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Freeze-drying of Upcon® conjugates
an alternative way to store the UCNPs in a powder form

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BACKGROUND AND MATERIALS

Motivation: Long shelf life of the product saves money in the industry

- Larger production batches and more flexible production scheduling
- Less disposal of unused expired material

Typical stability of the Upcon® conjugates (stored in aqueous suspension): a few months to one year

- Nanoparticle core: Very stable inorganic material ($NaYF_4$:Yb,Er) when stored with fluoride ions[1)]
- Biomolecules conjugated to the particles: More prone to aging
- Particle coating method and storage conditions also affect stability[2)]

Aim: Expanding the shelf-life of the Upcon conjugates by freeze-drying (storage in a powder form)

- Several Upcon coatings: Thin (<1 nm) or thick coating (12 nm) with either COOH- or N_3-functionality
- Different biomolecules: Robust streptavidin (SA) and two delicate antibodies (Ab)

Table 1. Follow-up of quality control parameters for Upcon-conjugates: Before / just after / 3–4 months after (SA- and Ab-conjugates) / 6 months after (only SA-conjugates) freeze-drying.

Observed changes over time (gray:4 months, green: 6 months)								
Coating type	Biomolecule	Stored	UCNP concentration	Aggregation	Detachment of biomolecule	Performance in assay		Preferable storage form
						Non-spesific binding	Specific signal	
C1 - Thick (12 nm) - COOH	SA	in solution	Increases	-	-	-	-	Ok
		as dry powder	-	-	-	-	-	Preferable
C4 - Thick (12 nm) - N_3	Ab (two different)	in solution	-	-	Increases	-	Ab1: Less active Ab2: -	Depends on Ab
		as dry powder	-	Less colloidal originally	-	-	Ab1: - Ab2: Less active	Depends on Ab
C5 - Thin (<1 nm) - COOH	SA	in solution	Increases	Colloidality decreases *	-	Increases	-	
		as dry powder	-	-	-	-	-	Preferable
	Ab	in solution	Increases	Colloidality decreases *	-	-	-	
		as dry powder	-	-	-	-	-	Preferable

*Aggregation can be broken off with high-power ultrasound

RESULTS

- **Performance and aggregation** after freeze-drying the Upcon conjugates **(Fig 1)**
  - Functionality of the biomolecules was retained during the drying process **(Fig 2)**
  - No particle aggregation was observed based on a sedimentation test
- **Stability comparison:** Powder form vs. aqueous suspension
  - Real-time stability testing: 4 months or 6 months (for Ab- or SA-conjugates, respectively)
  - All conjugates were functional after the storage period
  - Negative observations from each product were collected in **Table 1**
    - Thin coating (C5) aggregated strongly when stored in aqueous suspension while dried one remained colloidal
- **Storage in ambient temperature** instead of controlled storage condition (+4 °C):
  - Slightly compromised performance in heterogeneous assay at low analyte concentrations, but standard curve saturates at the same level

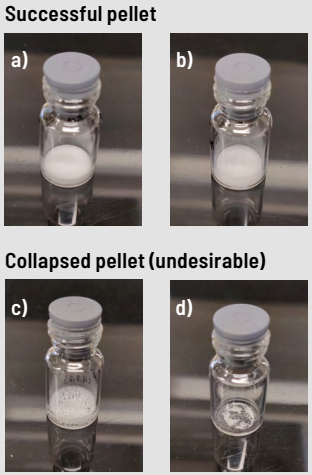

Fig 1. Upcon-SA conjugates freeze-dried from a simple buffer containing (a) BSA and sucrose, (b) only BSA, (c) only sucrose, (d) without any excipient.

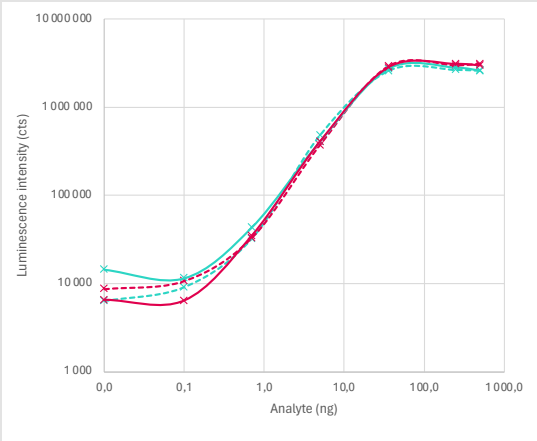

Fig 2. Heterogeneous sandwich assay to follow the performance of the Upcon-SA conjugates (with C5-coating) over time. Fresh (dashed line) and 6-months aged (solid line) conjugate stored in aqueous solution (green) or as dry powder (red).

CONCLUSION

- All freeze-dried Upcon conjugates remained functional in the process and rehydration step was easy and immediate
- Recommended excipients to add in simple buffered solutions for drying process:
  - Minimum requirement for a decent pellet formation was BSA
  - Additional sugar (e.g. sucrose) had a positive effect by slowing down the detachment of biomolecules
- Generally, freeze-dried Upcon conjugates undergo less changes during the storage period
  - Certain coatings benefit freeze-drying more (thin C5-coating) than the others

REFERENCES

[1] Lahtinen S. et al., *J. Phys. Chem. C* 2017, 121(1):656–665.
[2] Hlaváček A. et al., *Microchim. Acta* 2017, 184: 4159–4165.

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