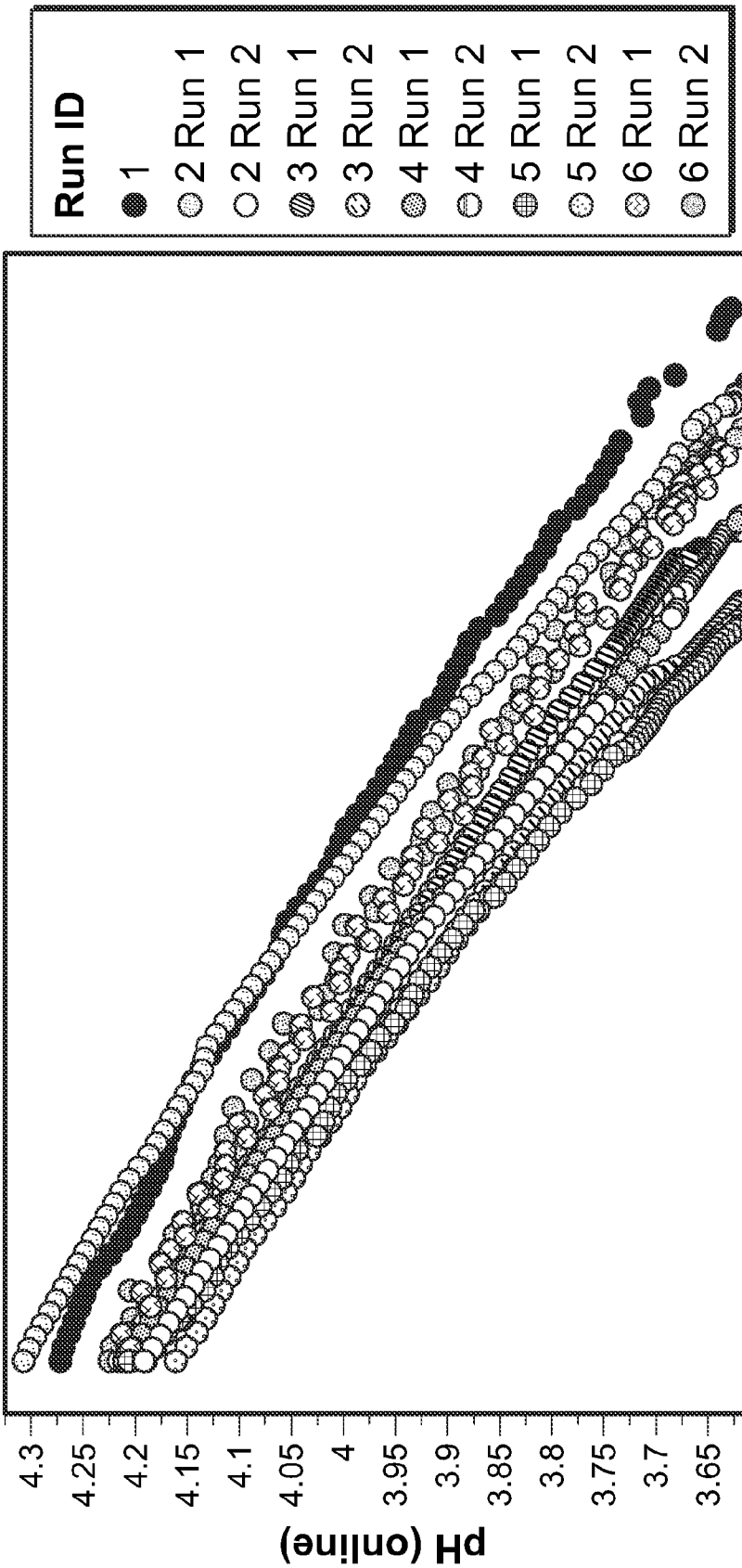

Run ID
1
2 Run 1
2 Run 2
3 Run 1
3 Run 2
4 Run 1
4 Run 2
5 Run 1
5 Run 2
6 Run 1
6 Run 2
pH (online)
4.3
4.25
4.2
4.15
4.1
4.05
4
3.95
3.9
3.85
3.8
3.75
3.7
3.65
3.6
Titrant Added (rot/kg)
0
1
2
3
4
5
6
7
8
9
10

FIG. 1

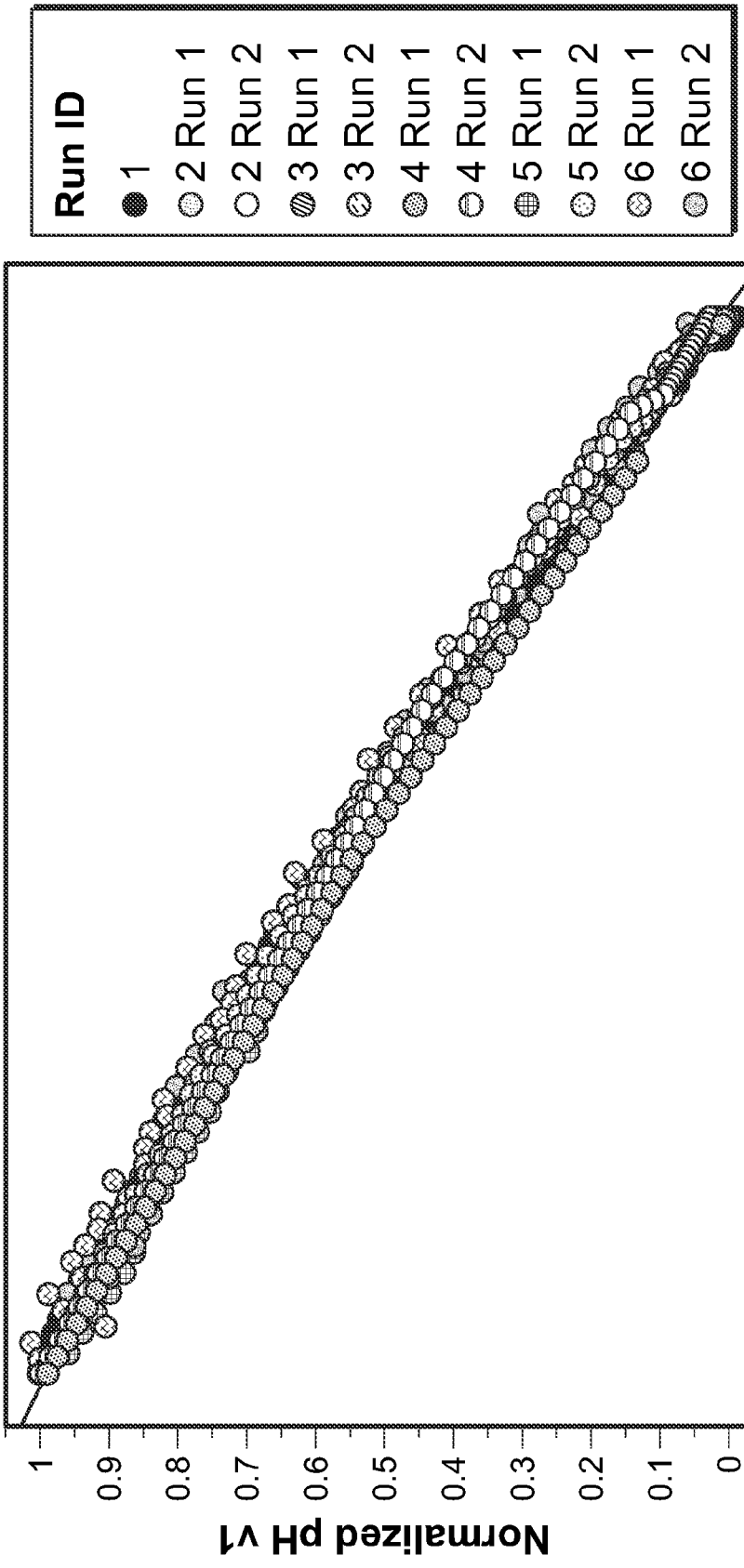

Run ID
1
2 Run 1
2 Run 2
3 Run 1
3 Run 2
4 Run 1
4 Run 2
5 Run 1
5 Run 2
6 Run 1
6 Run 2
Normalized pH v1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Normalized Titrant Addition v1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

FIG. 2

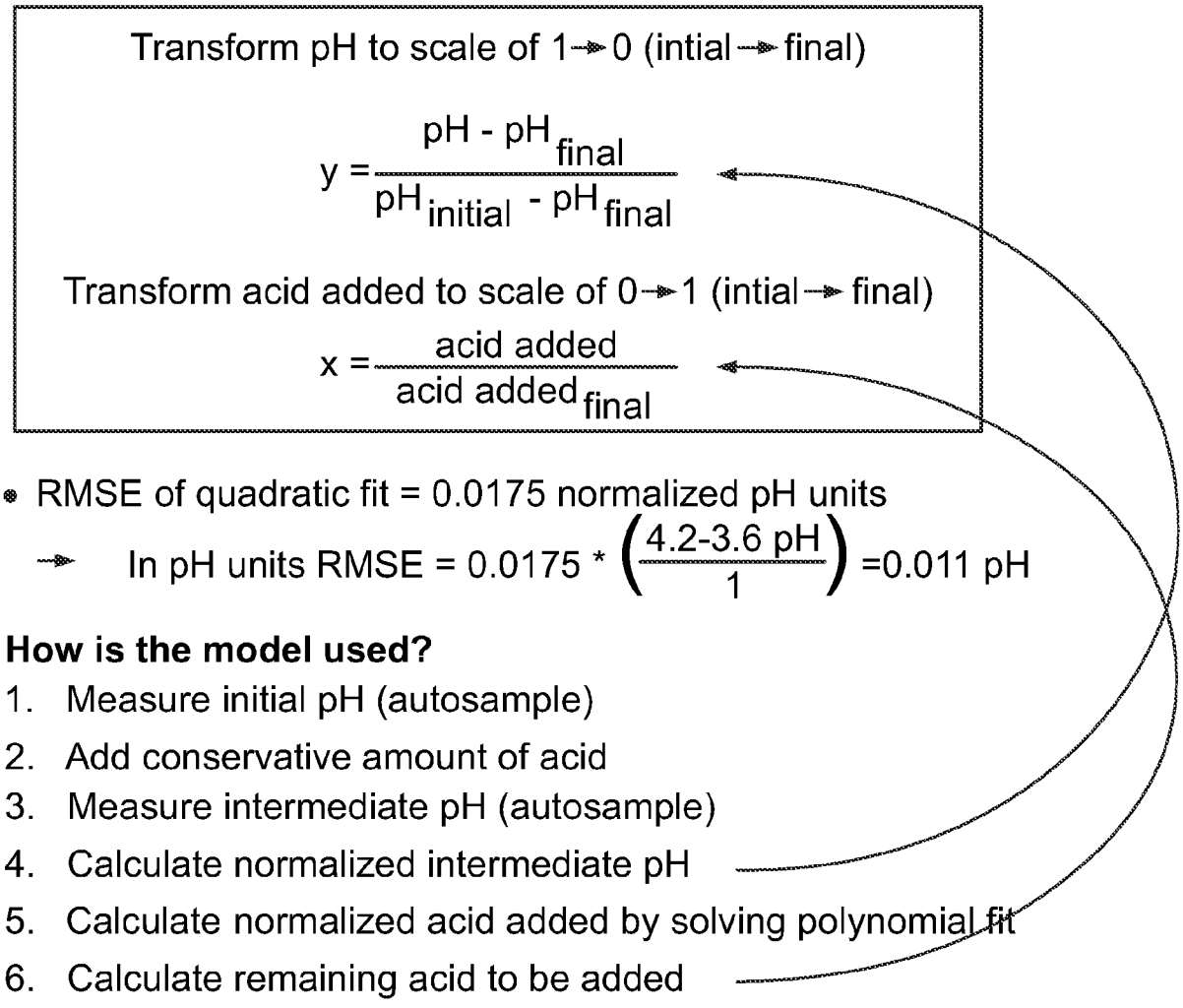

Transform pH to scale of 1→0 (intial→final)
$y = \frac{pH - pH_{final}}{pH_{initial} - pH_{final}}$
Transform acid added to scale of 0→1 (intial→final)
$x = \frac{acid\ added}{acid\ added_{final}}$
RMSE of quadratic fit = 0.0175 normalized pH units
In pH units RMSE = $0.0175 * \left(\frac{4.2\text{-}3.6\ pH}{1}\right)$ =0.011 pH
How is the model used?
1. Measure initial pH (autosample)
2. Add conservative amount of acid
3. Measure intermediate pH (autosample)
4. Calculate normalized intermediate pH
5. Calculate normalized acid added by solving polynomial fit
6. Calculate remaining acid to be added

FIG. 3

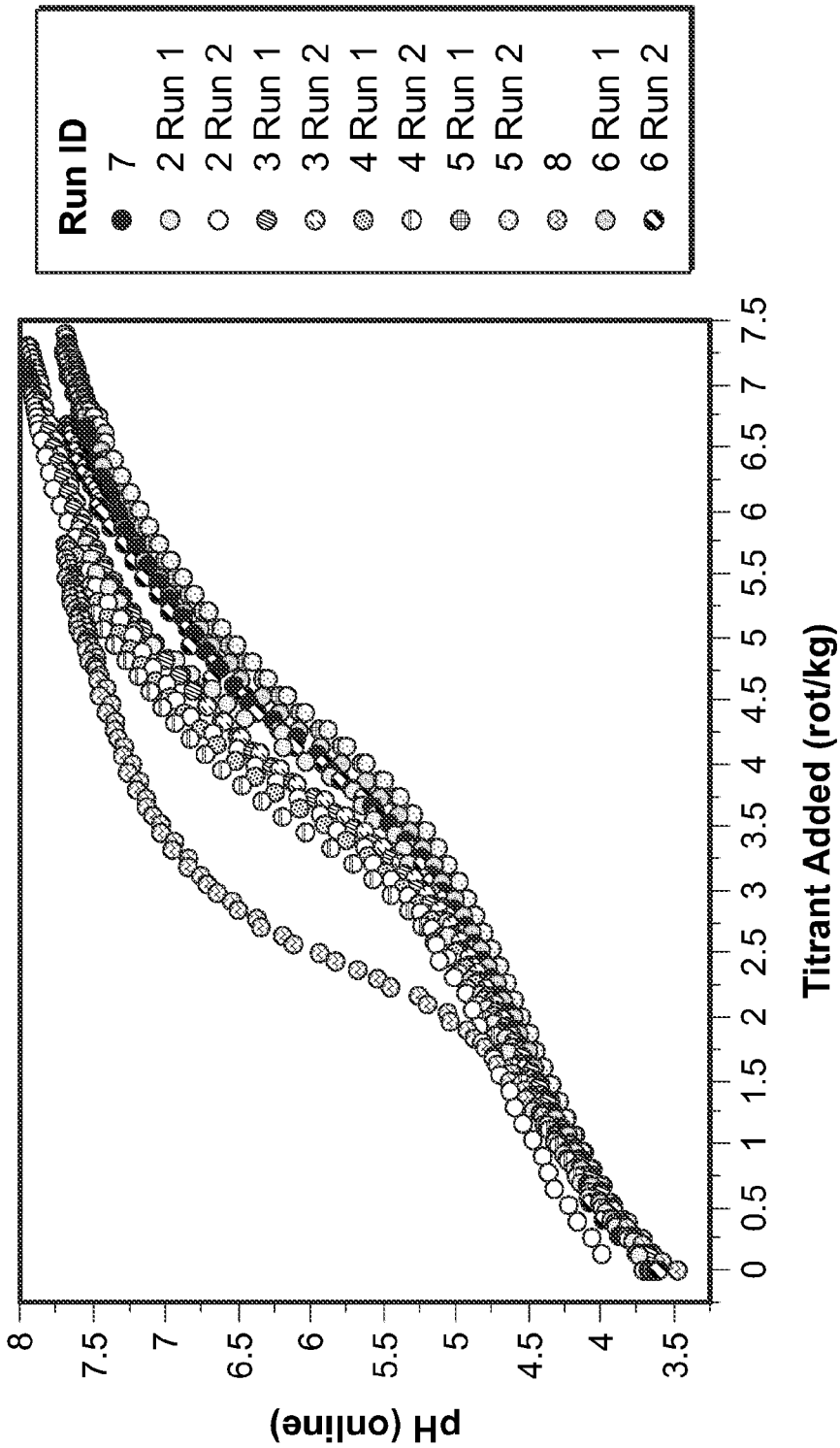

Run ID
7
2 Run 1
2 Run 2
3 Run 1
3 Run 2
4 Run 1
4 Run 2
5 Run 1
5 Run 2
8
6 Run 1
6 Run 2
pH (online)
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
Titrant Added (rot/kg)

FIG. 4

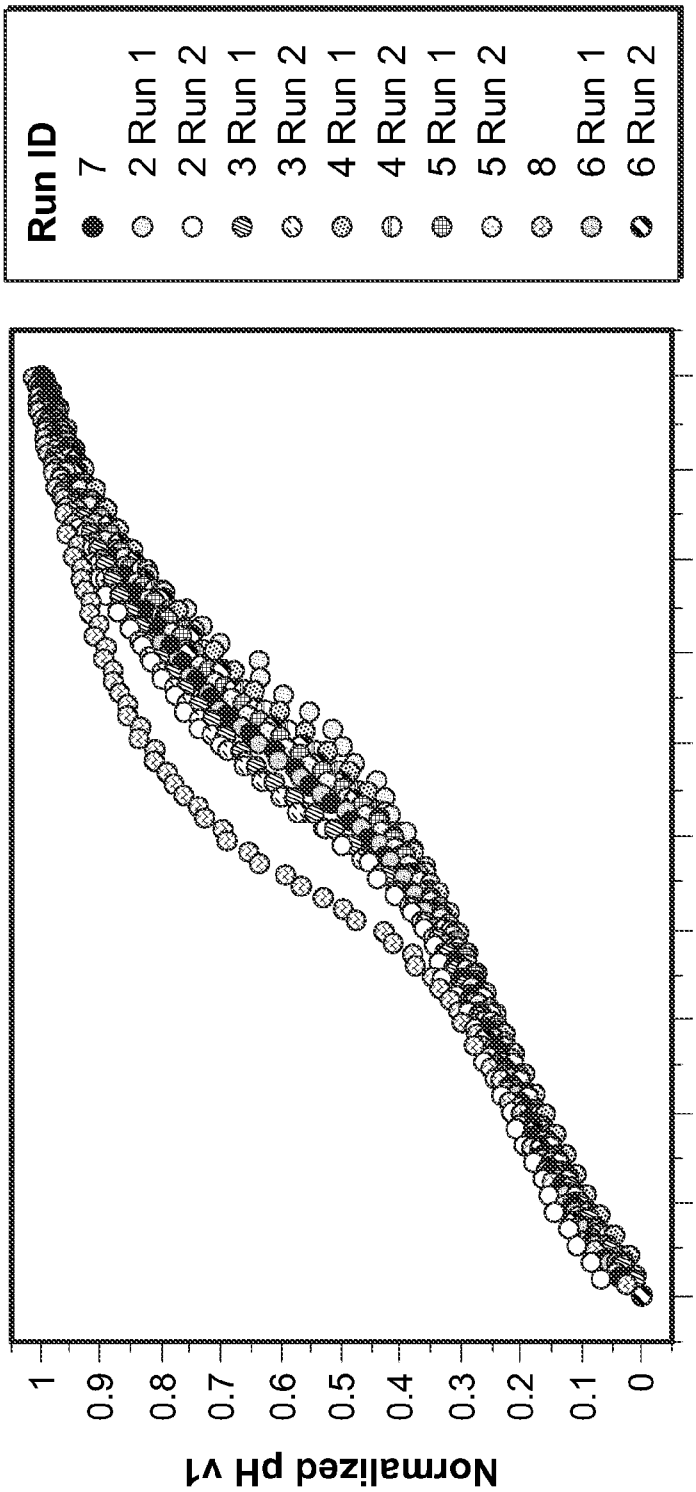

Run ID
7
2 Run 1
2 Run 2
3 Run 1
3 Run 2
4 Run 1
4 Run 2
5 Run 1
5 Run 2
8
6 Run 1
6 Run 2
Normalized pH v1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Normalized Titrant Addition v1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

FIG. 5

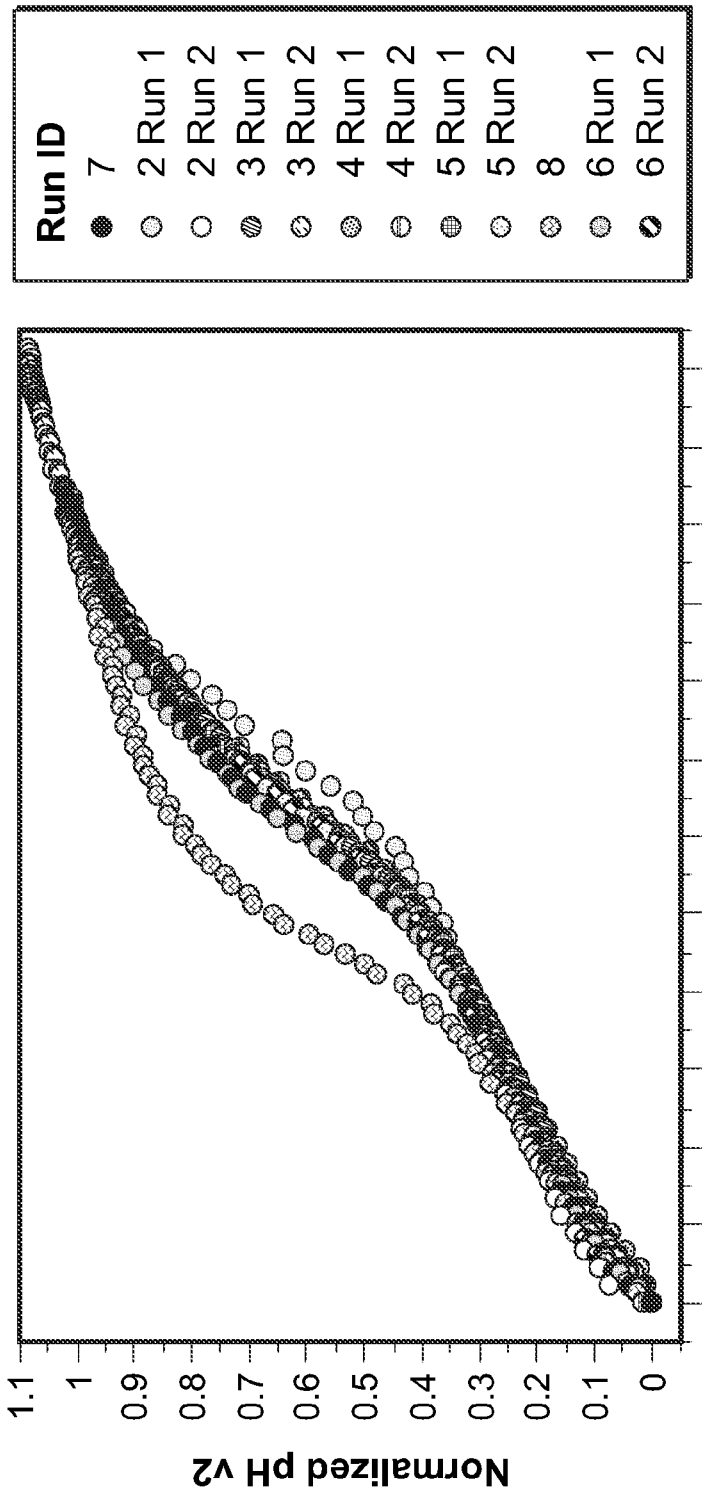

Run ID
7
2 Run 1
2 Run 2
3 Run 1
3 Run 2
4 Run 1
4 Run 2
5 Run 1
5 Run 2
8
6 Run 1
6 Run 2
Normalized pH v2
Normalized Titrant Addition v2
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2

FIG. 6

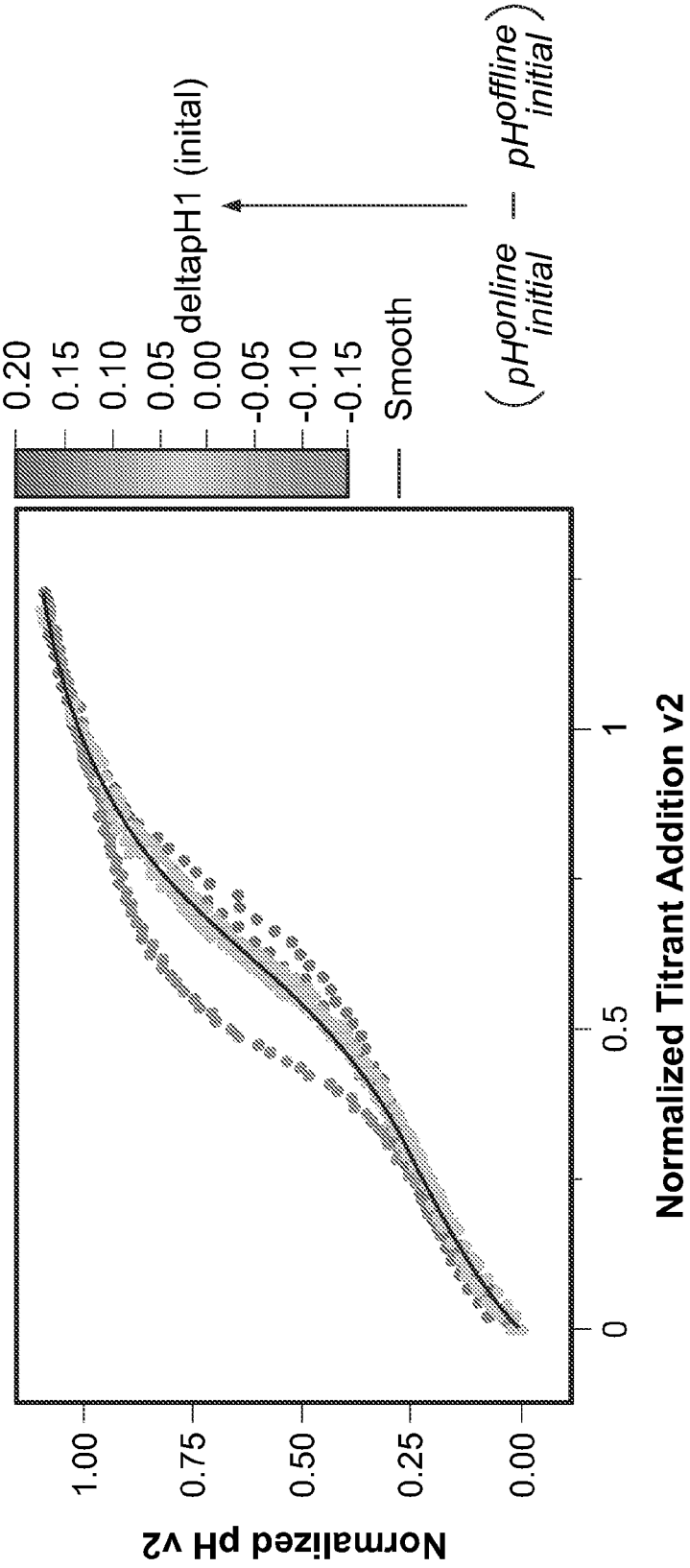

Normalized pH v2
1.00
0.75
0.50
0.25
0.00
0
0.5
1
Normalized Titrant Addition v2
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
deltapH1 (inital)
Smooth
$\left(pH^{online}_{initial} - pH^{offline}_{initial}\right)$

FIG. 7

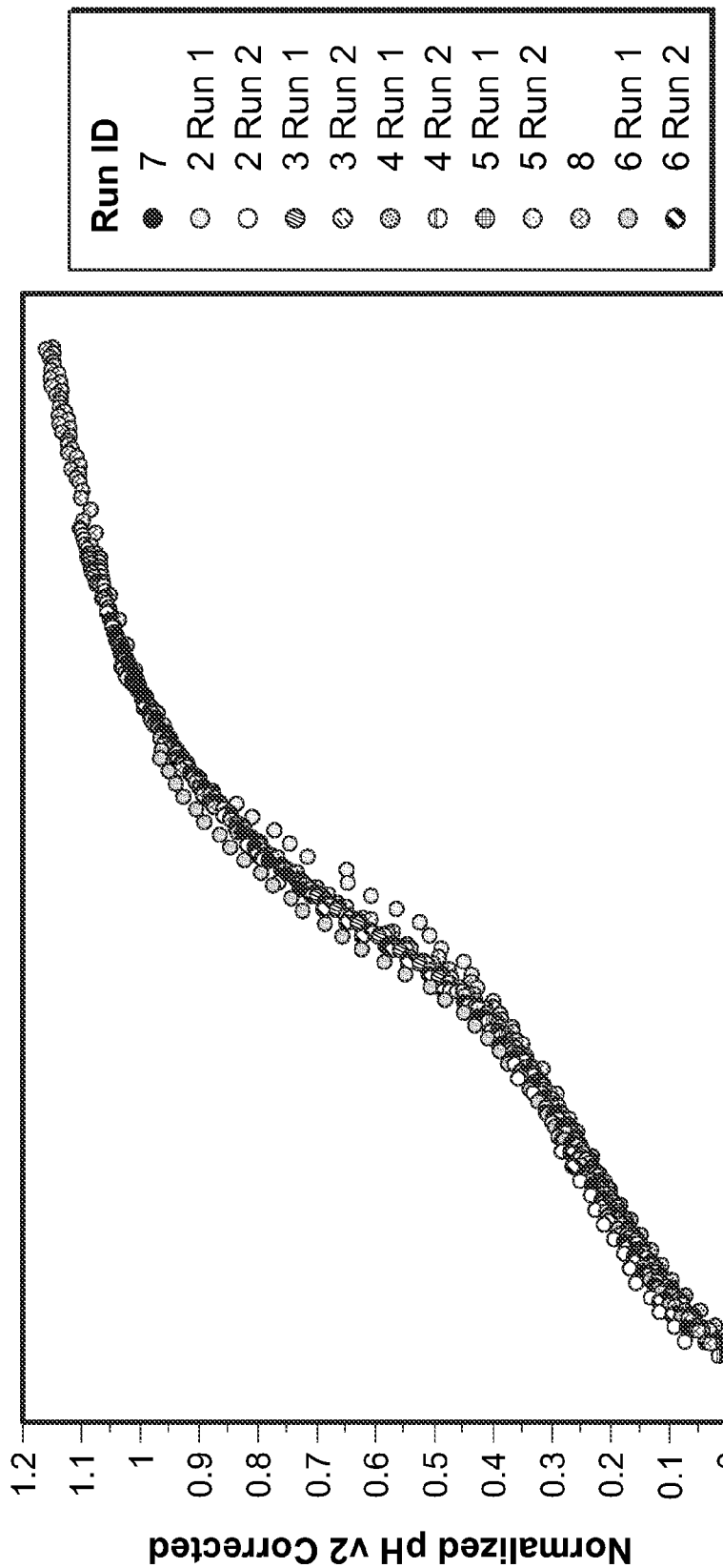

Run ID
7
2 Run 1
2 Run 2
3 Run 1
3 Run 2
4 Run 1
4 Run 2
5 Run 1
5 Run 2
8
6 Run 1
6 Run 2
Normalized pH v2 Corrected
Normalized Titrant Addition v2 Corrected
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6

FIG. 8

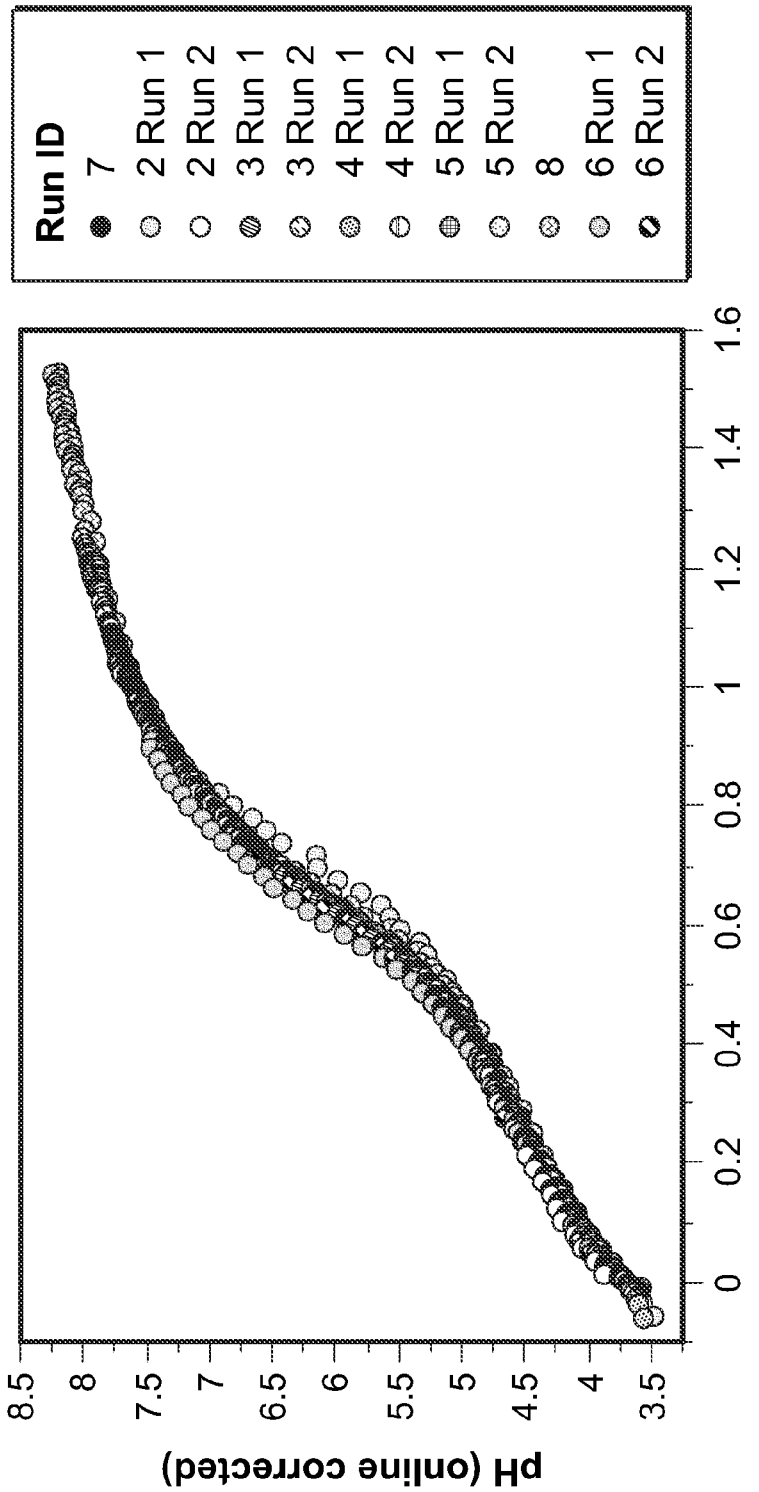

Run ID
7
2 Run 1
2 Run 2
3 Run 1
3 Run 2
4 Run 1
4 Run 2
5 Run 1
5 Run 2
8
6 Run 1
6 Run 2
pH (online corrected)
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
Normalized Titrant Addition v3 Corrected

FIG. 9

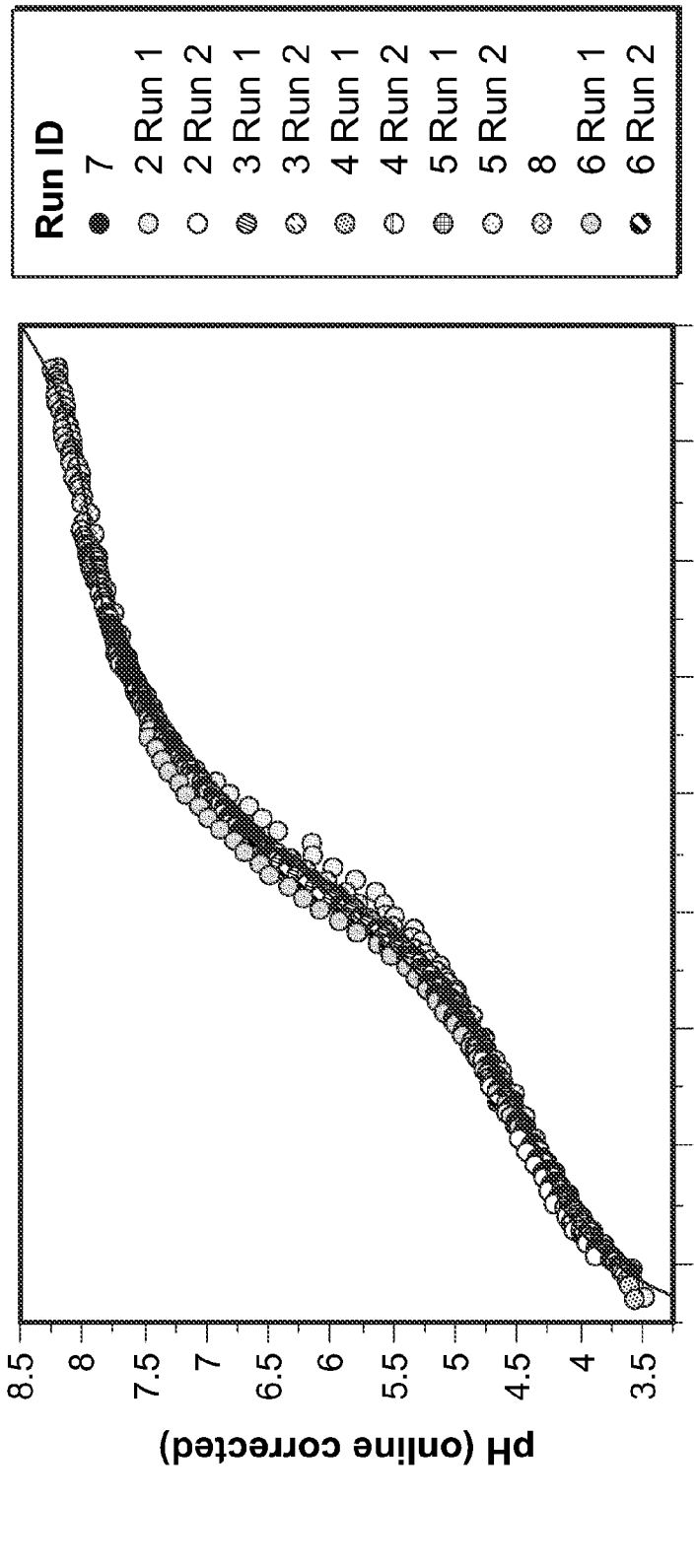

Run ID
7
2 Run 1
2 Run 2
3 Run 1
3 Run 2
4 Run 1
4 Run 2
5 Run 1
5 Run 2
8
6 Run 1
6 Run 2
pH (online corrected)
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
Normalized Titrant Addition v3 Corrected
Polynomial Fit Degree=6

FIG. 10

Components	Signals
2 x Watson Marlow 530 pumps	Speed Setpoint (AO) Speed Feedback (AI) On/Off (DO)
1 x Watson Marlow 120 pump	Speed Setpoint (AO) Speed Feedback (AI) On/Off (DO)
2 x Sonotec CO.55 ultrasonic flow meters	Autozero (DO) Volume Pulse (DI)
1 x Mettler M400 pH transmitter	pH (AI) Temperature (AI) mV (AI)
1 x Mettler pH flow cell	N/A
1 x imPULSE mixer control cable	Speed Setpoint (AO) On/Off (DO)
3 x Ethernet I/O modules	N/A

FIG. 11

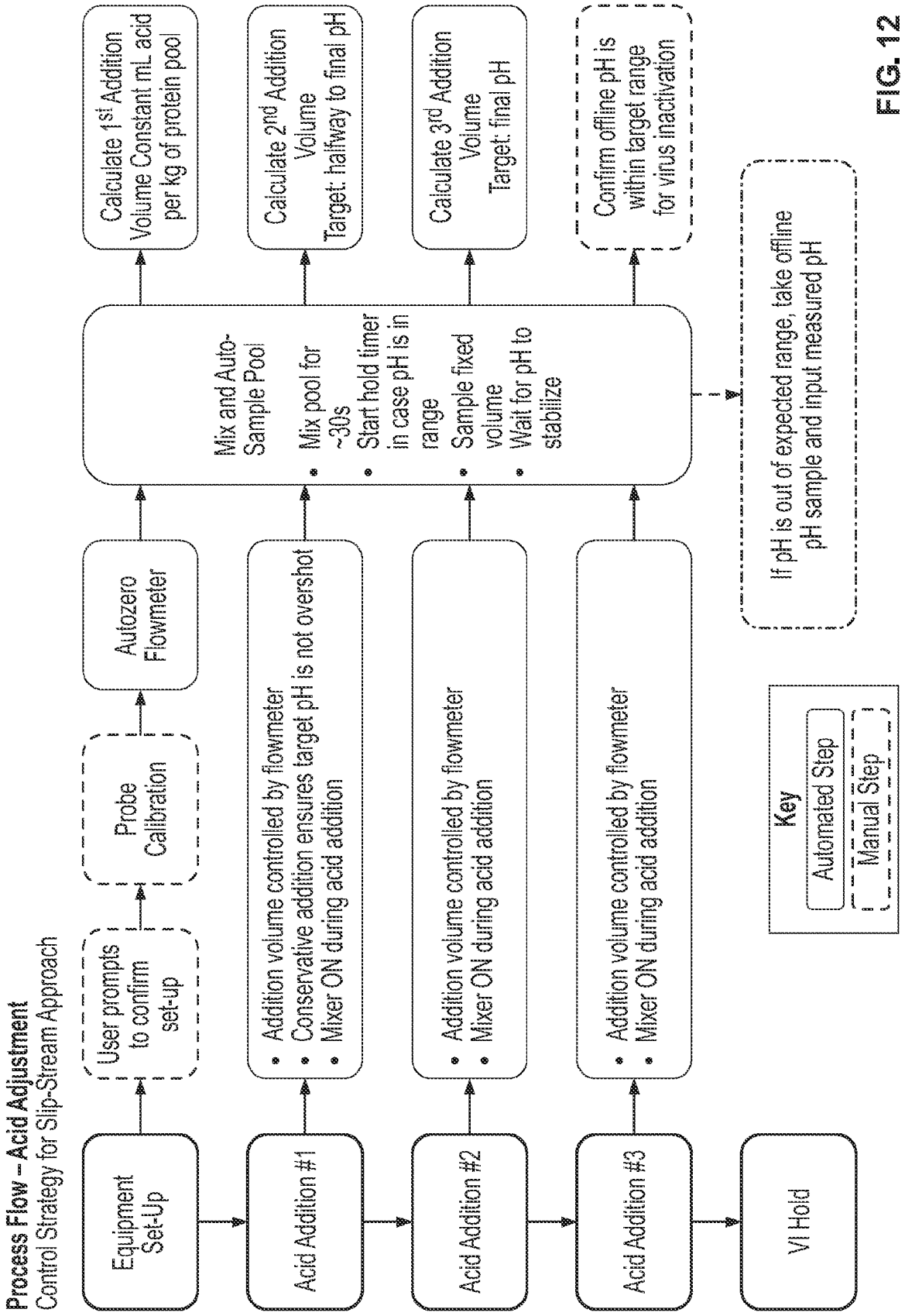

Process Flow – Acid Adjustment
Control Strategy for Slip-Stream Approach
Equipment Set-Up
User prompts to confirm set-up
Probe Calibration
Autozero Flowmeter
Acid Addition #1
• Addition volume controlled by flowmeter
• Conservative addition ensures target pH is not overshot
• Mixer ON during acid addition
Acid Addition #2
• Addition volume controlled by flowmeter
• Mixer ON during acid addition
Acid Addition #3
• Addition volume controlled by flowmeter
• Mixer ON during acid addition
VI Hold
Mix and Auto-Sample Pool
• Mix pool for ~30s
• Start hold timer in case pH is in range
• Sample fixed volume
• Wait for pH to stabiiize
Calculate 1st Addition Volume Constant mL acid per kg of protein pool
Caiculate 2nd Addition Volume Target: halfway to final pH
Calculate 3rd Addition Volume Target: final pH
Confirm offline pH is within target range for virus inactivation
If pH is out of expected range, take offline pH sample and input measured pH
Key
Automated Step
Manual Step

FIG. 12

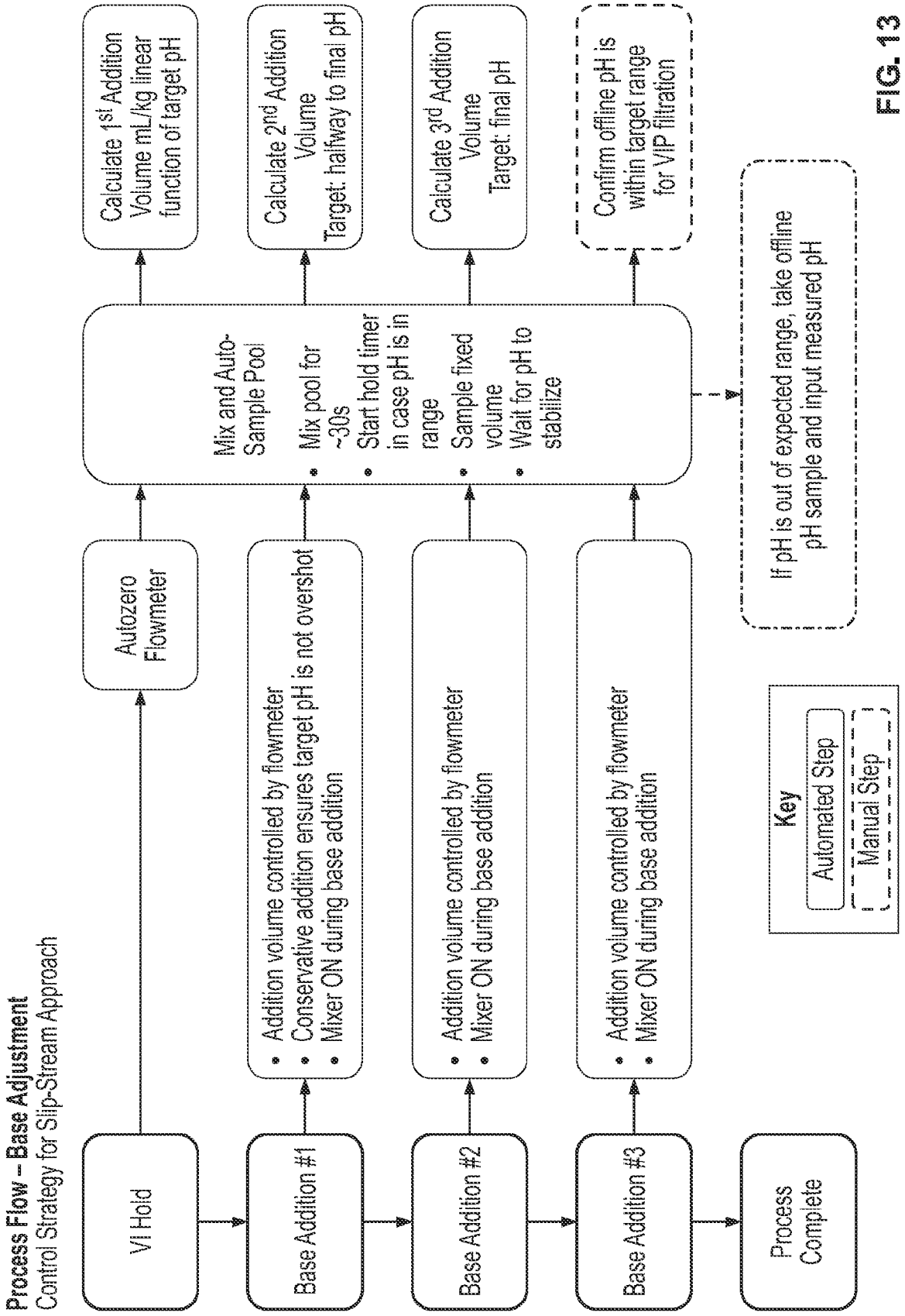

Process Flow – Base Adjustment
Control Strategy for Slip-Stream Approach
VI Hold
Base Addition #1
Base Addition #2
Base Addition #3
Process Complete
Autozero Flowmeter
• Addition volume controlled by flowmeter
• Conservative addition ensures target pH is not overshot
• Mixer ON during base addition
• Addition volume controlled by flowmeter
• Mixer ON during base addition
• Addition volume controlled by flowmeter
• Mixer ON during base addition
Mix and Auto-Sample Pool
• Mix pool for ~30s
• Start hold timer in case pH is in range
• Sample fixed volume
• Wait for pH to stabilize
Calculate 1st Addition Volume mL/kg linear function of target pH
Calculate 2nd Addition Volume Target: halfway to final pH
Calculate 3rd Addition Volume Target: final pH
Confirm offline pH is within target range for VIP filtration
If pH is out of expected range, take offline pH sample and input measured pH
Key
Automated Step
Manual Step

FIG. 13

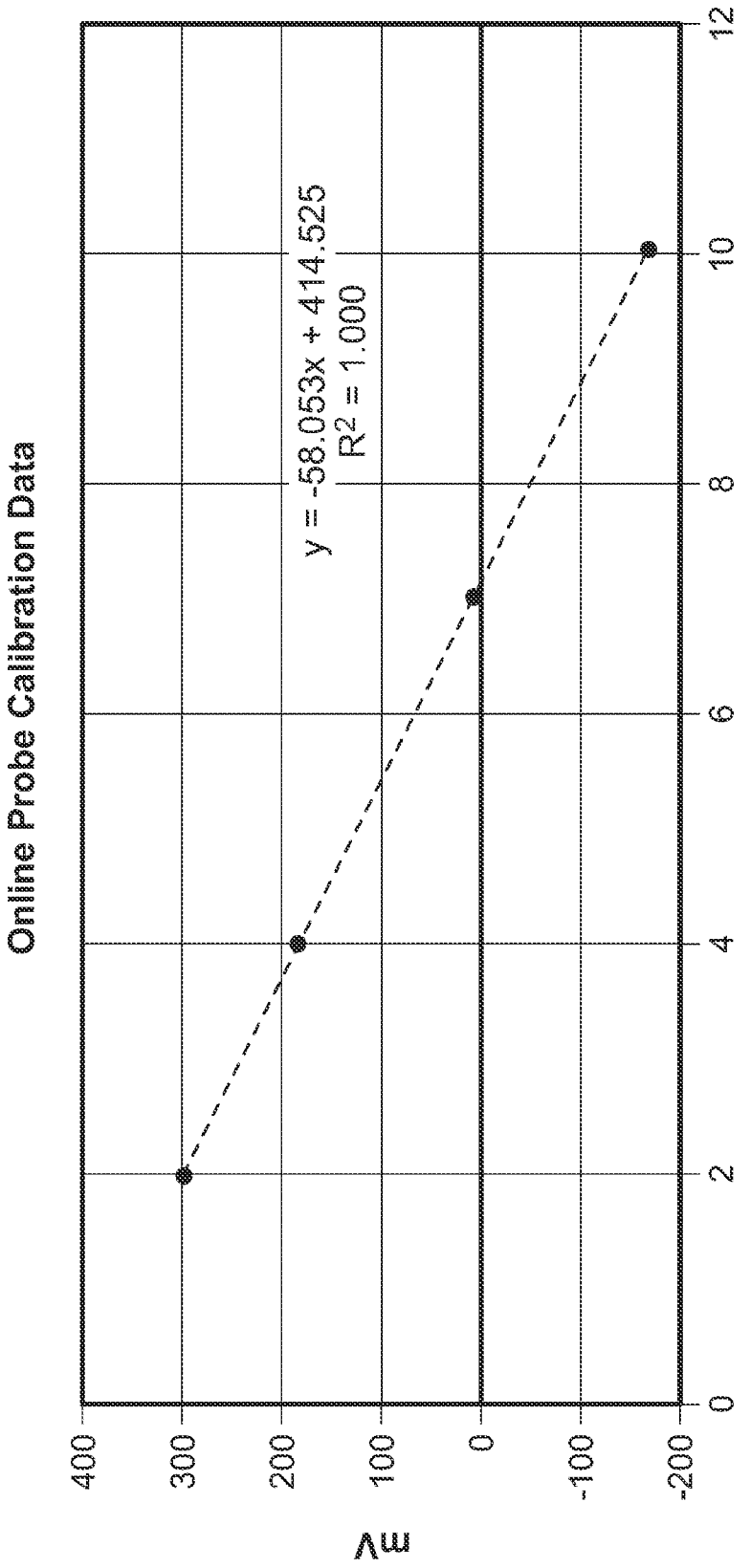

Online Probe Calibration Data
y = -58.053x + 414.525
R2 = 1.000
mV
pH
400
300
200
100
0
-100
-200
0
2
4
6
8
10
12

FIG. 14

Process Step	Test Run #1		Test Run #2		Test Run #3		Test Run #4		Test Run #5	
	ΔpH	Dosing Error (%)	ΔpH	Dosing Error (%)	ΔpH	Dosing Error (%)	ΔpH	Dosing Error (%)	ΔpH	Dosing Error (%)
Protein Pool	0.05	N/A	0.05	N/A	0.00	N/A	0.00	N/A	0.04	N/A
Acid Add #1	0.05	2.2	0.04	0.9	0.01	1.6	0.01	0.4	0.05	4.9
Acid Add #2	0.05	4.0	0.06	0.9	0.01	1.1	0.00	0.3	0.05	5.0
Acid Add #3	0.04	2.6	0.04	0.5	0.01	1.0	0.00	0.4	0.05	5.5
Base Add #1	0.01	0.6	0.02	2.2	0.01	1.7	0.02	0.2	0.02	-0.1
Base Add #2	0.00	0.3	0.02	1.8	0.01	0.4	0.02	-3.4	0.00	-0.6
Base Add #3	0.01	13.9	0.02	1.9	0.00	1.7	0.01	-0.1	0.00	0.0

$$\Delta pH = \text{Slipstream pH} - \text{Offline pH}$$

$$\text{Dosing Error} = \frac{(\text{Volume Dosed} - \text{Target Volume})}{\text{Target Volume}} * 100\%$$

FIG. 15

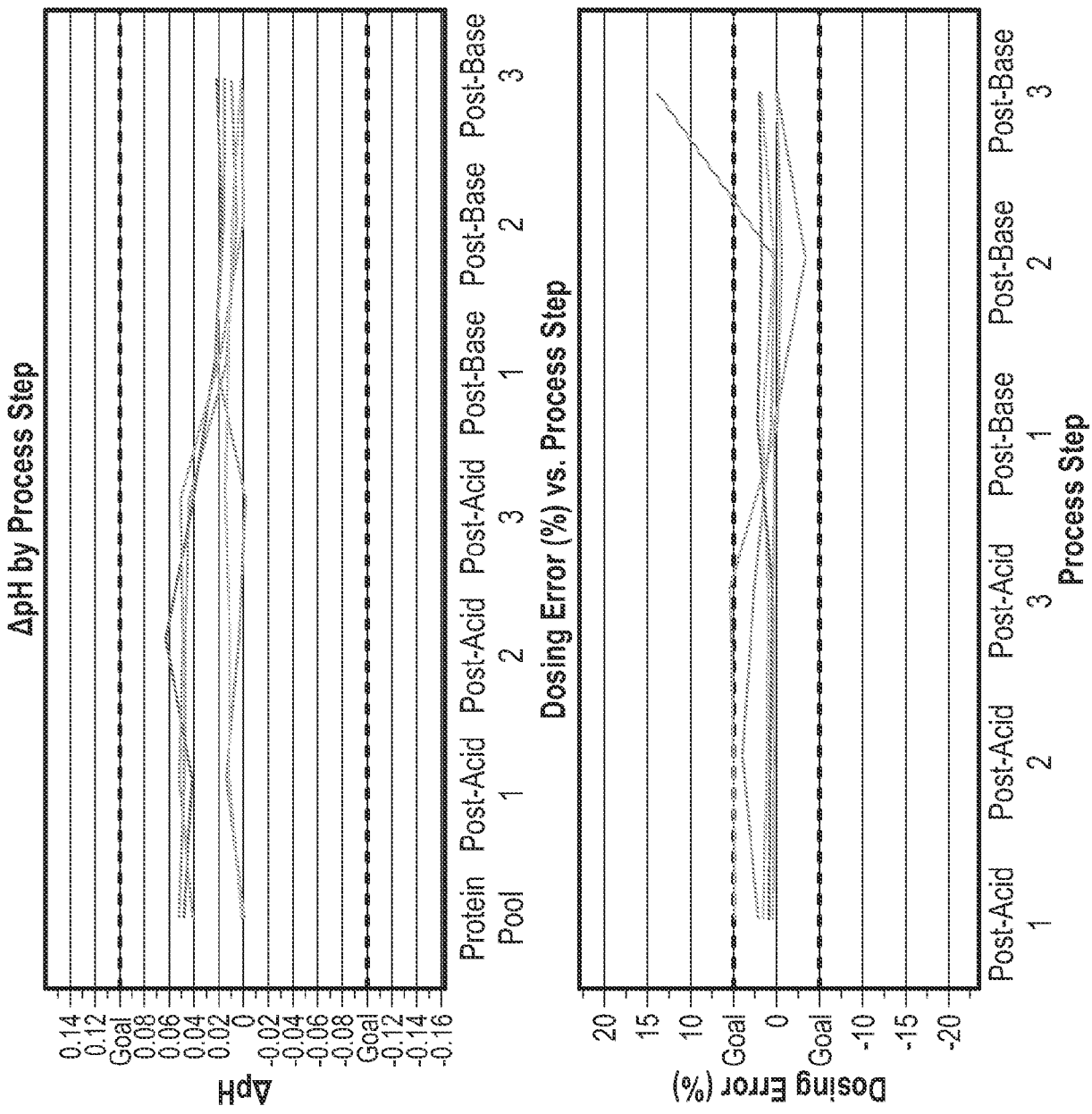

ΔpH by Process Step
ΔpH
0.14
0.12
Goal
0.08
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
-0.08
Goal
-0.12
-0.14
-0.16
Protein Post-Acid Post-Acid Post-Base Post-Base Post-Base
Pool 1 2 3 1 2 3
Dosing Error (%) vs. Process Step
Dosing Error (%)
20
15
10
Goal
0
Goal
-10
-15
-20
Post-Acid Post-Acid Post-Acid Post-Base Post-Base Post-Base
1 2 3 1 2 3
Process Step

FIG. 16

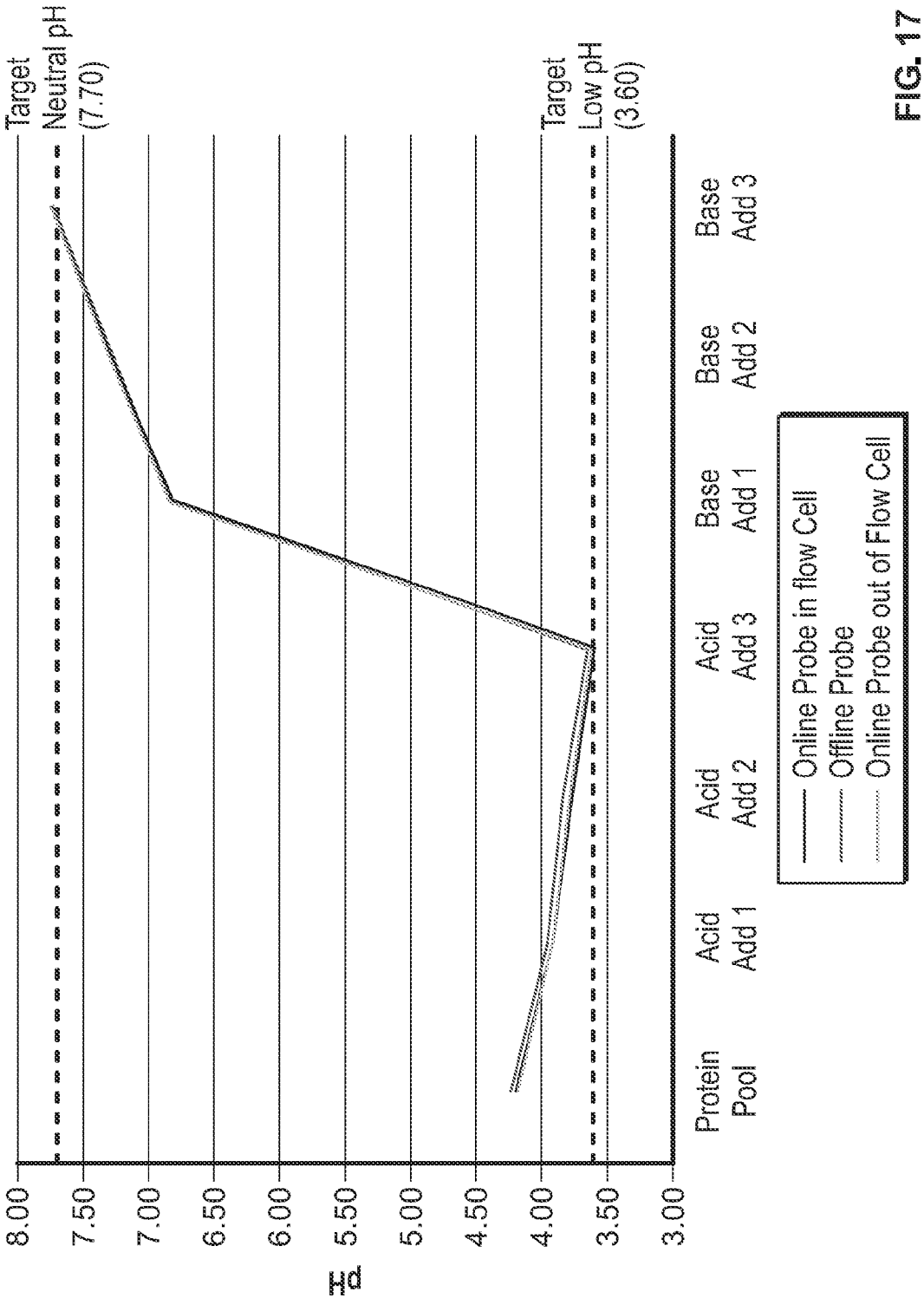

Target
Neutral pH
(7.70)
Target
Low pH
(3.60)
pH
8.00
7.50
7.00
6.50
6.00
5.50
5.00
4.50
4.00
3.50
3.00
Protein Pool
Acid Add 1
Acid Add 2
Acid Add 3
Base Add 1
Base Add 2
Base Add 3
Online Probe in flow Cell
Offline Probe
Online Probe out of Flow Cell

FIG. 17

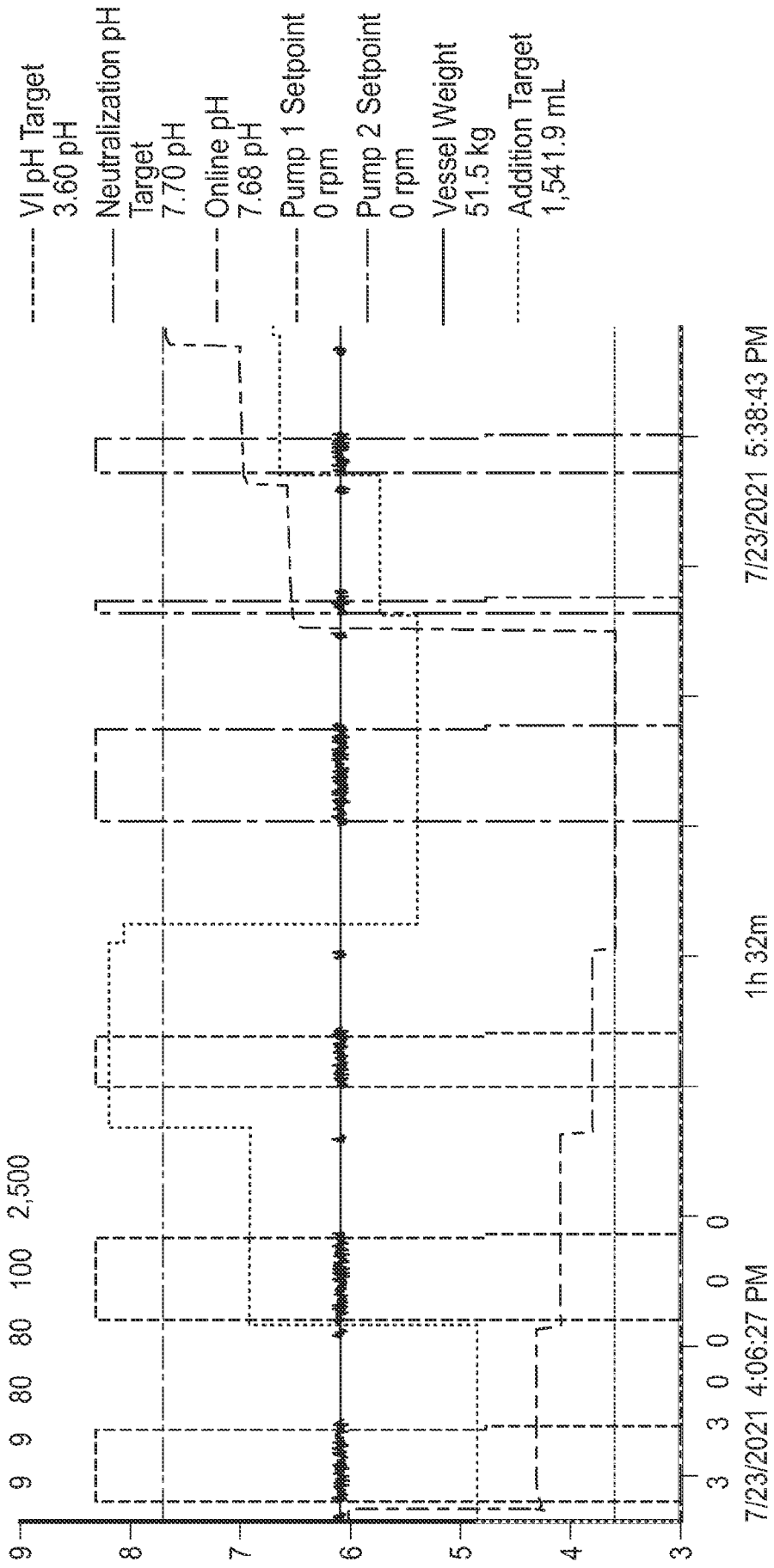

VI pH Target
3.60 pH
Neutralization pH
Target
7.70 pH
Online pH
7.68 pH
Pump 1 Setpoint
0 rpm
Pump 2 Setpoint
0 rpm
Vessel Weight
51.5 kg
Addition Target
1,541.9 mL
9
8
7
6
5
4
3
9 9 80 80 100 2,500
3 3 0 0 0 0
7/23/2021 4:06:27 PM
1h 32m
7/23/2021 5:38:43 PM

FIG. 18

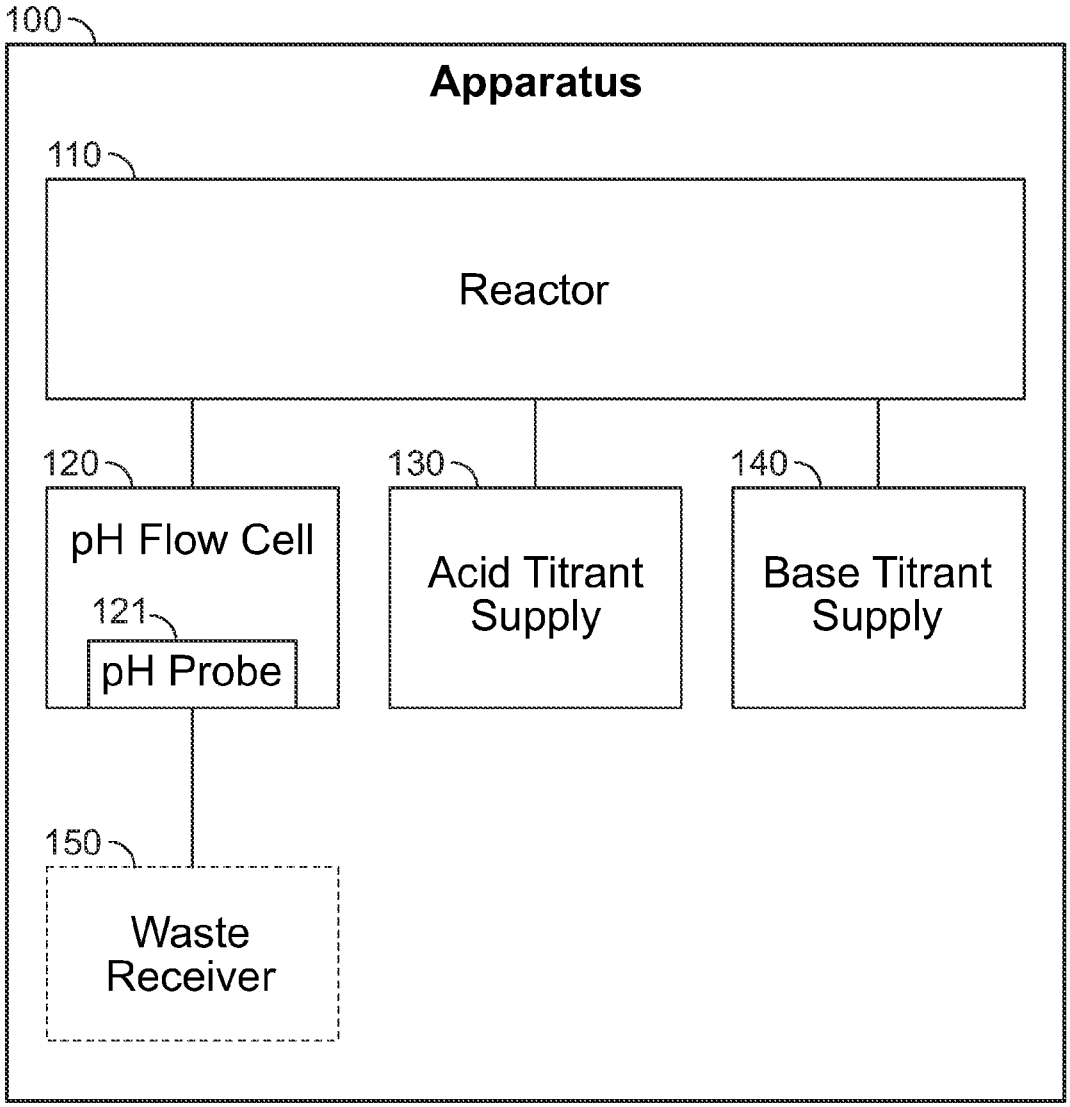

100
Apparatus
110
Reactor
120
pH Flow Cell
121
pH Probe
130
Acid Titrant Supply
140
Base Titrant Supply
150
Waste Receiver

FIG. 19

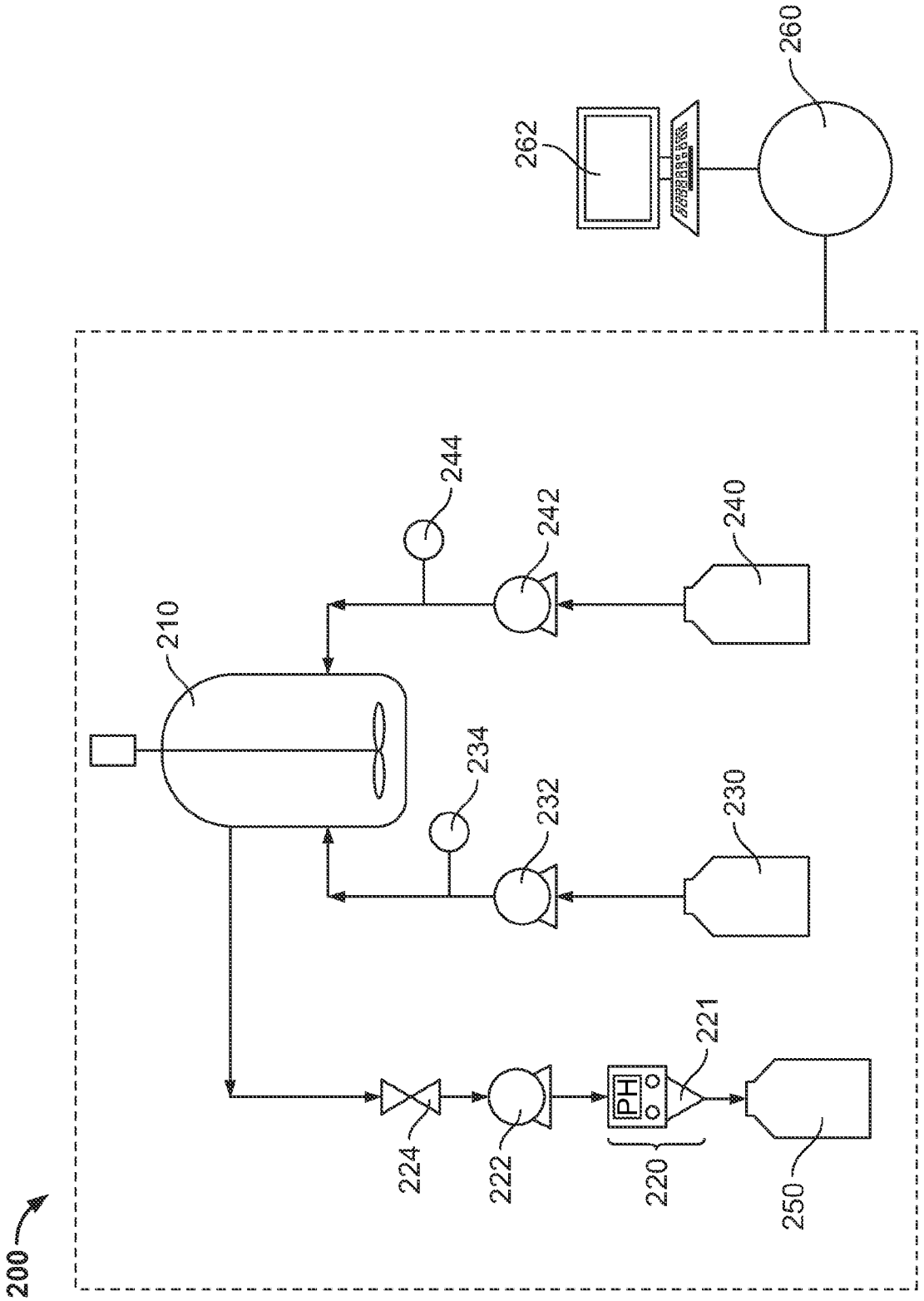


FIG. 20

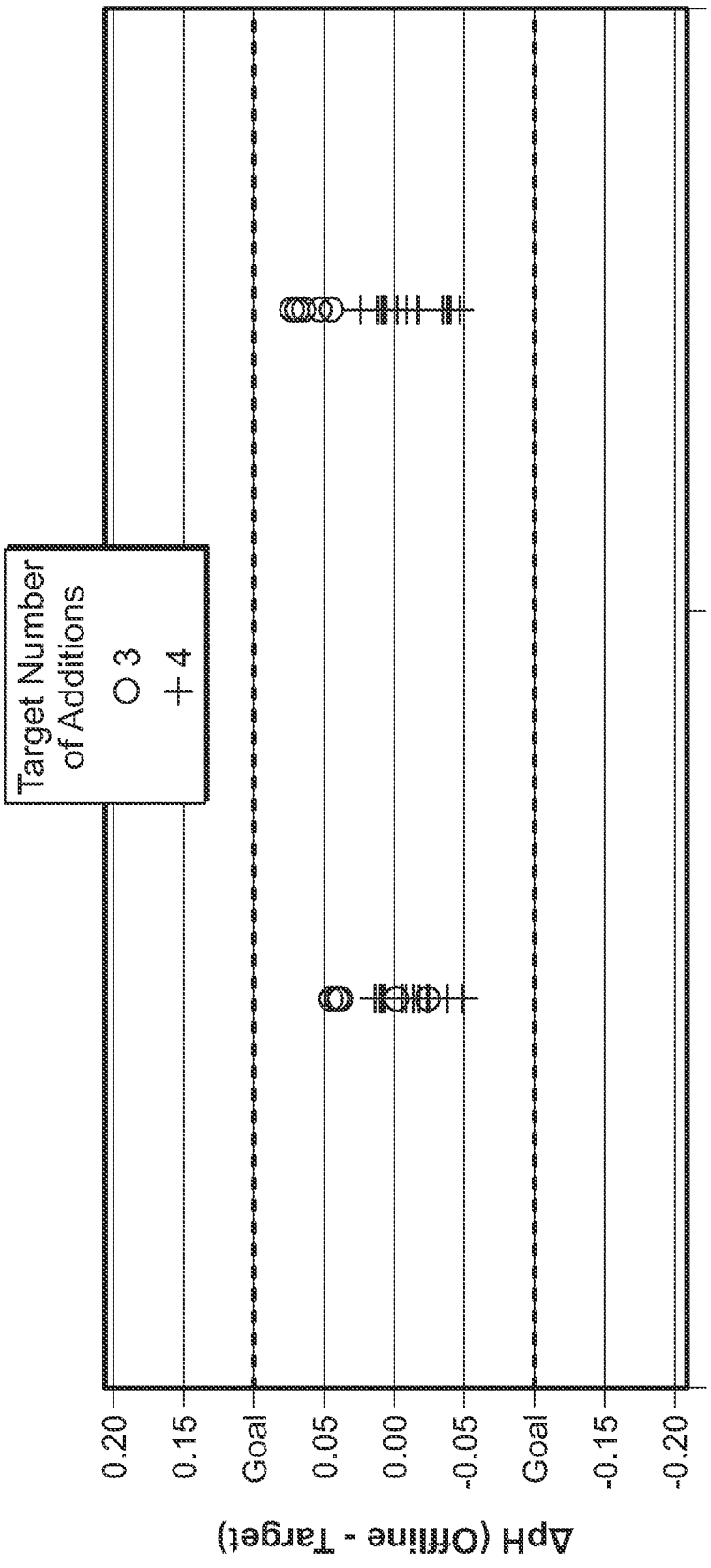

Target Number of Additions
3
4
0.20
0.15
Goal
0.05
0.00
-0.05
Goal
-0.15
-0.20
ΔpH (Offline - Target)
Acid
Base
Adjustment Step

FIG. 21

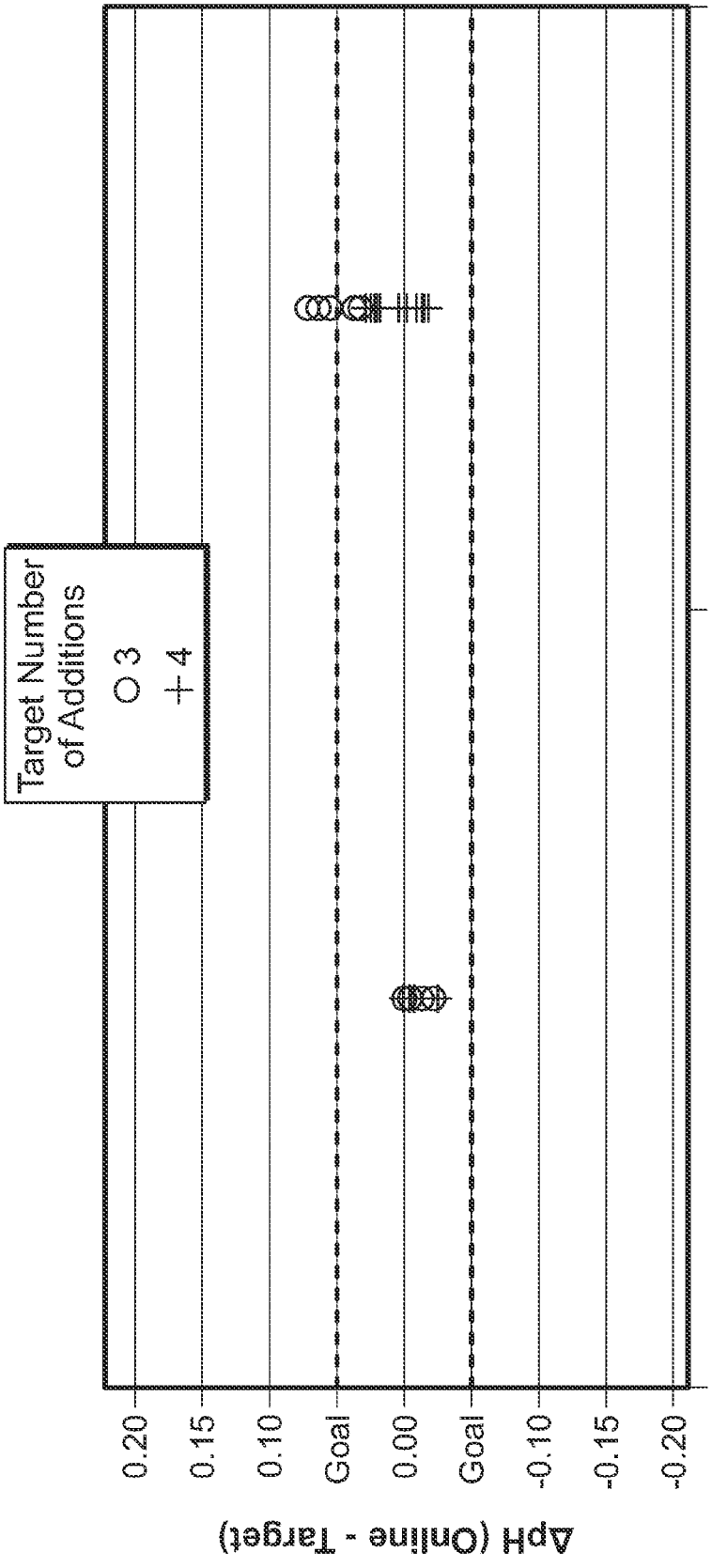

Target Number of Additions
3
4
0.20
0.15
0.10
Goal
0.00
Goal
-0.10
-0.15
-0.20
ΔpH (Online - Target)
Acid
Base
Adjustment Step

FIG. 22

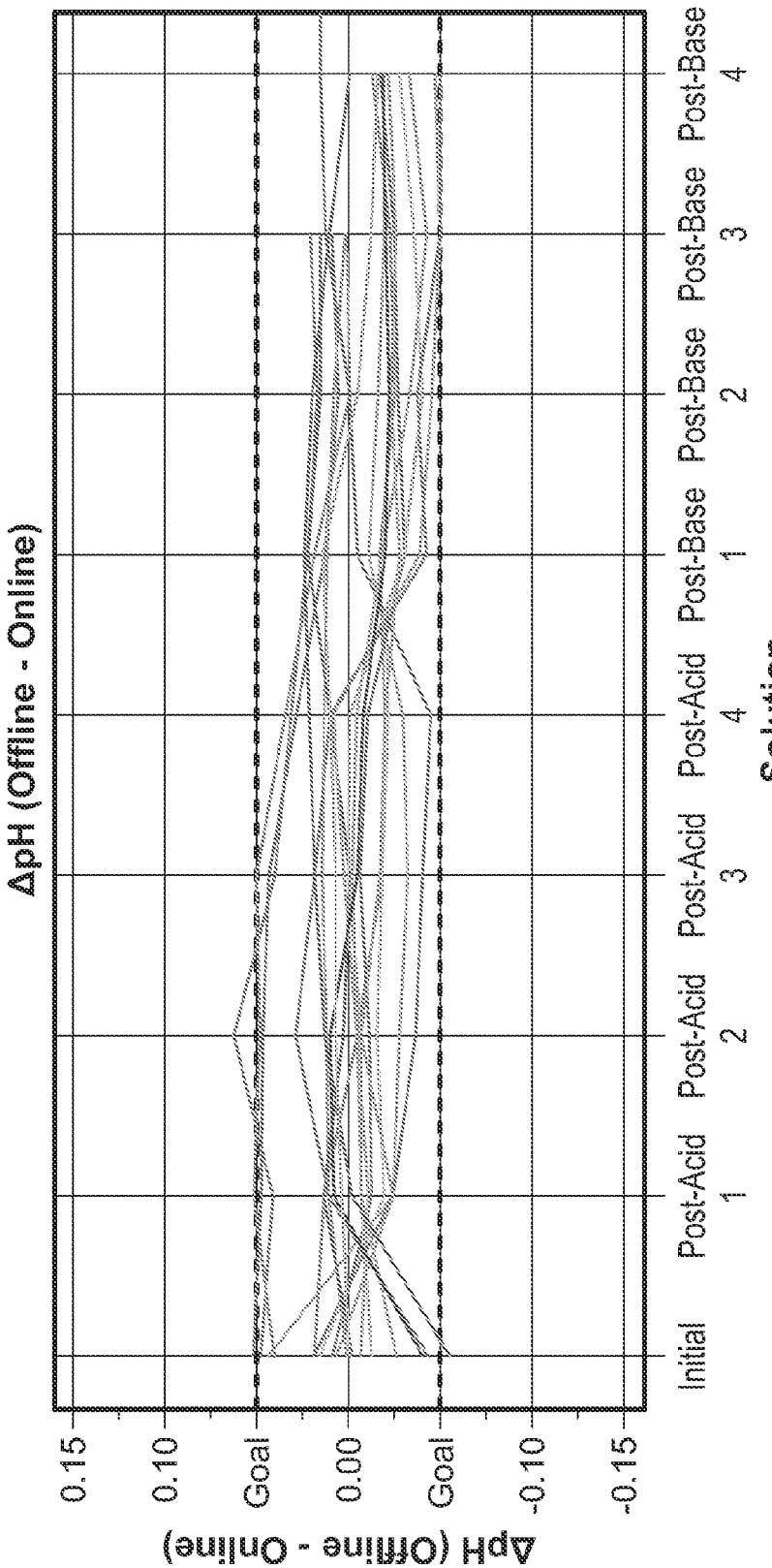

ΔpH (Offline - Online)
0.15
0.10
Goal
0.00
Goal
-0.10
-0.15
Initial
Post-Acid 1
Post-Acid 2
Post-Acid 3
Post-Acid 4
Post-Base 1
Post-Base 2
Post-Base 3
Post-Base 4
Solution

FIG. 23

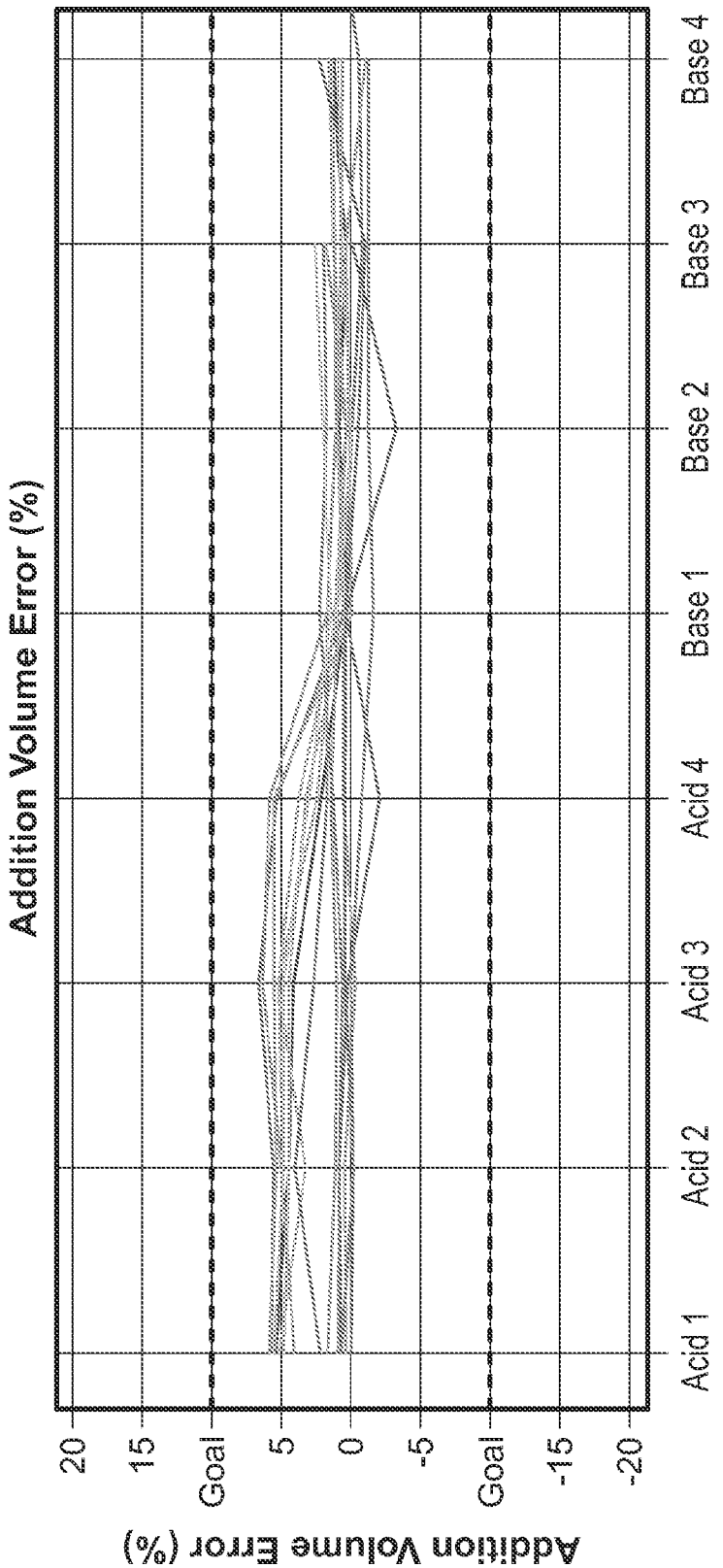

Addition Volume Error (%)
20
15
Goal
5
0
-5
Goal
-15
-20
Addition Volume Error (%)
Acid 1
Acid 2
Acid 3
Acid 4
Base 1
Base 2
Base 3
Base 4
Addition

FIG. 24