.*

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.*

MSAT Framework



Applied Design Principles

- Simplicity
- Risk Reduction
- Efficiency
- Risk Mitigation
- Cost Reduction
- Maintained product quality attributes
- Maintained productivity

Challenges faced by End Users

Powder hydration issues:
- Solubility
- Component Interactions
- Complexation
- Reduction/Oxidation

Error due to human intervention from:
- Handling and weighting
- Avoidable adjustments to volume(q.s.), pH, and osmolality

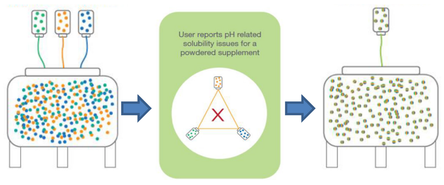
Abstract

Manufacturing Science and Technology (MSAT) Service was created to develop user-friendly cell culture media and other process solutions for the late stages of drug substance and cell and gene therapy process development when customers' efforts shift from speed to market to increasing efficiency and reducing risk. These three case studies presented describe how the MSAT service collaborates with FISI's customers to improve their cell culture media process workflows by simplifying their preparation methods and rebalancing the powder formulas to reduce errors associated with avoidable adjustments, handling, and weighing. Overall, the MSAT service improved the reliability of customer's media and preparation methods, resulting in significant reduction in labor and cost.

Case Study A

Goal: Simplify cell culture media preparation method

A. Complex/Insoluable — ABC Blend — Simple/Soluable



B.

Inorganic Salts:	mg/L		
CaCl2 (anhyd.)	165	99.1	33.2
CaCl2•2H2O			
CuSO4•5H2O		0.00125	0.0025
Fe(NO3)3•9H2O			
FeSO4•7H2O		0.415	0.83
KCl	330	376.8	223.6
KNO3	0.076	0.038	
MgSO4 (anhyd.)		28.61	57.22
MgCl2 (anhyd.)	28	48	
NaCl	4900	6049.5	7599
NaHCO3	3024	2100	1176
Na2HPO4		71	142
NaH2PO4•H2O	125	62.5	
NaSeO3	0.017	0.0085	
ZnSO4.7H2O		0.43	0.86
Other:	mg/L		
Adenine sulphate			
Adenosine-5-triphosphate			
Adenosine-5-phosphate			
Cholesterol			
2-Deoxy-D-ribose			
D-Glucose	4500	3151	1802
Glutathione (reduced)			
Guanine HCl			
HEPES	5958	2979	
Hypoxanthine Na		2.385	4.77
Linoleic acid		0.04	0.08
Lipoic acid		0.155	0.21
Phenol red	15 / None	8.1	12 / None
Putrescine 2HCl		0.0805	0.161
Ribose			
Sodium acetate			
Sodium pyruvate	110	110	110
Thymidine		0.35	0.7
Tween 80			
Uracil			
Xanthine Na			
Amino Acids:	mg/L		
L-Alanine	25	16.95	8.9
L-Arginine HCl	84	110	211
L-Asparagine	25	49.5	15
L-Aspartic acid	30	21.5	13
L-Cysteine HCl H2O		17.5	35
L-Cystine 2HCl	91.2	45.6	
Cystidine			
L-Glutamic acid	75	60.35	14.7
L-Glutamine	584	365	146
Glycine	30	59.75	7.5
L-Histidine HCl.H2O	42	31.5	21
L-Hydroxyproline			
L-Isoleucine	105	54.5	4
L-Leucine	105	59	13
L-Lysine HCl	146	91.25	36.5
L-Methionine	30	17.25	4.5
L-Phenylalanine	66	39.5	5
L-Proline	40	37.25	34.5
L-Serine	42	26.25	10.5
L-Threonine	95	53.5	12
L-Tryptophan	16	9	2
L-Tyrosine	104	55.9	7.8
L-Tyrosine 2Na.2H2O			
L-Valine	94	52.85	11.7

C.
- Combined components to balance the formula
- Engineered formula for solubility and reliable, easy preparation
- Reduced intervention
- Reduced time and labor
- Designed for scale-up

The total preparation time was reduced from 4 hours to around 1 hour

Final production medium powder composition:
- 45% Growth medium
- 20% Feed medium
- 35% Supplement
- 100x liquid supplement

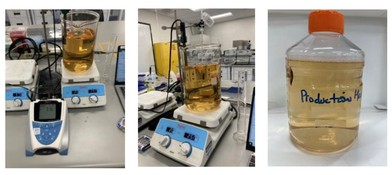
Simplified medium and preparation method
- Single powder
- No adjustments to pH or osmolality during hydration
- No q.s. step
- Glutamine and $NaHCO_3$ added as separate powders

Customer better prepared for adopting automated Oceo Rover™ methods – based on the same basic chemistry.

Received positive customer feedback: "The media preparation was a wonderful experience."

Customer stated:
- *"We managed to complete 10L preparation and filtering within an hour. In contrast to the 3 to 4 hour process of manually weighing individual component."*
- *"The pH and osmolality fall within right range without adjusting."*
- *"Metabolite measurement are close to the one manually prepared."*
- *Finally, our customer "confirmed that the cells are growing well with the MSAT medium."*

Three liter medium preparation



- pH shifts from ~5 to 7 after the addition of the 100x liquid supplement, leading to full powder dissolution
- Targeted pH is achieved after sodium bicarbonate is added
- No q.s. step required by starting with the total vrequired volume

Case Study B

Goal: Identify the cause of precipitation and implement reliable preventative method

Feed medium stability failing the required 12-day storage hold. Observed in three powder qualification batches.

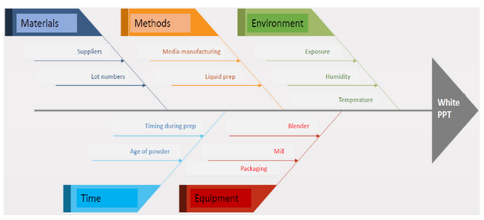
White precipitate forming during storage hold at 4°C.

Observed powder clumping after just 30 minutes on the bench.

Powder shown after two hours of exposure.

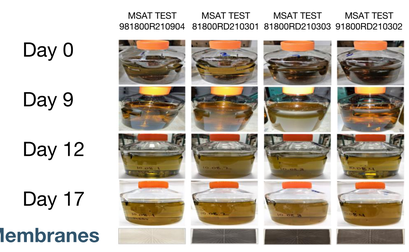
- Batch record data
- Method
- Equipment
- Parameters
- Time
- Environment
- Raw materials
- Sources of materials

Ishikawa cause-and-effect diagram



The powder was slightly darker near the surface and the storage container emitted sulfur-like odor

Side-by-side EMS powders screen



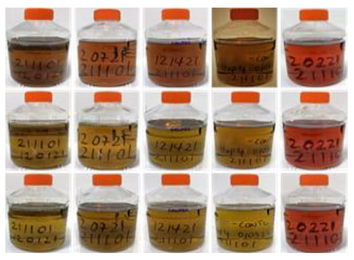
Data Summary of seven EMS tested powders

Lot#	981800RD 210301	981800RD 210303	981800RD 210302	981800RD 210904	981800RD 211005	981800RD 211006	981800RD 211101
Name	Test #1	Test #2	Test #3	MSAT Control	L-Cysteine source Test 1	L-Cysteine source Test 2	(-) L-Cysteine deficient Control
Date of manufactured	03/17/21	03/18/21	03/17/21	09/02/21	10/28/21	10/28/21	11/04/21
Number of days before used to prepare LF31 at FISI (Passed 12-day storage test: Yes or No)	205 (No)	204 (Yes)	205 (No)	2 (Yes)	8 (Yes)	8 (Yes)	2 (Yes)
				3 (Yes)			
				8 (Yes)			
				36 (Yes)	27 (Yes)	27 (No)	
				67 (No)			
Day of storage when precipitate was first noticed	10	17	8	None (30+)	18	18	None
				None (30+)			
				None (30+)	7	15+	
				38			
				10			
L-Cysteine supplier	Supplier A	Supplier A	Supplier A	Supplier B	Supplier A	Supplier B	NA

Effect of moisture on GMP powder

Preparation #	1	2	3	4	(-) L-Cystein
Powder storage day	30	36	43	67	31

Liquid media storage day



Powder instability was due to exposure: time + moisture during storage and handling

Powder degradation leads to precipitation.

Changes implemented at FISI
- Purge mill with dry Nitrogen (currently practiced in cGMP)
- Control humidity in manufacturing areas (currently practiced in cGMP)
- Add desiccants in packaging
- Additional moisture barriers (bags)
- Identify and then implement a superior custom container/ closure system

Recommendations for Company B
- Allow packages to warm up to ambient temp prior to opening and use
- Minimize time containers are open
- Do not save/ reuse opened containers (use complete contents or discard excess powder if any remaining)
- Frequent ordering of right-sized batches to minimize storage times

Conclusions:
- Tighter control of age and exposure of powder
- Powder solubility diminish during storage after
  - 67 days in large lined bucket (5 kg)
  - 27 days in small container (1 kg)
- Hygroscopic components in powder- Choline Chloride
- Strong sulfide like odor indicates a degradation reaction
- Precipitate was observed after more than 30 days of storage

Case Study C

Goal: Investigate if factors in preparation method cause the aberrant cell culture performance

Altered metabolism and growth rate observed with liquid medium supplemented with L-Glutamine solution at point-of-use.

Slower L-Glutamine utilization.
Lag in growth.
Premature decline in viability.
Increase osmolality due to:
- Elevated lactate level-> increases base demand
- Reduced uptake of the main feed

Identified factors for Design-of-Experiment (DOE)

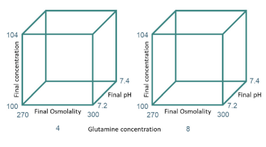
Factor Name	Units	Low	High
Final pH		7.2	7.4
Final osmolality	mOsm/Kg	270	300
Final concentration	%	100	104
Glutamine concentration	m/M	4	8

Two factors identified for OFAT experiments

Factor Name	Tested Levels	
Copper concentration	2x	
Glutamine addition	Liquid	Powder

Variable space for full factorial design of four factors.

The fractional factorial design has only 8 out of the possible sixteen conditions.



Fractional factorial experiment designed to evaluate the effect of the four selected factors

Generated nine prototypes for customer to conduct 20 run screening study.

Run	Final pH	Final osmolality (mOsm/Kg)	Final concentration (%)	Glutamine concentration (mM)
1	7.2	270	100	4
2	7.2	270	100	4
3	7.2	270	104	8
4	7.2	270	104	8
5	7.2	300	100	8
6	7.2	300	100	8
7	7.2	300	104	4
8	7.2	300	104	4
9	**7.3**	**285**	**102**	**8**
10	**7.3**	**285**	**102**	**8**
11	**7.3**	**285**	**102**	**8**
12	**7.3**	**285**	**102**	**8**
13	7.4	270	100	8
14	7.4	270	100	8
15	7.4	270	104	4
16	7.4	270	104	4
17	7.4	300	100	4
18	7.4	300	100	4
19	7.4	300	104	8
20	7.4	300	104	8

Nine unique replicated conditions
Resolution IV, alpha = 0.05

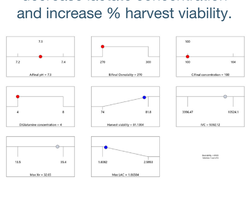
Cell culture data generated using the Ambr®15 system and analyzed using Design-Expert® software.

Higher levels of glutamine and osmolality appear to negatively affect the % Harvest Viability

ANOVA - % Harvest Ability

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	68.61	6	11.44	4.98	0.0047	significant
A-Final pH	7.29	1	7.29	3.17	0.0938	
B-Final Osmolality	29.70	1	29.70	12.93	0.0024	
C-Final concentration	0.0025	1	0.0025	0.0011	0.9741	
D-Glutamine concentration	29.16	1	29.16	12.70	0.0026	
AC	2.25	1	2.25	0.9798	0.3370	
AD	0.0025	1	0.0025	0.0882	0.7703	
Residual	36.74	16	2.30			
Lack of Fit	3.10	3	1.03	0.3995	0.7557	not significant
Pure Error	33.64	13	2.59			
Cor Total	105.35	22				

Optimal settings predicted to decrease lactate concentration and increase % harvest viability.



Higher levels of glutamine appear to increase the observed lactate concentration

ANOVA - MAX Lactate

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.4360	4	0.1090	3.15	0.0397	significant
A-Final pH	0.0008	1	0.0008	0.0233	0.8804	
B-Final Osmolality	0.0991	1	0.0991	2.86	0.1079	
C-Final concentration	0.0212	1	0.0212	0.6117	0.4443	
D-Glutamine concentration	0.3149	1	0.3149	9.10	0.0078	
Residual	0.6230	18	0.0346			
Lack of Fit	0.1855	5	0.0371	1.10	0.4051	not significant
Pure Error	0.4375	13	0.0337			
Cor Total	1.06	22				

The optimal set points are:
Osmolality - 270 mOsm/kg
Glutamine concentration - 4 mM
pH - 7.3
Final concentration - 100%.

Characterized media preparation process to fine tune method's set points and avoid edges of failure

Lowest growth observed in condition with all factors at the higth levels of final pH, osmolality, concentration, and Glutamine concentration.
- Higher lactate seen in conditions with lower growth
- Low osmolality leads to lower lactate production.
- High osmolality overall negative effect
- High Final concentration appears to be beneficial to growth
- Interaction between pH and other factors appear to negatively affect growth when tested at their high levels
- High variation seen in midpoint's lactate production
- Copper effect seems to be cell line specific