



# IMPACT OF THE PREPARATION OF THE 1.0 MG/ML NIVOLUMAB CLINICAL SOLUTION ON THE PARTICULATES (AGGREGATION) MEASURED BY DYNAMIC LIGHT SCATTERING: NaCl AND GLUCOSE CONCENTRATION AND AGITATION EFFECT



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## Background and importance

Nivolumab (Opdivo®) is a human immunoglobulin G4 (IgG4) monoclonal antibody (mAb) that binds to programmed death receptor 1 (PD-1) and blocks its interaction with PD-L1 and PD-L2. As a complex protein, routine handling or unintentional mishandling of its solutions may cause degradation that could remain unnoticed but could potentially compromise the clinical safety and efficacy of the drug product [1].

To assess the impact on nivolumab (Opdivo®) aggregation process promoted by slight modification in the concentration of the compound (NaCl 0.9% or glucose 5%) used to prepare the clinical diluted solution of nivolumab at 1.0 mg/mL. Also, to assess the impact on the aggregation on nivolumab clinical diluted solutions (1.0 mg/mL, in NaCl 0.9% and glucose 5%) promoted by agitation stress.

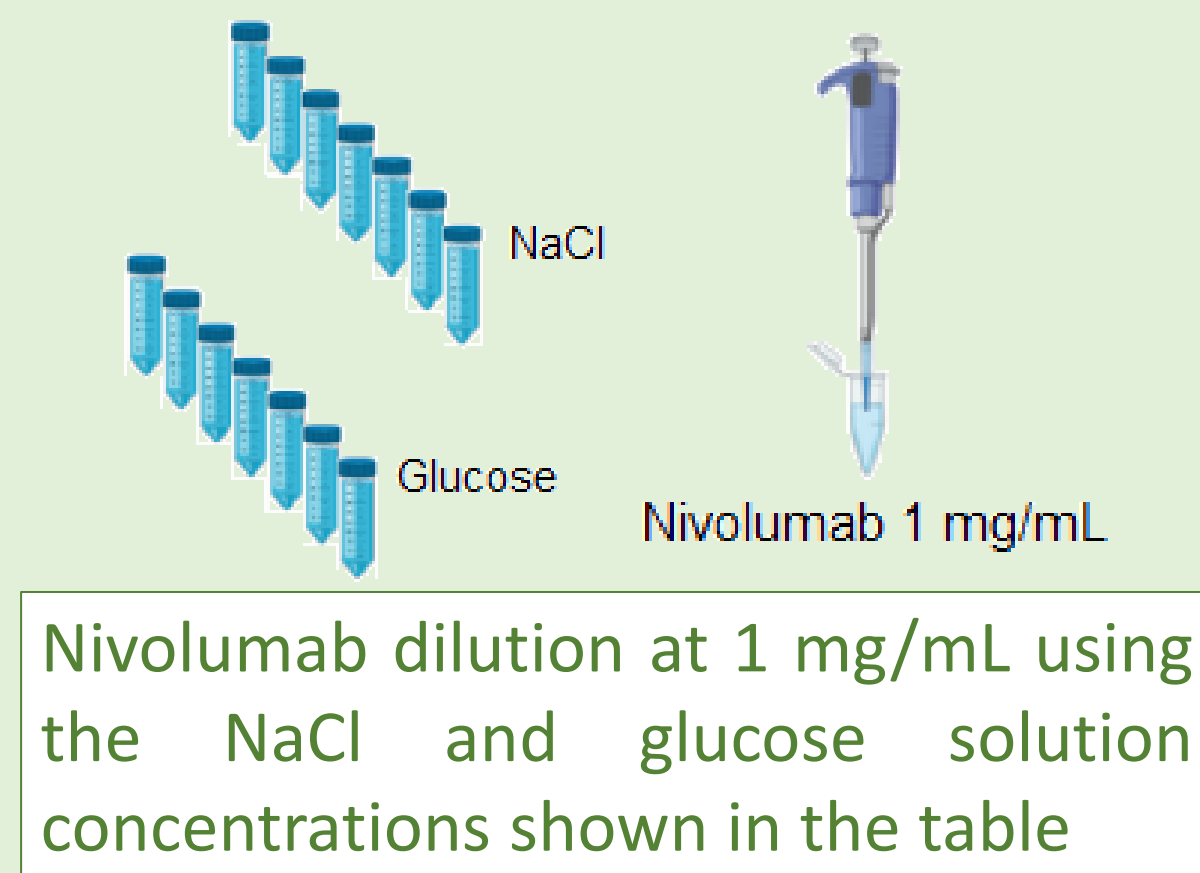
## Aim and objectives

## Materials and methods

### Impact on the aggregation on nivolumab promoted by the variation in the diluent concentration

NaCl (%)	Glucose (%)
0.5	1
0.7	2
0.8	3
<b>0.9</b>	<b>5</b>
1	7
1.3	9
1.5	10

Clinically employed solutions

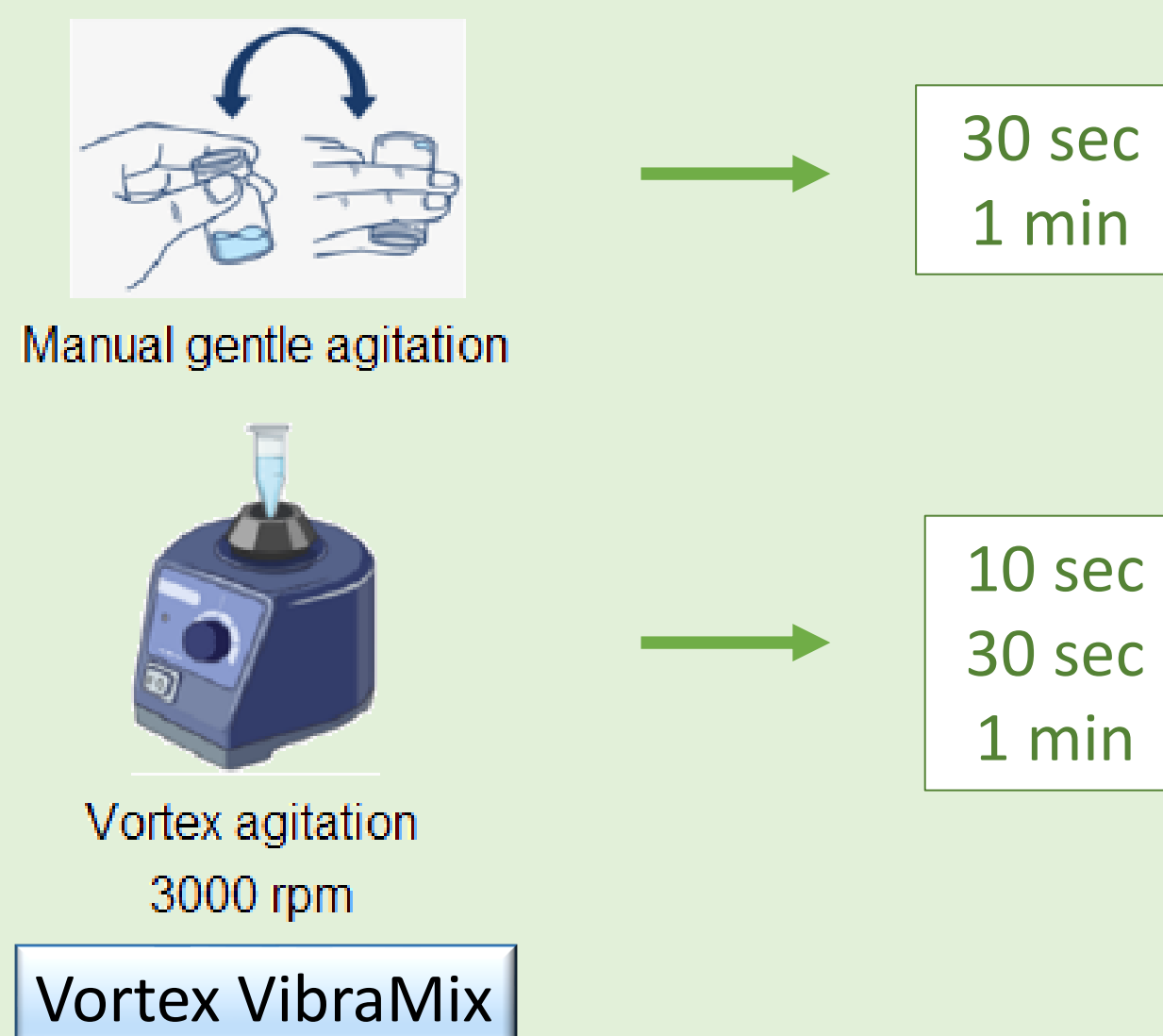


### Impact on the aggregation on nivolumab promoted by agitation stress

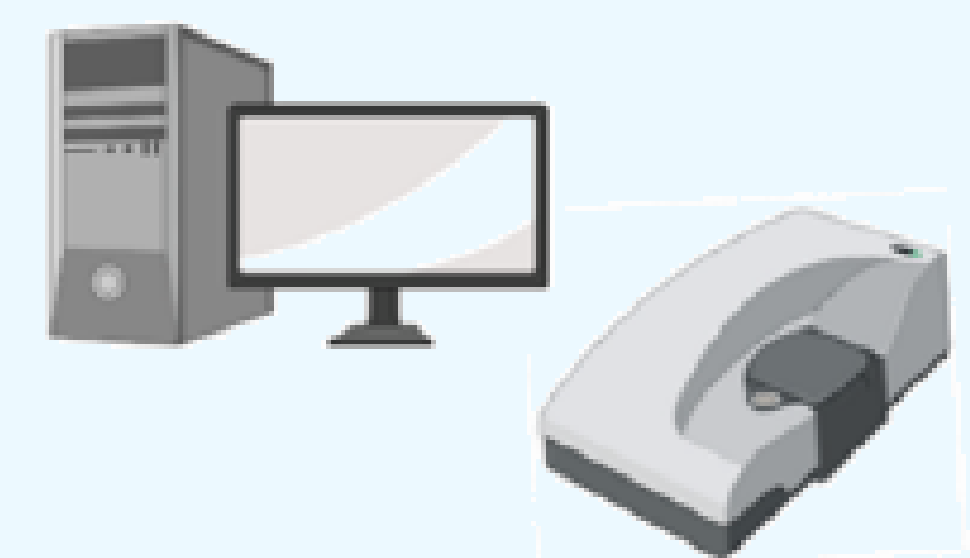
NaCl 0.9%  
Glucose 5%

Nivolumab 1 mg/mL

Nivolumab dilution at 1 mg/mL using NaCl 0.9% and glucose 5% clinical solutions



Particulate was tracked by Dynamic Light Scattering (DLS)



Malvern Zetasizer Nano ZS90

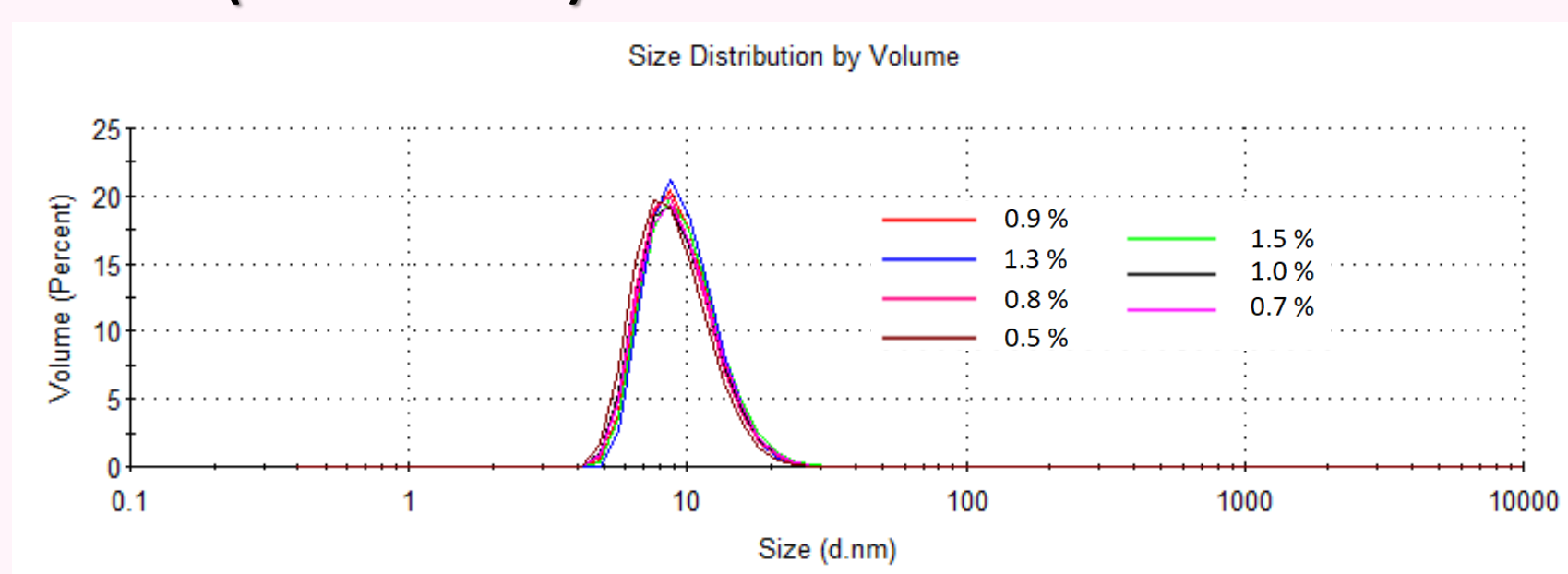
### DLS parameters

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

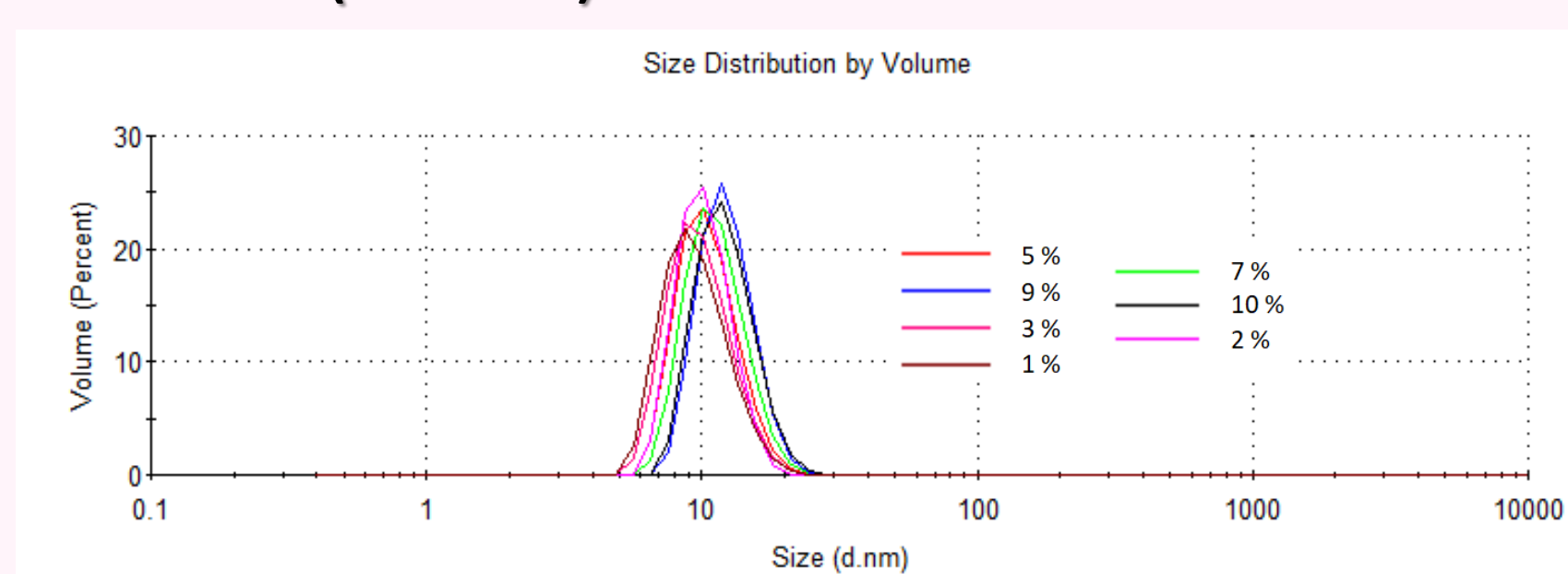
## Results

### DLS size distribution by volume graphs: variation of the diluent concentration

#### NaCl (0.5-1-5%)

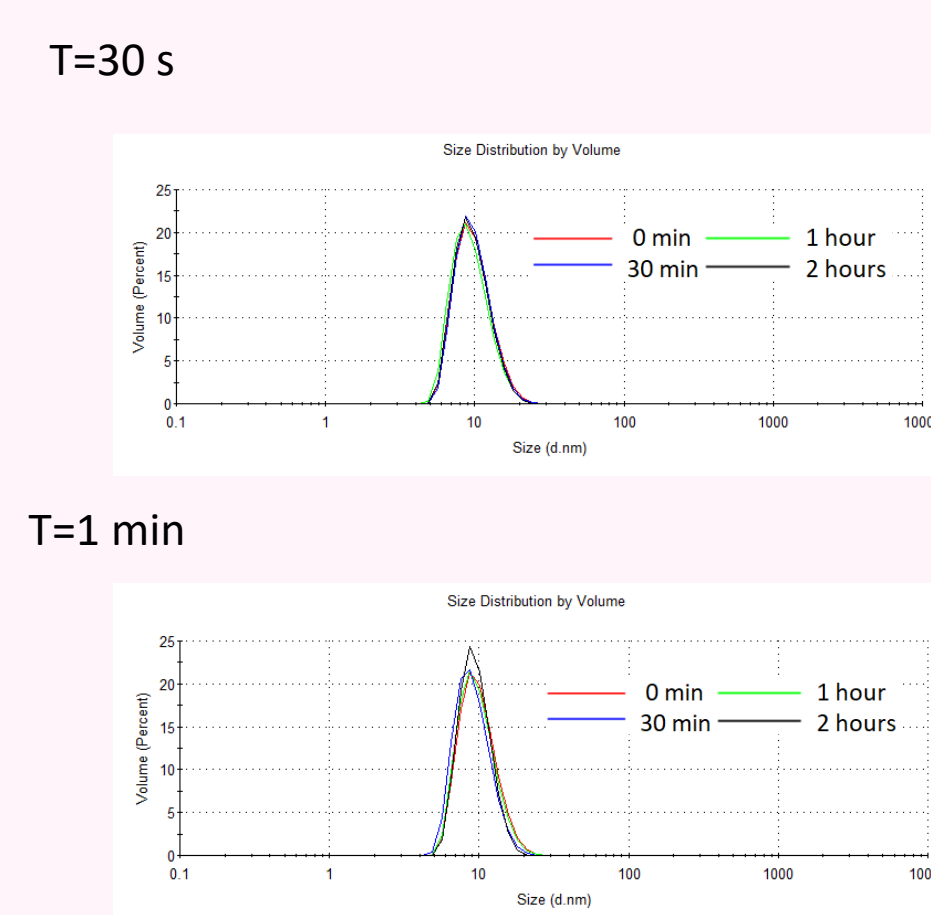


#### Glucose (1-10%)

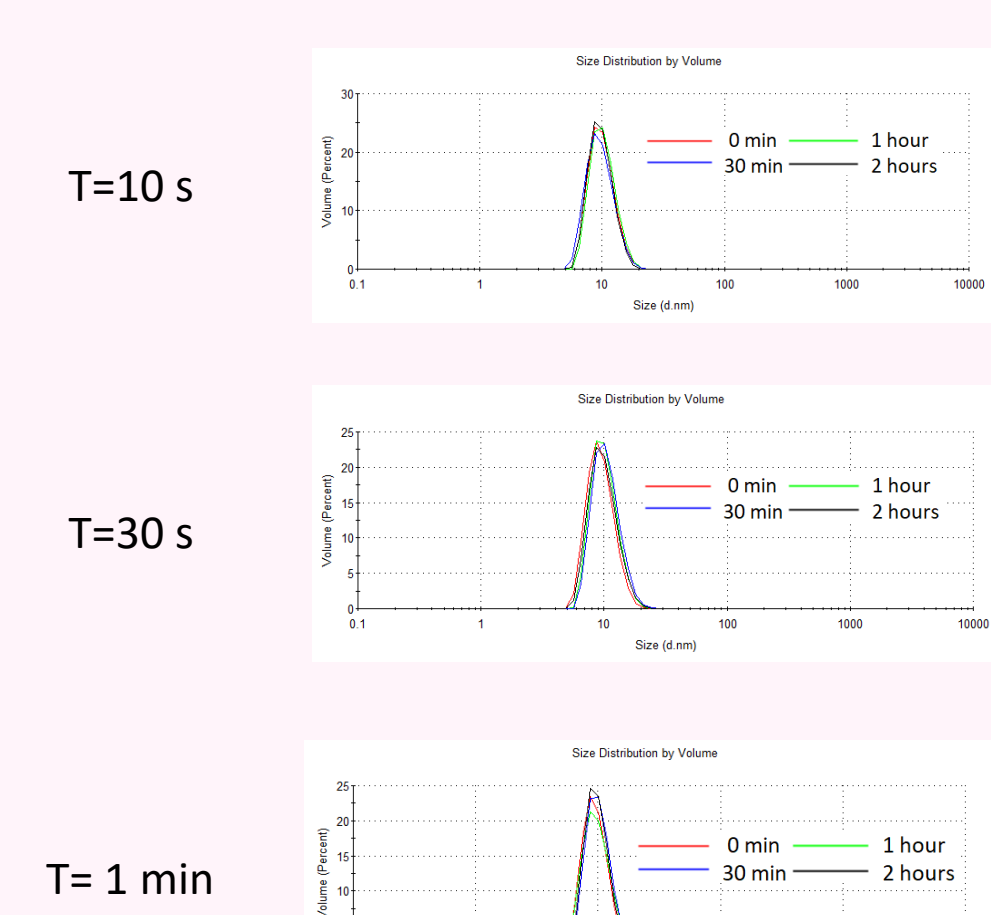


### DLS size distribution by volume graphs: manual and vortex agitation on 0.9 % NaCl samples

#### Manual agitation

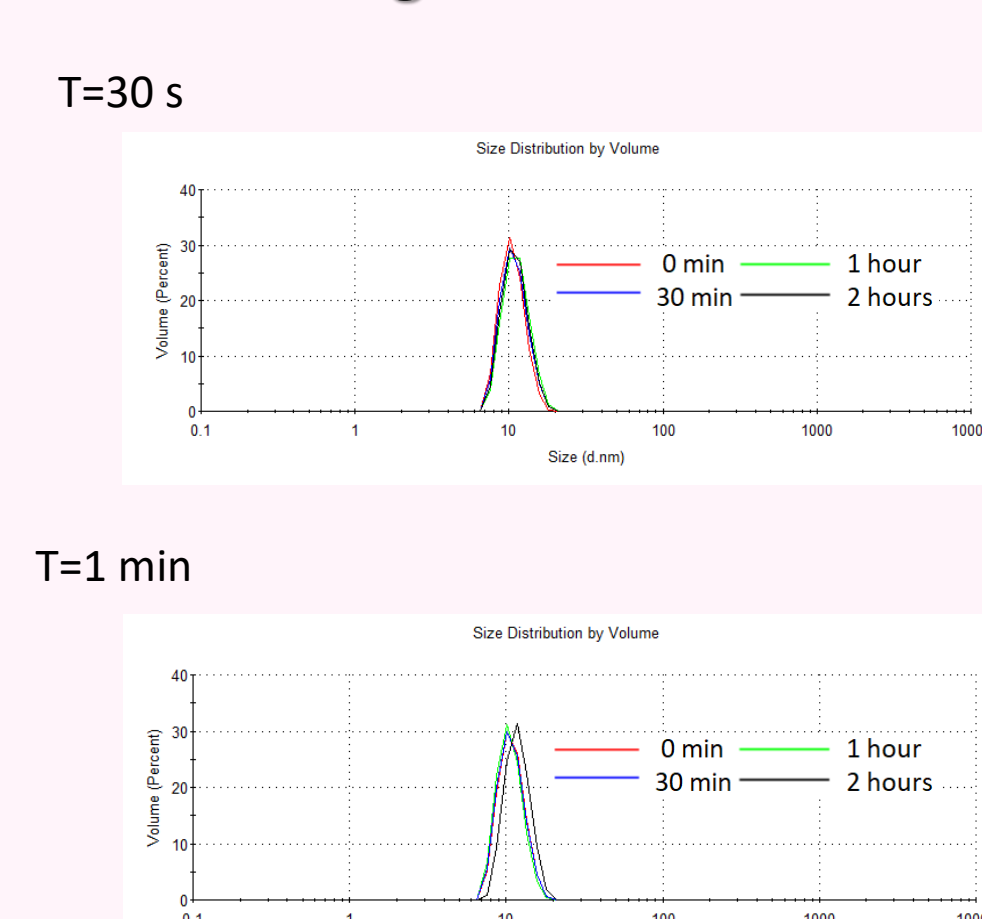


#### Agitation by vortex

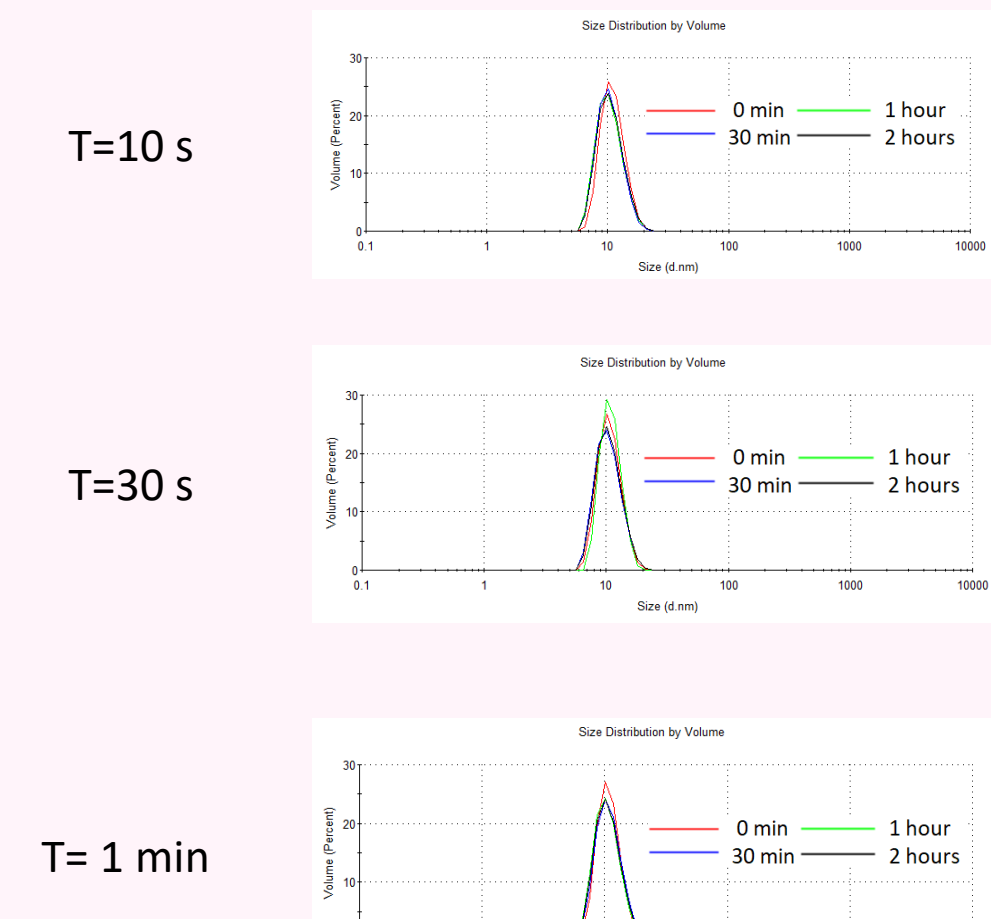


### DLS size distribution by volume graphs: manual and vortex agitation on 5% glucose samples

#### Manual agitation



#### Agitation by vortex



Reference samples of diluted nivolumab at 1.0 mg/mL in NaCl 0.9% and 5% glucose showed a single particulate population with hydrodynamic diameter (HD) of  $9.66 \pm 2.96$  nm and  $10.67 \pm 2.68$  nm respectively, attributed to nivolumab monomers. No significant changes were obtained in the HD when concentration of the diluents was changed. Also, no significant changes were observed after the stress by agitation, with the HR values within the interval corresponding to the size of the monomers of the nivolumab standard solution (reference samples).

## Conclusion and relevance

Variation on NaCl and glucose concentration around that indicated for clinical use, i.e. 0.9% and 5% respectively, does not promote aggregation or increase on the particulate size on 1 mg/mL nivolumab solution that could be detected by DLS (0.1 nm - 10 µm). Also, gentle hand shaking and vortex shaking have no impact on aggregation on these clinical nivolumab solutions by increasing the particulate size, similarly measured by DLS.

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

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