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Challenges in Optimizing Formulations for Multi-Antigen Vaccines

Lakshmi Khandke, PhD.
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Vaccines Research,
Pfizer Global R&D

May 22, 2012

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Introduction

- Ideal combination or multi-antigen vaccines
  - Safe and effective
  - Stabile individual components
  - Provide broader coverage in immune response and protection
  - Physiological or sub species diversity
  - Reduce number of injections.
  - Improves timeliness of immunizations.

- Case Study: *Staphylococcus aureus tetra antigen* vaccine
  - *Combination of two proteins and two polysaccharide – protein conjugates*

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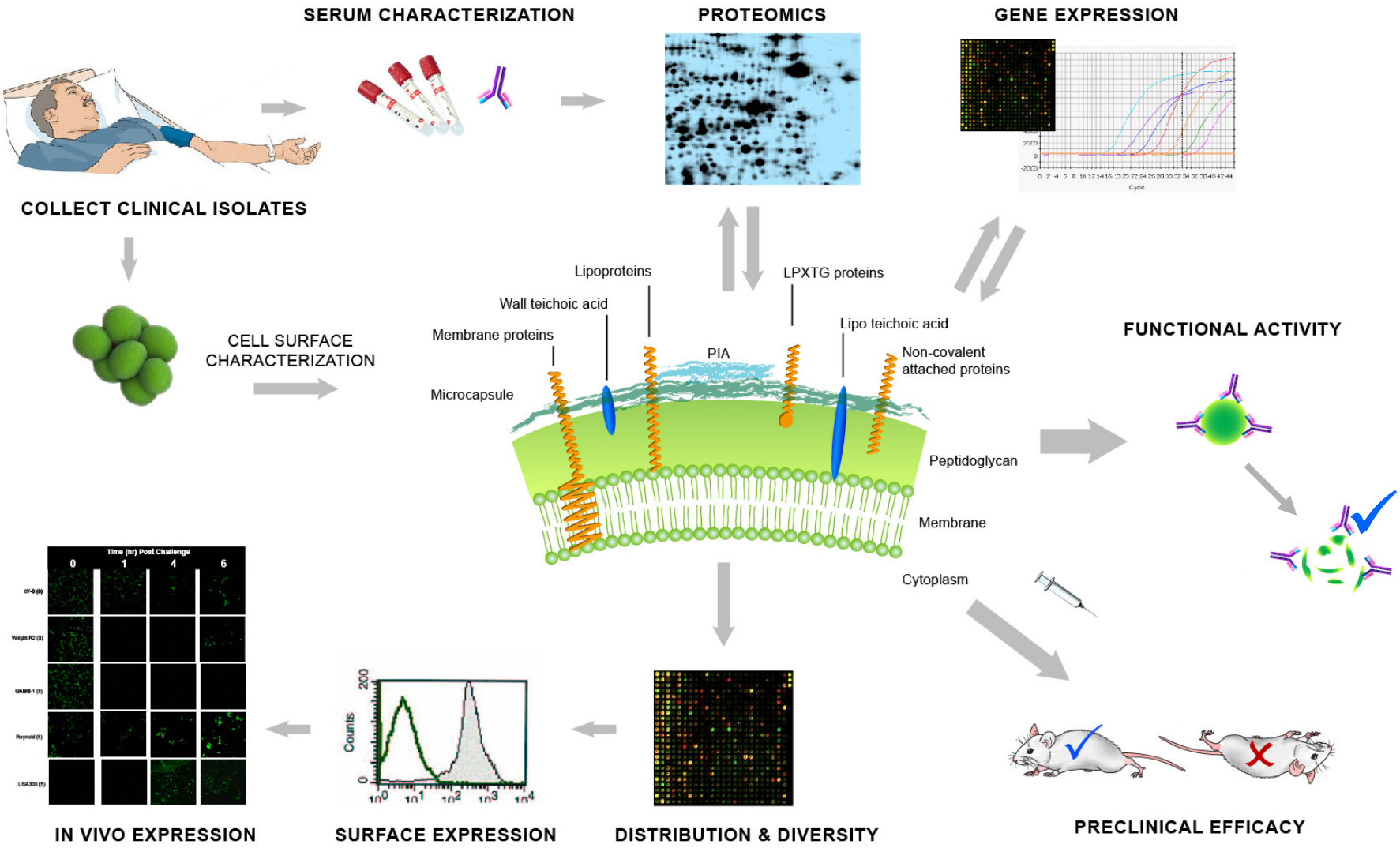
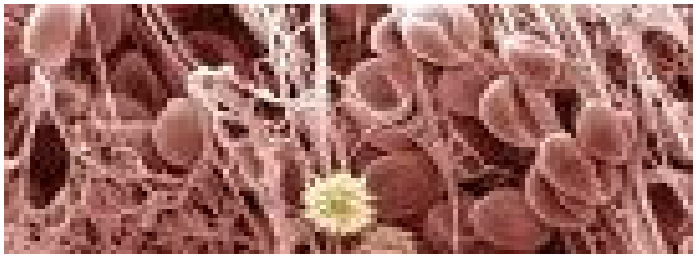
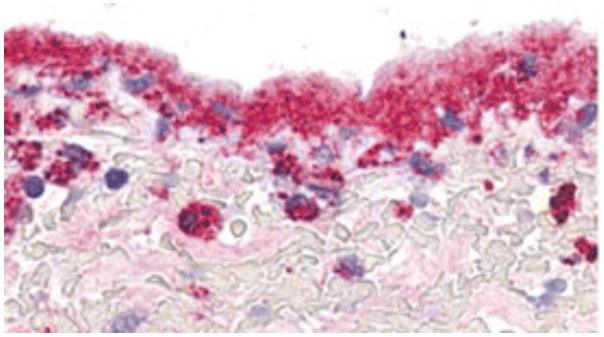
Vaccine antigen discovery and validation



Important pathogenesis mechanisms

- Divalent cation scavenging
- Attaches to cell surfaces and host components resulting in
  - Cell destruction by lytic enzymes
  - Biofilms recalcitrant to antibiotic treatment
  - Blood clots
- Evasion of opsonization and phagocytosis
- Toxemia





Challenges with multi-antigen formulations

- Compatibility with antigens
  - Physical characteristics such as pH, density, viscosity, size and size distribution, surface charge
  - Different mechanism of degradation for each component
  - Biochemical characteristics (e.g. adsorption, binding or coupling of an antigen).
  - Varying levels of stability
- Conformational changes of antigens
  - Surfactants can change conformational epitopes leading to loss of epitopes and decreased *in vitro* potency
  - Lipid components can alter hydrophobicity
- Analytical challenges
  - Difficult to quantitate individual components
  - Separation techniques are limited
  - Tight interaction between adjuvant and antigen
- Stability of the vaccine – shelf life
- Dosage form and delivery

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Optimizing a formulation requires understanding of mechanisms for instability from Drug Substance to Product

Utilize Biophysical, Physical, Chemical and HPLC techniques based assays to optimize formulation process

Process related

1. Freeze /Thaw
2. Temperature
3. pH
4. Downloading
5. Mechanical stress
    1. Agitation
    2. Shear effect
6. Filtration-membrane pressure
7. Container-Closures
8. Light
9. Oxygen
10. Metal ions

Chemical

1. Oxidation
2. Deamidation
3. Hydrolysis
4. Disulfide exchanges

Physical

1. Aggregation
2. Denaturation
3. Precipitation
4. Adsorption

Select optimum solution conditions potential interactions can involve other components of the vaccines, including buffers, stabilizers, surfactants, adjuvants and preservatives

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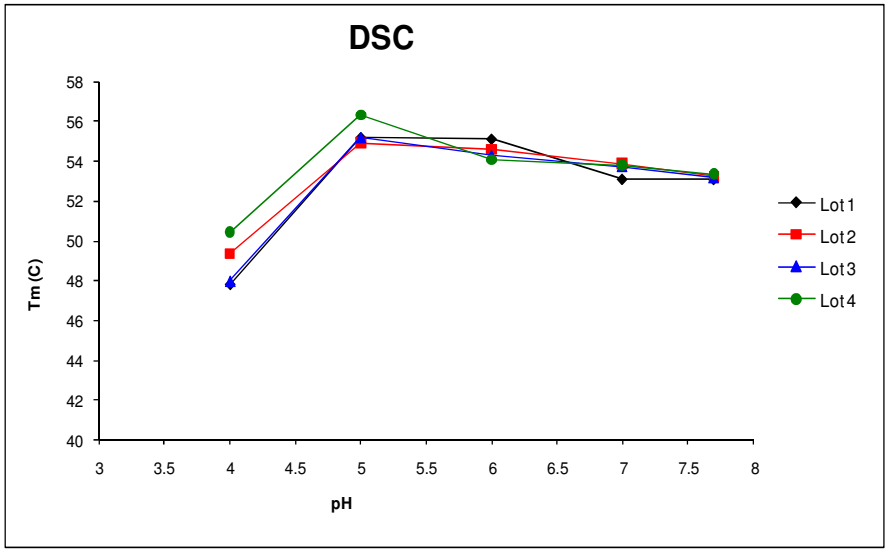
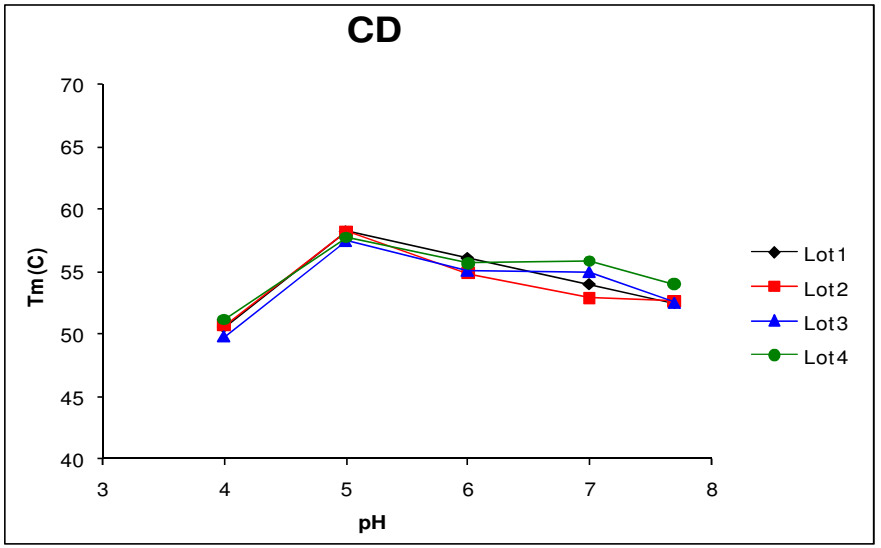
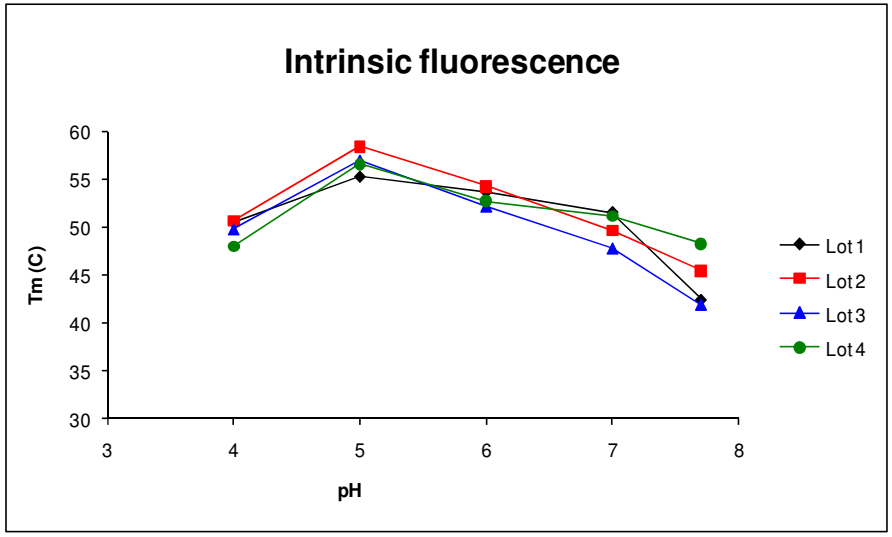
Characterization of protein antigens

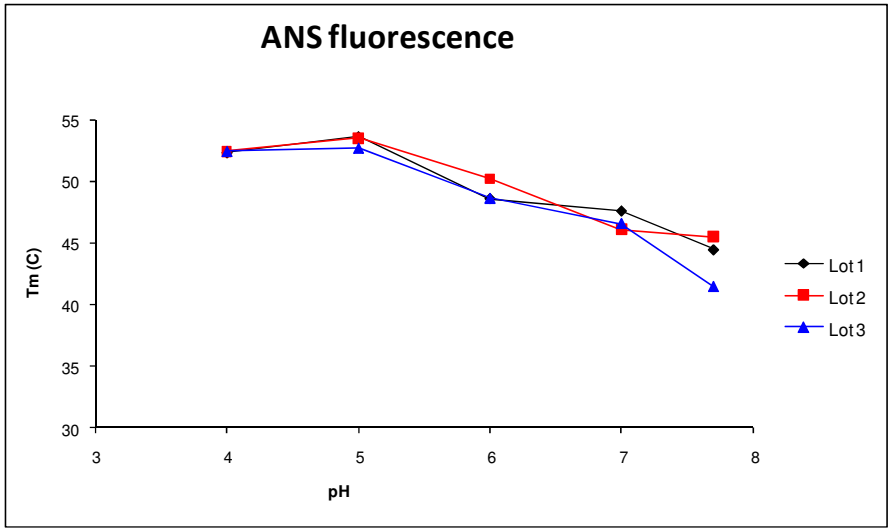
- Thermo stability
  - Determine as a function of pH - buffer
  - This data will help guide the selection of formulation pH and buffer system
- Biophysical Methods
  - Fluorescence spectroscopy: tertiary structure
    - Intrinsic: Trp
    - Extrinsic: ANS dye
  - Circular dichroism: secondary structure
  - Differential scanning calorimetry: monitor overall protein unfolding
  - OD_{350}: detection of aggregation
- Real time and accelerated stability using various stress conditions
  - Analysis based on HPLC analysis such as reverse phase or IEX or SEC

Biophysical analysis of Protein 1





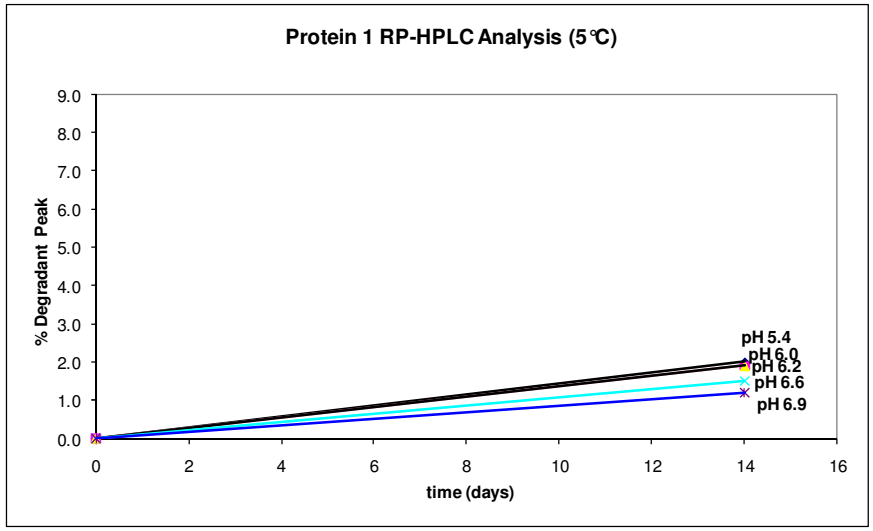


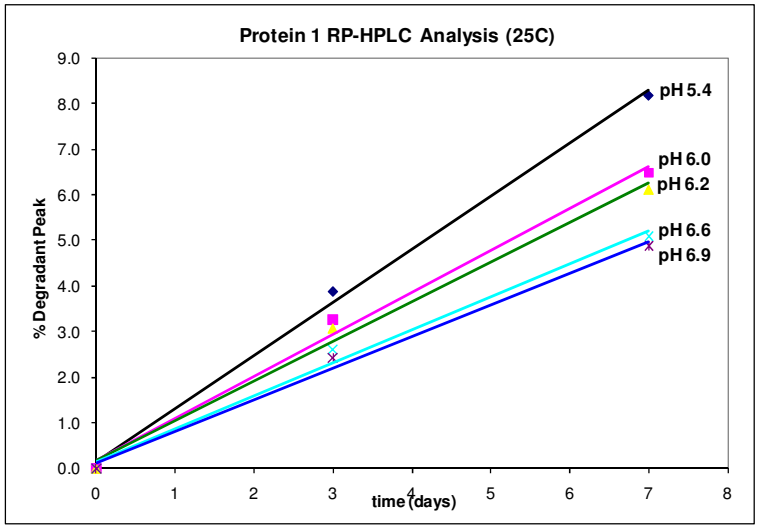


- Optimal pH around 5.0 to 6.0

Vaccine Research

Protein 1: clips in solution





5°C Prediction

Days	pH 5.4	pH 6.0	pH 6.2	pH 6.6	pH 6.9
0	0	0	0	0	0
1	0.1	0.1	0.1	0.1	0.1
2	0.3	0.3	0.3	0.2	0.2
3	0.4	0.4	0.4	0.3	0.3

25°C Prediction

Days	pH 5.4	pH 6.0	pH 6.2	pH 6.6	pH 6.9
0	0	0	0	0	0
1	1.3	1.1	1.0	0.9	0.8
2	2.5	2.0	1.9	1.6	1.5
3	3.6	2.9	2.8	2.3	2.2

Major mechanism of degradation of Protein 1 is clipping in solution
Protein cannot be stabilized under aqueous conditions

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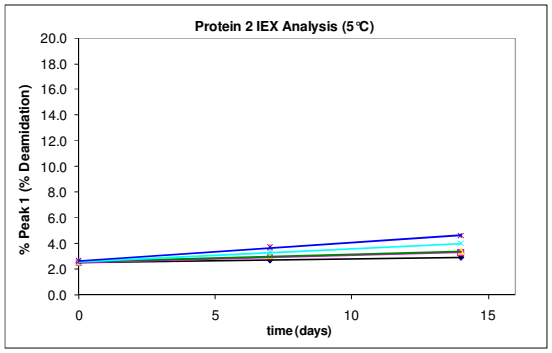
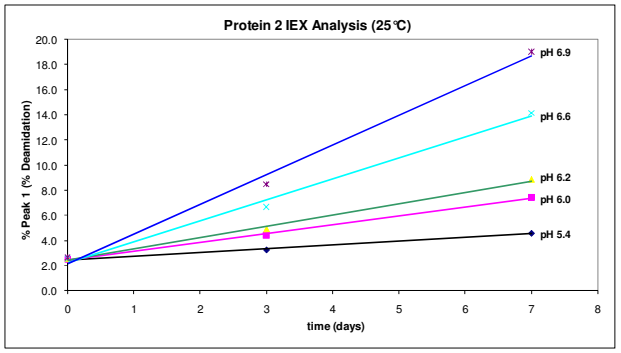
Protein 2: can deamidate under process conditions





5C prediction

Days	pH 5.4	pH 6.0	pH 6.2	pH 6.6	pH 6.9
0	2.5	2.5	2.6	2.6	2.6
1	2.5	2.5	2.6	2.7	2.8
2	2.6	2.6	2.7	2.8	2.9
3	2.6	2.7	2.8	2.9	3.1

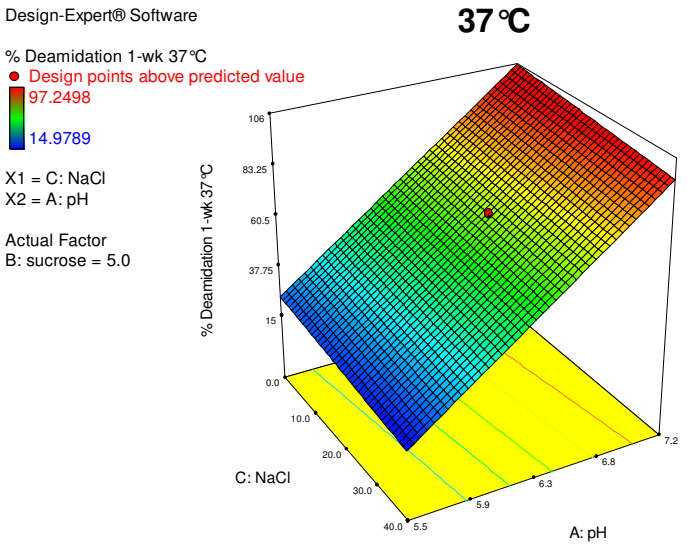
25C prediction

Days	pH 5.4	pH 6.0	pH 6.2	pH 6.6	pH 6.9
0	2.5	2.5	2.6	2.6	2.6
1	2.7	3.1	3.3	3.9	4.5
2	3.0	3.8	4.2	5.6	6.9
3	3.3	4.5	5.1	7.2	9.2

DOE Formulations

Sample No.	pH	Sucrose (%)	NaCl (mM)
1	5.5	0	40
2	7.5	0	40
3	6.5	5	20
4	5.5	10	40
5	7.5	0	0
6	6.5	5	20
7	7.5	10	0
8	5.5	10	0
9	5.5	0	0
10	6.5	5	20
11	7.5	10	40

Based on multiple studies; pH, salt and temperature are dominant factors for stability



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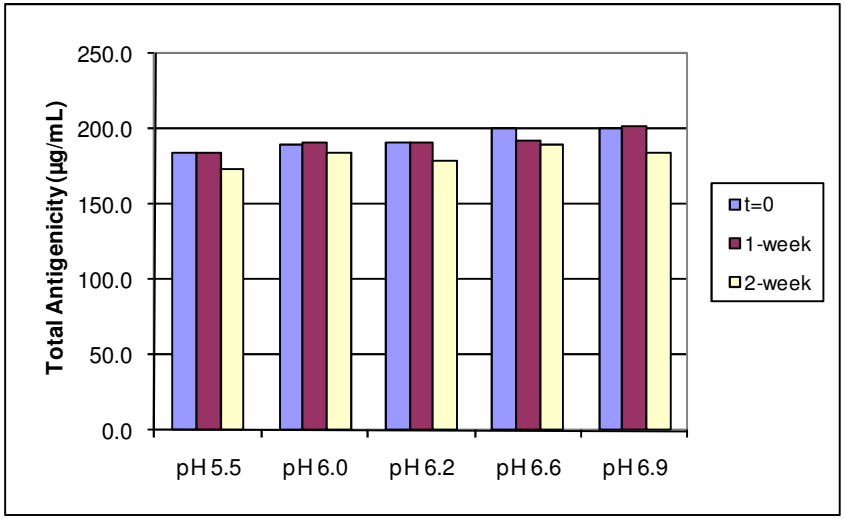
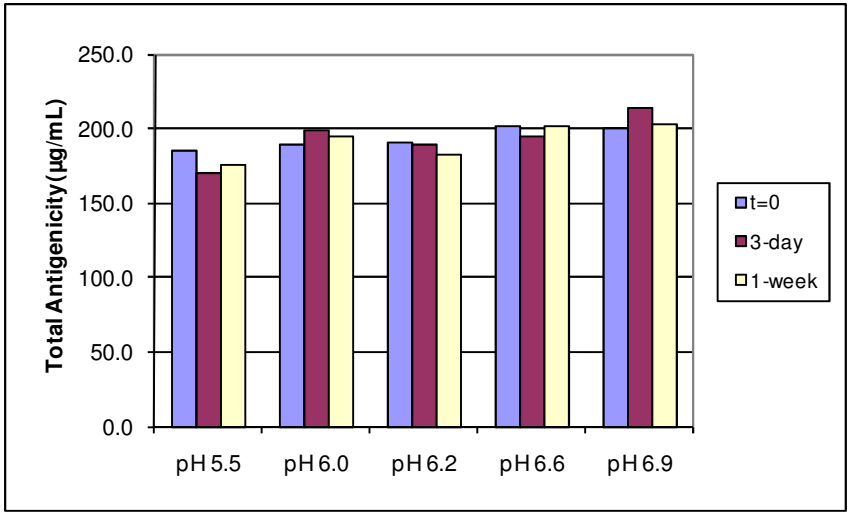
Conjugate 1 and 2 are stable at pH 6.5 or above

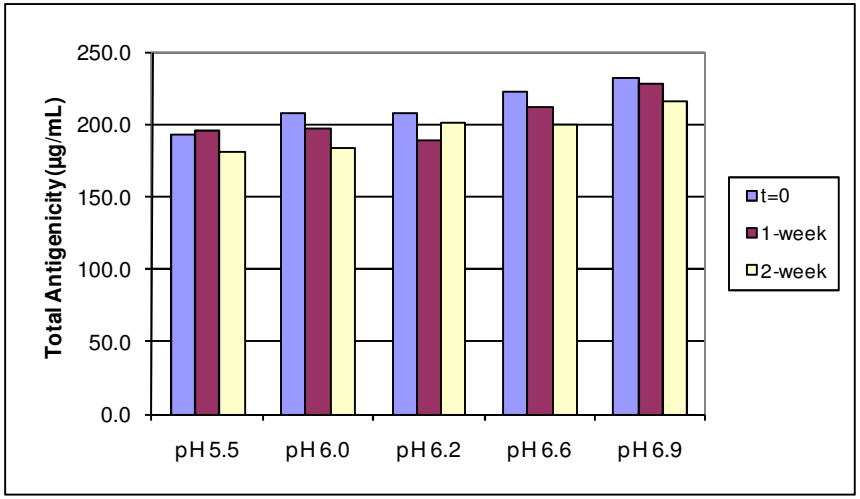
Conjugate 1 @ 5ºC



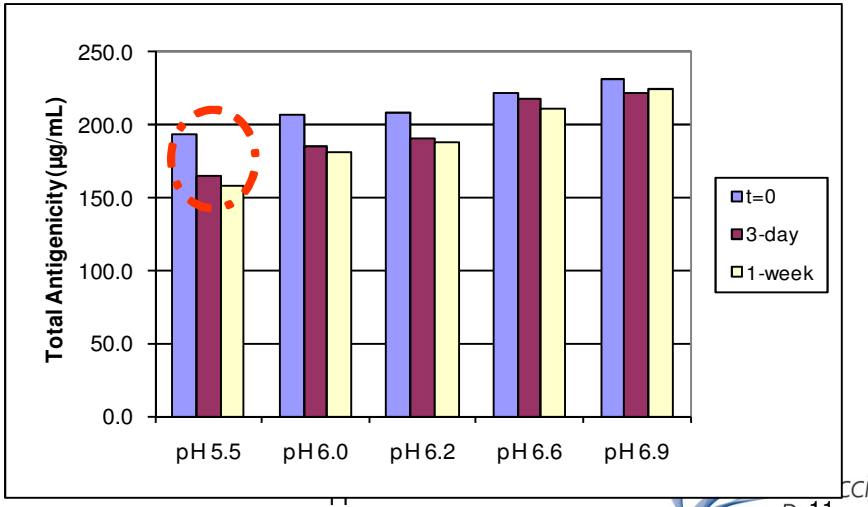
Conjugate 1 @ 25ºC



Conjugate 2 @ 5ºC



Conjugate 2 @ 25ºC



Drug product formulation rationale is based on stability

	Conjugate 1	Conjugate 2	Protein 1	Protein 2
Buffer matrix Drug Substance matrix	10mM His 6.7	10mM His 6.7	10mM His 6.5	10mM His 6.0 (5.8 to 6.2)
Key driver to stability	pH > 6.5	> pH 6.5	Absence of water	pH 6.0 Absence of salt and water
Buffer	Histidine most optimal for stability and lyophilization			
Degradation mechanism	Aggregation Filterability	Aggregation Filterability	Clipping	Deamidation
Stability	Typically for conjugates change is decrease in size followed by increase in free sugar		Stable	Stable
Drug Product : Target 6.5 ± 0.3 to maximize stability of all four components				

Combined vaccine unstable in solution - Need to freeze dry

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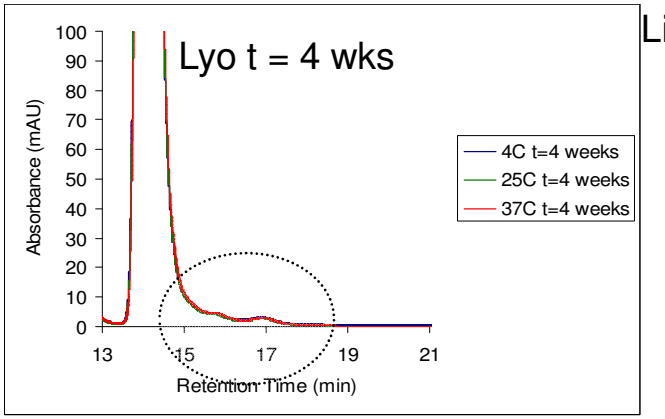
When product is unstable in solution - Freeze dry

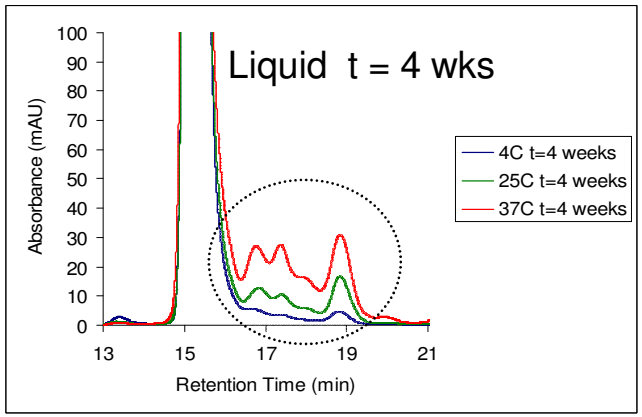
- Lyophilization
  - Selection of excipients/bulking agents
  - Glass transition
  - Eutectic temperature
  - Crystallization temperature
  - Process times
    - Freeze temperature
    - Annealing
    - Primary drying
    - Secondary drying time
- Success factors include
  - Cake appearance
    - Depends on excipients
  - Biophysical analysis
  - Moisture
  - Recon time
  - Osmolality
  - Binding to adjuvants
  - Long term product stability
- Technologies applied
  - Freeze microscopy to determine collapse temperature
  - Modulated DSC
  - X-ray crystallography
- **Analytics**
  - Biophysical analysis
    - CD/ Flourescence /DSC/FTIR
  - Karl Fischer
  - Visual appearance
  - pH
  - Osmometry
  - Nephelometry
  - Protein chemical based assays
  - HPLC
  - *in vitro* potency

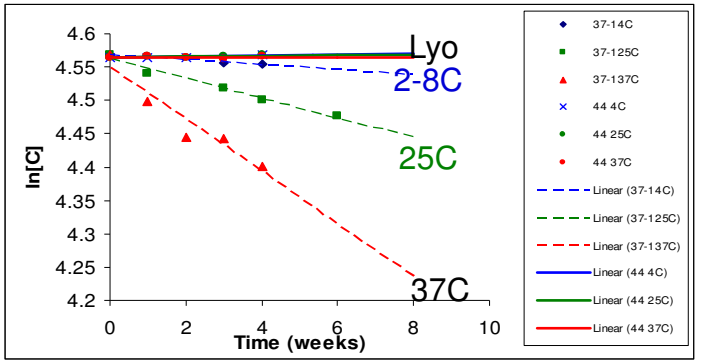
Rationale for lyophilization

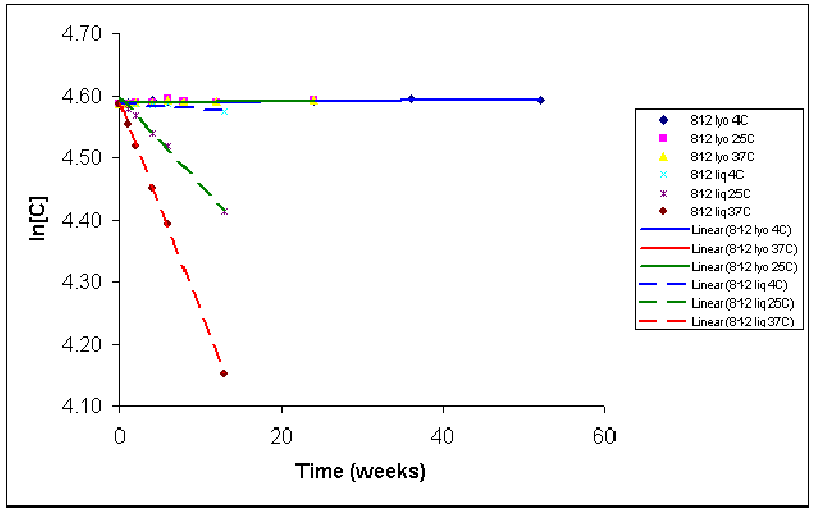
Kinetic plot of Protein 1: Unstable Liquid Formulation vs a Freeze-Dried Product



Liq







Degradation occurs upon storage when formulated as a liquid

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How do we characterize antigen-adjuvant formulations

- Formulation
  - Co-formulate with antigen
  - Mix and shoot at the clinic
- Stability
  - Degradation mechanisms may be affected by an adjuvant
  - Compatibility
- Long term stability of adjuvants
  - Compatibility of antigen-adjuvant
    - Solution conditions such as pH, buffer and other excipients Low dose stability
- Stability of vaccine antigen and adjuvant after reconstitution
  - Integrity of Antigen in combination
- Analytics
  - HPLC methods
    - SEC, IEX, Reversed-phase
    - Confirm multiple components do not interfere with each other
  - Size of Particulate Adjuvants
    - Dynamic Light Scattering, Mastersizer
  - Antigen-adjuvant interactions
    - Isothermal Titration Calorimetry
    - % binding assays (if applicable)
    - Zeta Potential: can predict binding based on electrostatic interactions
  - Antigen Conformation
    - Biophysical methods such as Fluorescence, CD or DSC can show if adjuvant is changing the antigen conformation
  - *In vitro* potency

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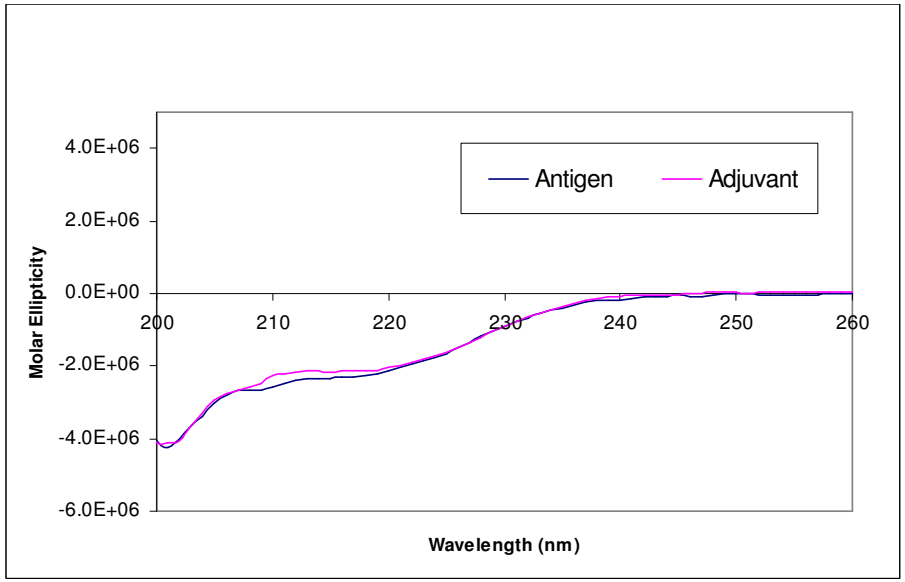
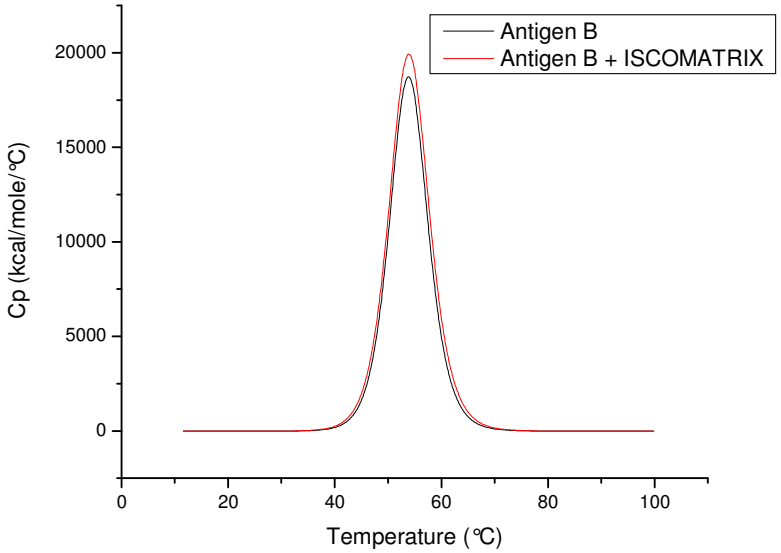
An example of Biophysical methods used to evaluate effect of adjuvant on protein structure

Circular Dichroism



Differential Scanning Calorimetry



- These analysis provide confidence that there are no conformational changes in the antigen.
- These analysis need to be conducted on individual antigens only

Vaccine Research

Differential binding of antigens to aluminum salts

Al conc. mg/mL	% Antigen Bound to Al as $AlPO_4$			
	Protein 1	Protein 2	Conjugate 1	Conjugate 2
0.75	2.6	100	18.1	13.8
0.5	3.5	100	15.6	11.1
0.25	0	100	14.6	11.4
0.125	4.0	100	12.9	10.5

Al conc. mg/mL	% Antigen Bound to Al as Al(OH)3			
	Protein 1	Protein 2	Conjugate 1	Conjugate 2
0.75	100	100	100	100
0.5	100	100	100	100
0.25	100	100	95.6	95
0.125	100	100	71.2	87.6

Antigens bound to Al(OH)3 cannot be recovered easily

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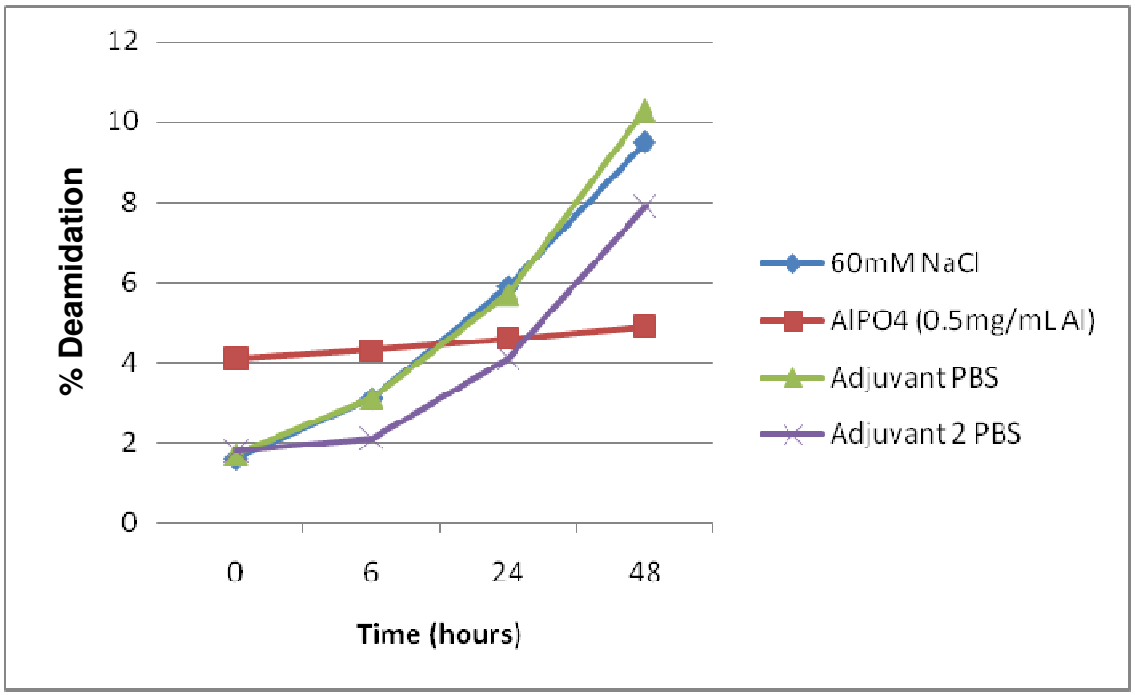
Demonstrate purity of antigen in the presence of adjuvants



The protein binds tightly to AlPO4. *Could this help prevent deamidation?*

Storage time of the vaccine may be limited at room temperature after reconstitution of the vaccine.
Recommendation : deliver vaccine within four hours

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Summary – The challenges

- Formulation challenges
  - Selection of antigen candidates to maximize the immunological outcome
  - With multiple antigens the final vaccine formulations may be optimized based on a compromise – *a sweet spot*
  - Lyophilization may be the optimal dosage form but is expensive
  - Product stability
  - Limited time between reconstitution of the vaccine and delivery
  - Interaction with adjuvants
  - Optimizing doses for each antigen in a clinical study
- Analytical challenges
  - Multiple assays to quantitate each antigen and or monitor purity
  - Example- If one of the antigens aggregates – SEC HPLC cannot be applied with multiple components
- Combination vaccines are critical to the success of vaccination programs, and each new combination must be carefully studied to ensure comparable safety and immunogenicity of the individual components.

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- Ozgur Akcan
- Leena Bagle
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