Efficient Cadmium Removal via EDTA Chelation

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Nucleic Acid Therapeutics by LGC

Introduction

Cadmium is a highly toxic heavy metal equivalent with lead, mercury, and arsenic. Exposure to cadmium is well-known to cause several severe health complications, and therefore it is classified as a class I impurity in new drug products (as defined by ICH Q3D(R2)). During manufacturing, it is possible to introduce cadmium via raw materials, and in rare cases, the manufactured oligonucleotide may become nonviable for its intended use. The process development team at Axolabs, Petaluma designed a series of experiments utilizing the chelating agent EDTA, to remove cadmium from spiked samples mid- and post-manufacture.

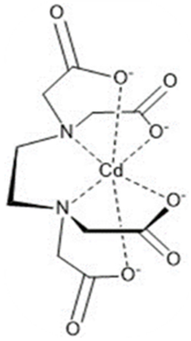

Figure 1. Theoretical EDTA coordination with cadmium. [1]

Dangers of Cadmium

Cadmium is a heavy metal found naturally in soil. Humans can be exposed to cadmium via diet, smoking or in some industries including battery manufacturing. The health impacts of cadmium exposure are numerous as cadmium accumulates in the body over one's lifetime with a half-life of up to 38 years in the kidneys. Renal disease, cancer and demineralization of the bones are just a few of the possible effects of cadmium toxicity. [2]

Initial EDTA Experiment

The chelating agent ethylenediaminetetraacetic acid (EDTA) was utilized to bind and remove cadmium from a solution of oligonucleotide.

- Oligonucleotide dissolved in phosphate buffer at a concentration of 45 OD/mL was spiked with 3 ng/g Cd.
- A large molar excess of EDTA was charged to the reaction and mixed for 1 hour at room temperature.
- The solution was desalted to remove EDTA-Cd via ultrafiltration diafiltration.
- The product was lyophilized.

Cd + Oligo → **0.1 mM EDTA, 1 hr, RT** → **Desalt (UFDF)** → **Lyophilized Product**

Results

- The concentrated sample out of UFDF was below the detection level for Cd.
- The lyophilized product demonstrated **a 98% decrease** in absolute levels of Cd.

Table 1. Sample corrected ICP-MS results for initial cadmium removal experiment.

Sample	Cd (ng/g)	Amount	Density (g/mL)	Total Cd (ng)
Pre-EDTA	2.98	930 mL	1.018	2821
Post-EDTA	29.15	2.225 g	N/A	65

Plan for Optimization

A scale-down model of UFDF was created using centrifuge tubes fit with 1 kDa PES membranes in order to maximize experimental throughput. A Design of Experiment (DoE) using JMP software was implemented to assess critical chelation parameters towards optimal cadmium removal. The three discrete factors evaluated were time, oligonucleotide concentration, and EDTA concentration, with an output of cadmium concentration.

Figure 2. PALL Macrosep Advance Centrifugal Device

Small-Scale EDTA Experiments

- Solid oligonucleotide at 228 ng/g Cd was rehydrated in phosphate buffer.
- EDTA was introduced at the listed concentrations.
- Solutions were shaken for 0.5 – 4 hours.
- Solutions were diafiltered via spin tube until permeate met conductivity spec.

Table 2. JMP generated DoE with results.

Sample	Time (hrs)	[Oligo] (OD/mL)	[EDTA] (mM)	Cd (ng/g)
6	0.5	25	0.5	0.341
13		75	0.05	0.513
8		100	0.5	0.697
12	1	25	0.1	0.166
3	2	50	0.2	0.276
4		50	0.2	0.239
9		75	0.5	0.442
1		100	0.2	0.559
5	4	25	0.05	0.153
2		25	0.5	0.259
7		75	0.2	0.532
10		100	0.05	0.536
11		100	0.5	0.483
Control 1: Buffer + EDTA (0.5mM)				ND
Control 2: Buffer + Oligo (100 OD/mL)				0.582
Starting Material				228

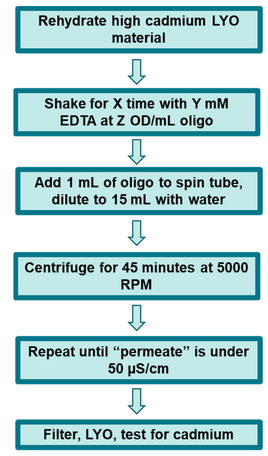

Figure 3. Experimental flowchart.

Results

- Control 2 (with no EDTA) had significantly reduced levels of cadmium.
- No relationship found between [Cd] and any of the variables.

Conclusions

- EDTA can effectively remove cadmium from oligonucleotides solutions.
- Spin tubes are not a one-to-one scale-down model of UFDF.
- Spin tubes can be used in small-scale manufactures for the removal of cadmium from solution.

References

[1] Jess, Guajardo, et al. 'A Comparative Study of the Chelating Effect Between Textured Soya Aqueous Extract and EDTA on Fe3+, Pb2+, Hg2+, Cd2+ and Ni2+ Ions'. Soybean Physiology and Biochemistry, InTech, 2 Nov. 2011. Crossref, doi:10.5772/21343.
[2] Bernard A. Cadmium & its adverse effects on human health. Indian J Med Res. 2008 Oct;128(4):557-64. PMID: 19106447.