# IMPACT OF PH MODIFICATION ON THE AGGREGATION OF NIVOLUMAB (OPDIVO®) IN CLINICAL SOLUTIONS AT 1 AND 5 MG/ML MEASURED BY DYNAMIC LIGHT SCATTERING (DLS) AND SIZE EXCLUSION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-DIODE ARRAY DETECTION (SE/ HPLC-DAD)

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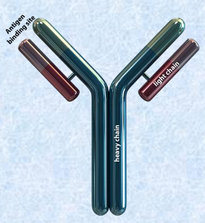
## Background and importance




Nivolumab (Opdivo®) is a human IgG4 monoclonal antibody (mAb) that binds to programmed death receptor 1 (PD-1). As a complex protein, routine handling or unintentional mishandling of its solutions may cause unnoticed physical aggregation that could potentially compromise the clinical safety and efficacy of the medicine [1].

## Aim and objectives

To assess the impact on nivolumab (Opdivo®, pH 6) aggregation process promoted by pH modification (from pH 4 to 8) in two clinical diluted solutions at 1 and 5 mg/mL. Also, to assess the influence of such pH modifications on nivolumab stability over time (up to 72h, stored at 4°C and protected from light).


## Materials and methods


Nivolumab 10 mg/mL

NaCl 0.9%

Nivolumab dilution using NaCl 0.9%

Nivolumab 1 mg/mL

Nivolumab 5 mg/mL

### Impact on the aggregation on nivolumab promoted by pH modification

Nivolumab 1 mg/mL

Nivolumab 5 mg/mL

pH modifications of both diluted samples

| pH |
|---|
| 4 |
| 5 |
| 5.5 |
| **6** |
| 6.5 |
| 7 |
| 8 |

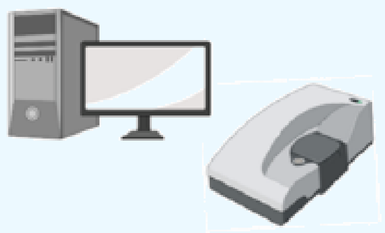
0.1M HCl

Control sample

pH of the original medicine

0.1M NaOH

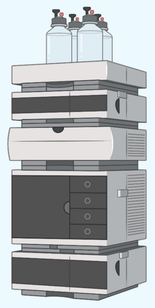
### Particulate was tracked by Dynamic Light Scattering (DLS)



Malvern Zetasizer Nano ZS90

| DLS parameters | |
|---|---|
| Cell description | Plastic cuvette |
| Temperature | 20 °C |
| Material | Protein |
| Dispersant | Water |
| Dispersion optics | 90° |
| Number of records | 100 |
| Duration of every record | 10 sec |

### Aggregate profile was determined by Size-Exclusion High-Performance Liquid Chromatography (SE/HPLC-DAD)



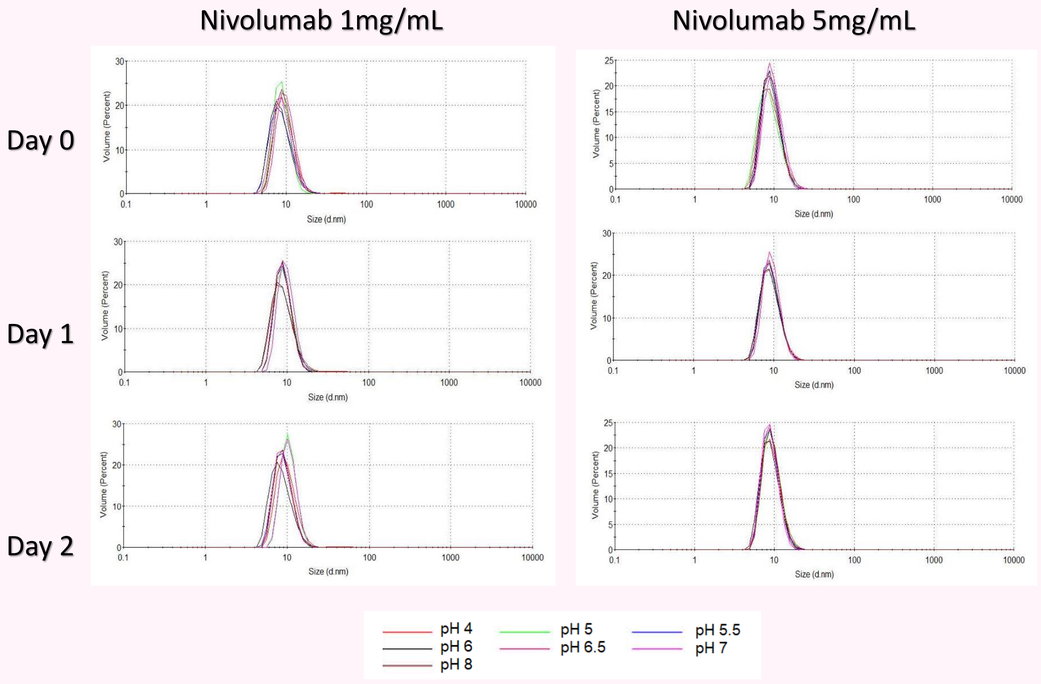
- Agilent 1100 chromatograph
- Agilent Bio SEC-5, 5 µm, 300A, 4.6 x 300 mm column

| SE/HPLC-DAD conditions | |
|---|---|
| Column temperature | Room temperature (25°C) |
| Flow | 3.5 mL/min |
| Mobile phase | 150 mM phosphate buffer pH 7 |

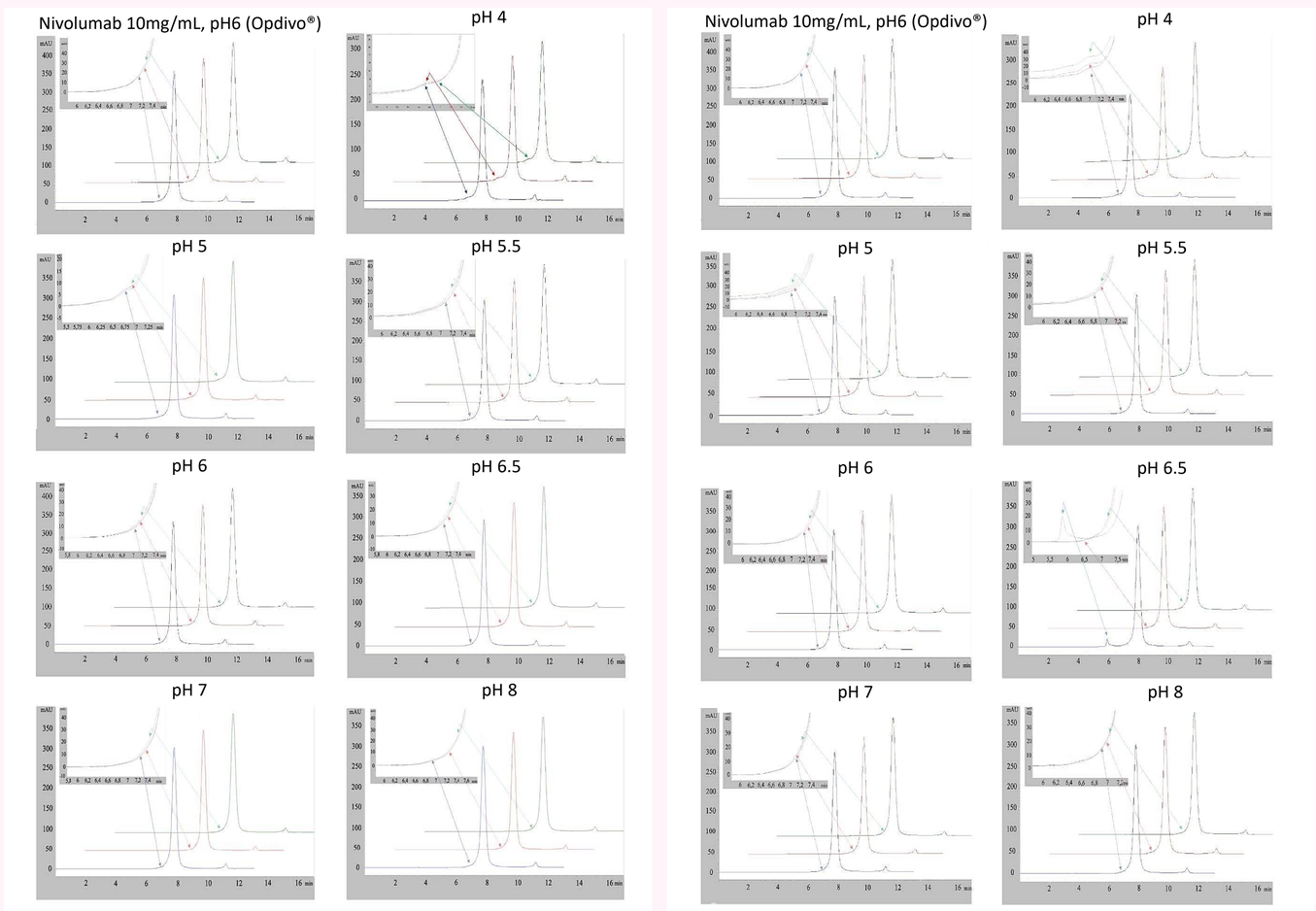
Measurements were carried out on **day 0**, **day 1** and **day 2** in order to evaluate the stability of the freshly prepared solutions to pH changes as well as their stability over three days.

## Results

### DLS size distribution by volume graphs



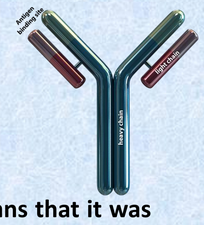

### SE/HPLC-DAD chromatograms




Control samples of diluted nivolumab at 1 and 5 mg/mL showed a single particulate population with hydrodynamic diameter (HD) of 10.02±2.54 nm and 9.32±2.43 nm respectively, attributed to nivolumab monomers; and the chromatographic profiles indicated the presence of a single peak which also corresponded to monomers. After pH modifications, SE/HPLC-DAD indicated a slight onset of dimers formation in samples at pH 4 and 5 for both diluted clinical solutions, keeping this situation over time with no more dimer increase. No significant changes were observed in any of the samples studied either by DLS or when visually checked.

## Conclusion and relevance

Variations of the pH around the medicine's pH value (pH 6) promoted a very slight dimer formation at pH 4 and 5 either in 1 or 5 mg/mL nivolumab solutions. Neither particles smaller than 10 µm nor visible particles were detected.




[1] M.R.Nejadnik et al. J.Pharm.Sc.107(2018)2013-2019.

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