Investigation of albumin adsorption on DK-I-56-1 nanocrystals by dynamic light scattering

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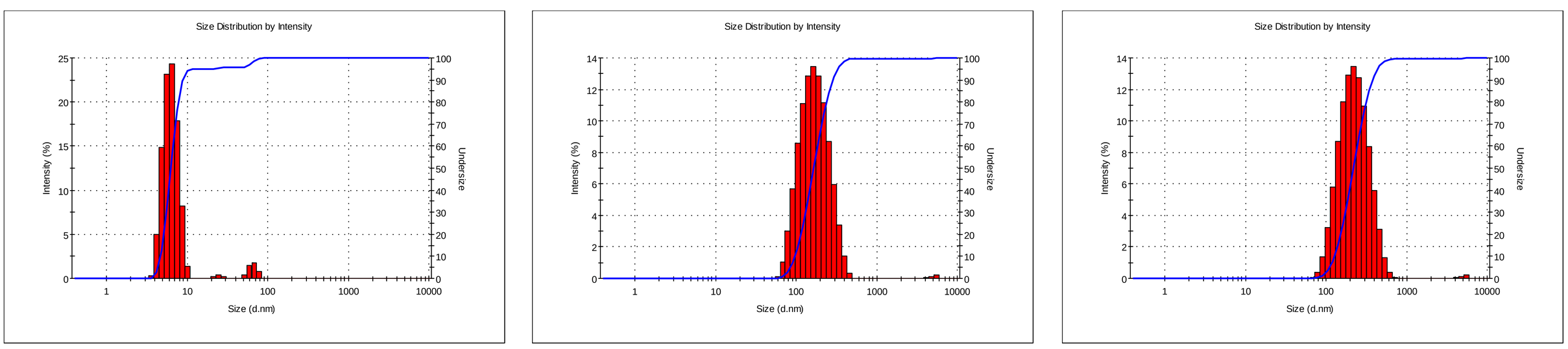
Introduction

After parenteral administration, nanoparticles interact with different proteins, forming a shell called corona, which further influences nanoparticles' biodistribution. Protein adsorption is affected by particle size and shape, but also by molecular interactions of chemical groups from the particle surface and amino-acid residues of the proteins. In human plasma, albumin is the most abundant protein so it is frequently used for the investigation of protein-nanoparticle interactions (1).

Methods

In this study we investigated the attachment of bovine serum albumin (BSA) to recently developed nanocrystals (2) of DK-I-56-1 (7-methoxy-2-(4-methoxy-d3-phenyl)-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one), stabilized by polysorbate 80 (NS2) or the combination of polysorbate 80 and poloxamer 407 (NS4). Nanocrystal dispersion was incubated in medium containing 0.1% or 1% BSA in phosphate buffer saline (pH 7,4, PBS) at 37 °C for 1 h. Particle size analysis was conducted by dynamic light scattering (DLS) in 10 min interval, at 37 °C on Zetasizer ZS90 (Malvern Instruments Ltd., Worcestershire, UK).

Results



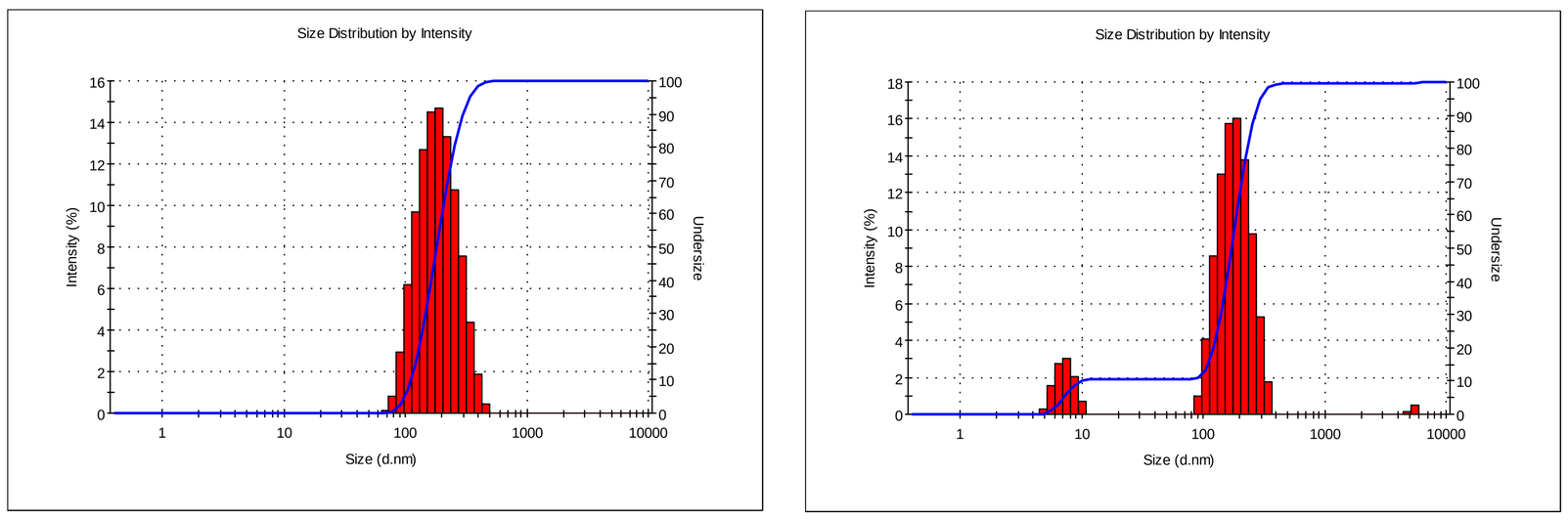
a) b) c)

Figure 1: DLS results for a) BSA in PBS, b) NS2 in PBS and c) NS4 PBS.

Peak 1: 6.3 ± 0.3 nm
Peak 2: 65.3 ± 0.3 nm

✓ The adsorption of albumin (BSA) was influenced by the nanocrystal formulation and albumin concentration, but not incubation time.

✓ In a medium with 0.1% BSA, no particle size difference was noticed in either formulation, when compared to particle size in PBS.



a) b)

Figure 2: DLS results for a) NS2 and b) NS4 in 1% BSA/PBS

Table 1: Mean hydrodynamic diameter (z-ave) and polydispersity index (PDI) before and after incubattion of NS2 iand NS4 in different media

Formulation / Particle size	0 h		1 h	
	z-ave (nm)	PDI	z-ave (nm)	PDI
NS2 in PBS	153.9 ± 1.6	0.202 ± 0.008	163.0 ± 0.5	0.187 ± 0.009
NS2 in 0.1% BSA/PBS	153.8 ± 1.2	0.181 ± 0.019	161.6 ± 2.1	0.186 ± 0.016
NS2 in 1.0% BSA/PBS	161.0 ± 1.3	0.251± 0.020	165.2 ± 0.6	0.250 ± 0.009
NS4 in PBS	206.9 ± 5.5	0.205 ± 0.032	205.2 ± 2.1	0.201 ± 0.002
NS4 in 0.1% BSA/PBS	199.3 ± 4.5	0.224 ± 0.034	186.9 ± 2.2	0.188 ± 0.010
NS4 in 1.0% BSA/PBS	146.1 ± 8.9	0.457 ± 0.068	145.2 ± 0.8	0.358 ± 0.004

✓ Formulation NS2: after the addition of 1% albumin, particle size and particle size distribution has increased, which indicated albumin binding.

✓ In formulation NS4, with higher albumin concentration two peaks were visible, one from the free albumin, and one from nanocrystal particles.

Conclusion

The affinity of albumin was influenced mainly by the interaction with the nanocrystal stabilizers. Additional experiments should be performed to investigate the nature of observed interactions, as well as its relevance after nanocrystals administration.

References

1. Agrahari V, et al. Adv Drug Deliver Rev 2019; 148: 146-180.
2. Mitrović JR, et al. Eur J Pharm Sci 2020; 152: 105432.

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