

# Characterization of Protein Antigens by Light Scattering Techniques

Lubomir Kovac  
Wyeth Vaccines Early Phase Programs  
401 N. Middletown Rd.  
Pearl River, NY, USA  
(845) 602-3063  
Fax (845) 602-1074  
E-mail: kovac@wyeth.com

Lubomir Kovac, Bruce Green and April Xu  
Wyeth Vaccines Early Phase Analytical Sciences, Pearl River, NY

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## ABSTRACT

Development of a successful drug requires extensive analysis of the product. Analytical tests fall into broad categories of characterization and comparability assays. Size exclusion chromatography (SEC) in combination with light scattering techniques is presented as a tool for the characterization of three protein antigens as vaccine candidates.

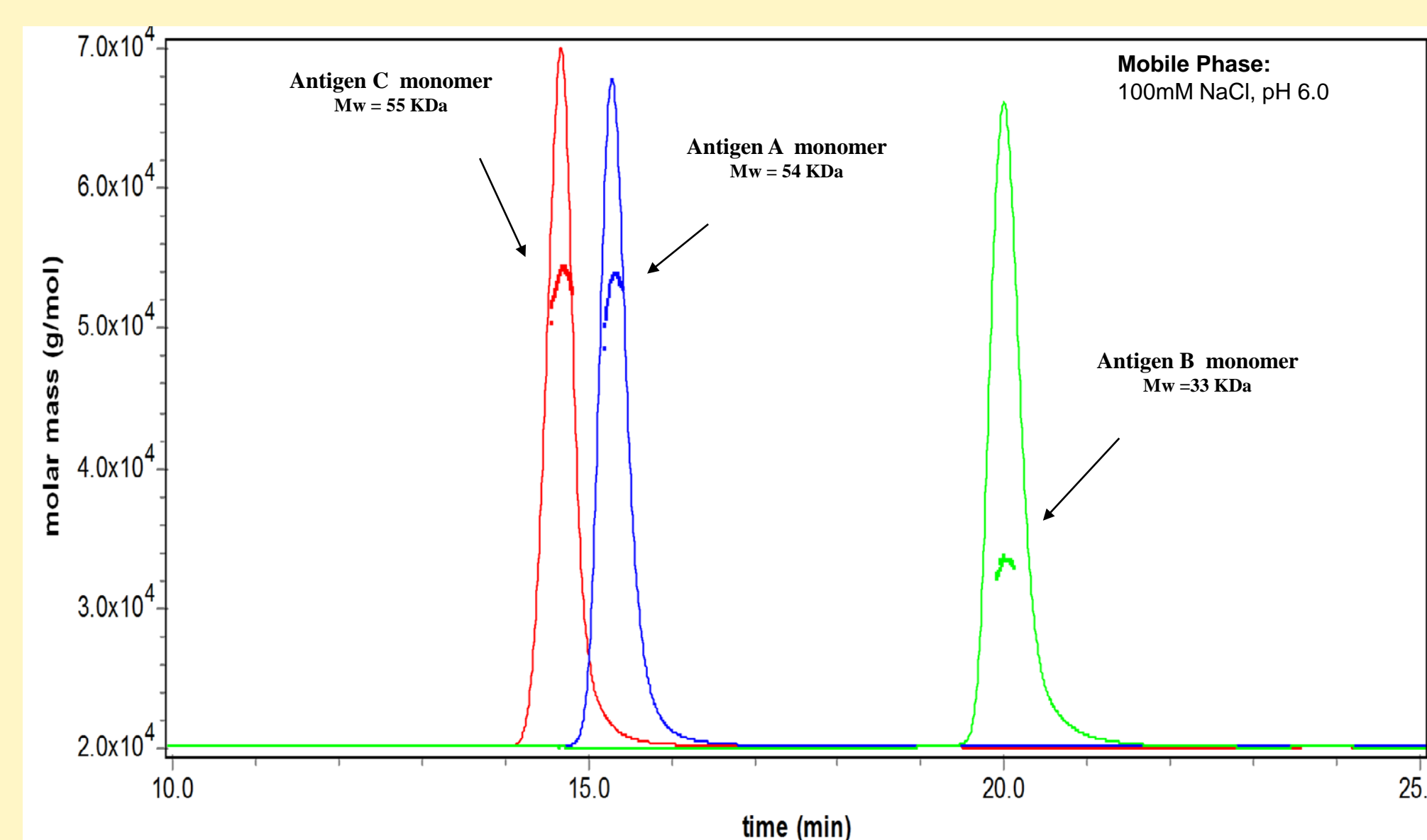
An SEC assay has been developed to characterize three protein antigens of interest. Forced oligomerization experiments were performed to understand assay's stability indications. Presented data revealed differences among protein antigens in their response to pH and ionic strength. SEC alone is not sufficient to characterize behavior of antigens based on elution times. The elution times can be dramatically affected by solution conditions and are not always indicative of protein self-association.

## BACKGROUND

- Several protein antigens are selected as vaccine candidates. Appropriate analytical control strategies must be designed to demonstrate the quality of the product.
- One of the commonly used techniques for characterizing protein antigens is SEC. SEC coupled with an on-line light scattering detector can be used to monitor the stability of proteins and the oligomerization state of proteins.
- However, the elution profile from SEC analysis can be complicated by several factors: protein self-association/aggregation states, interaction with the column matrix and non-equilibrium conditions used for SEC analysis.
- It is important to understand the interactions driving self association/aggregation as these can be perturbed by solution properties i.e. pH, ionic strength, temperature, co-solutes, etc. This information is needed for understanding and interpreting SEC results as well as assay's stability indications.

## RESULTS

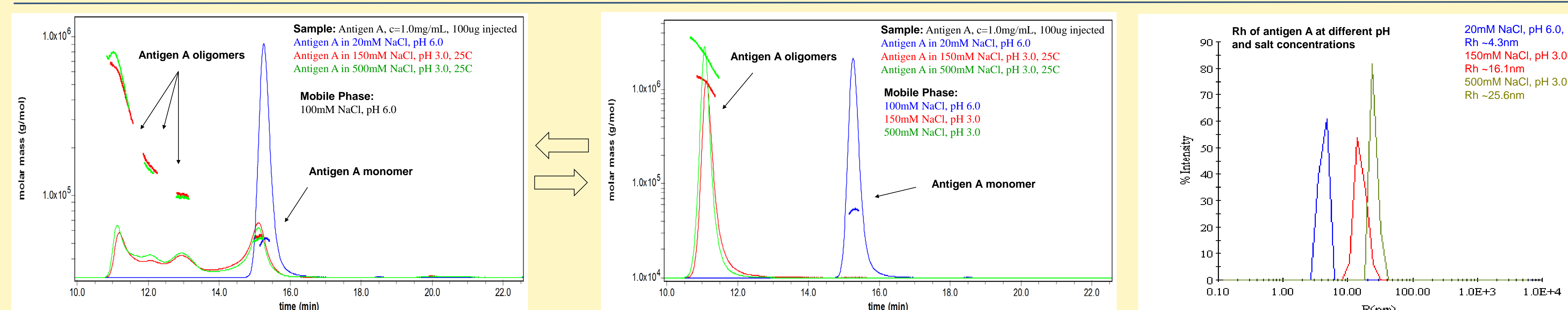
### SEC-MALLS of Protein Antigens A, B and C at pH 6.0 on G3000SWxl column



- SEC-MALLS confirms that all protein antigens are in monomeric form at pH 6.0.

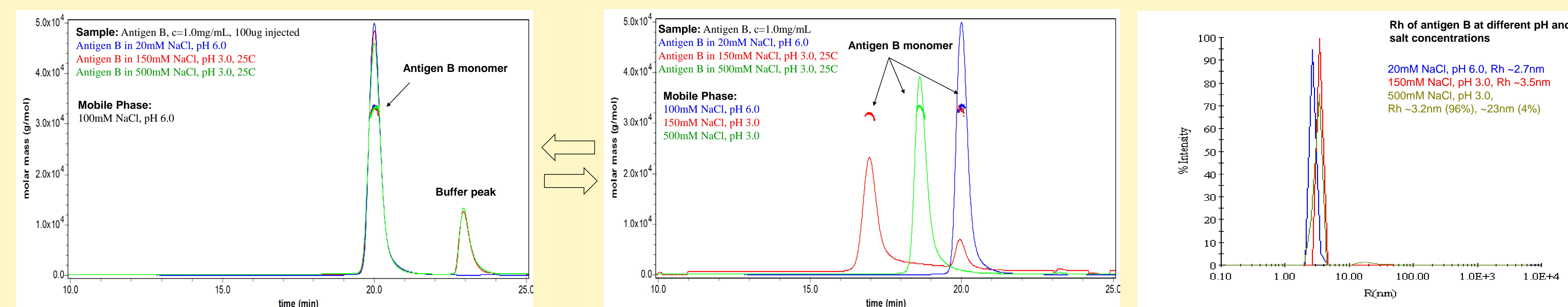
## RESULTS

### Forced Oligomerization of Protein Antigen A: SEC-MALLS on G3000SWxl Column and Mass Distribution of Particles by DLS



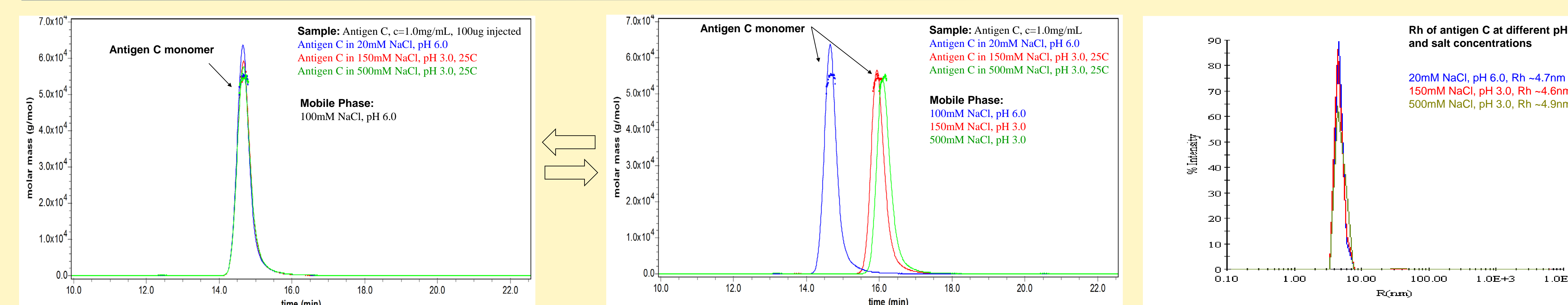
- SEC-MALLS profiles indicate formation of oligomers at low pH. Oligomerization of antigen A appears to be partially reversible under the given conditions.

### Forced Oligomerization of Protein Antigen B: SEC-MALLS on G3000SWxl Column and Mass Distribution of Particles by DLS



- Observed shift in elution time of stressed samples on SEC column is likely due to conformational change and is completely reverted when pH of mobile phase is adjusted to 6.0.

### Forced Oligomerization of Protein Antigen C: SEC-MALLS on G3000SWxl Column and Mass Distribution of Particles by DLS



- Observed shift in elution time of stressed samples on SEC column is likely due to conformational change and/or nonspecific protein interactions with column matrix and is completely reverted when pH of mobile phase is adjusted to 6.0.

## METHODS

- Size exclusion chromatography with on-line light scattering detection

Agilent 1100 HPLC System with diode array detector (UV) was connected to a DAWN HELEOS light scattering instrument and Optilab rEX differential refractometer (Wyatt Technology, Santa Barbara, CA). The detectors were connected in the following order: UV-LS-RI.

TSK-Gel G3000SWxl 7.8mm x 30cm column from TOSOH Corporation was used in all separations. Mobile phases consisting of 10mM Sodium Phosphate, 5mM diethylenetriamine (DIEN) and various concentrations of NaCl at pHs as indicated on figures were delivered at 0.5mL/min. DIEN was used as an additive to suppress nonspecific interactions of proteins with the column matrix.

Astra V software version 5.3.2.16 (Wyatt Technology, Santa Barbara, CA) was used to collect and process experimental data.

- Dynamic light scattering analysis

Dynamic light scattering (DLS) measurements were performed on a DynaPro Plate Reader Plus (Wyatt Technology, Santa Barbara, CA) using 384-well plates (Thermo Electron Corporation). Sample volume per well was 55µL. All DLS measurements were performed at 25 °C.

Dynamics software, version 6.9.0.7 (Wyatt Technology, Santa Barbara, CA) was used to collect and process experimental data.

## SUMMARY

- Forced oligomerization experiments show differences among protein antigens in their response to the low pH and high NaCl concentrations. Only one of three antigens (antigen A) undergoes oligomerization under the given stress conditions.
- SEC alone is not sufficient to characterize the behavior of protein antigens under tested conditions. Elution times, while significantly affected by solution conditions, are not always indicative of protein self-association. The observed shift in elution times of stressed antigens B and C is likely due to conformational change.
- Differences among antigens in their response to various solution properties have to be considered when developing appropriate analytical tests for their characterization.

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