# Encapsulation Efficiency of DiI

**Aim:** To investigate encapsulation efficiency achieved within nanoprecipitation of PLGA-PEG nps with DiI.

**Materials and Methods:**

Nanoparticles with encapsulated DiI (and nps without DiI) are used to assess the EE of the dye.

NPS with DiI: batch 22FEB18C, S/AS = 0.1; DiI (SolventPhase): 7.1 µM

NPS w/o dye: 18JAN18A, S/AS = 0.1;

**Concentration of NPS**

1. NPs concentration, using Amicon Ultra-0.5 Centrifugal Filters, 100 kDa, RC:
   1. Filter the vortexed nps with 0.45 µm sterile filter.
   2. Add 400 μL of nps suspension into the filter placed inside the microcentrifuge tube (with previously washed glycerin preservative), cap the tube.
   3. Place the tube into the centrifuge holder, make sure to align the cellulose membrane in parallel to the centrifuge circumference.
   4. Set the following centrifugation parameters: 14 kG (rcf), 10 min, 17°C
   5. Repeat the procedure for 3 times (each time remove the supernatant and add 400 µL of non-concentrated nps suspension into the filtering unit).
2. Collect the supernatant (sn) in a clean Eppendorf
3. Add 80 μL of supernatant to the filter unit and resuspend nps in the sn by pipetting it up and down ~30x. Avoid bubbles/foam.
4. NPs recovery using the same device:
5. Place the filter device upside down in the microcentrifuge tube.
6. Place the tube in the centrifuge, aligning the open cap toward the center of the rotor.
7. Set the following centrifugation parameters: 1 kG (rcf), 2 min, 17°C
8. Collect the reconstituted nps into new Eppendorf.

Repeat the same procedure for the other batch.

**Sample prep for absorbance measurement**

Obtained stock of concentrated nps:

22FEB18C and 18JAN18A is diluted x 10 in ACN.

Following dilutions (in ACN) were prepared of each sample

15x, 10x, 5x and 3x (the last one only for the batch 22FEB18C: nps with DiI).

**Calibration std prep**

To mimic the sample composition the following calibration std. was prepared:

|  |  |  |
| --- | --- | --- |
| Ingredient | Amount | Conc. |
| PLGE-PEG | 11.7 mg | 10.09 mg/mL |
| ACN | 1.16 mL |  |
| DiI | 7.7 µL (stock 1 mg/mL) | 7.1 µM |

And further dilutions were prepared in ACN

|  |  |  |
| --- | --- | --- |
| Dilution | Pol [mg/mL] | DiI [µM] |
| 20x | 0.509 | 0.356 |
| 15x | 0.678 | 0.474 |
| 10x | 1.017 | 0.711 |
| 5x | 2.035 | 1.422 |
| 3x | 3.3913 | 2.370 |

**Spectrophotometry**

Absorbance was measured in ACN (neglecting the small water content (nmt 3% v/v) present in the nps sample), using Spectrophotometer Shimadzu UVmini 1240.

Selected wavelength: 545 nm

Cuvette: High Precision Cell Quartz SUPRASIL, Hellma Analytics, art. no. 115-F-10-40; 10 mm light path + cap.

**Results:**

Concentration of the nps

Concentration factor: 1.2 mL/0.08 mL = 15x

Process yield factor: 0.9

Concentration recovery factor: 0.9

* 22FEB18C

DiI after the concentration step: 7.1 µM / 6.9 x 0.9 x 0.9 x 15 = 12.5 µM

Polymer after the concentration step: 1.46 mg/mL x 0.9 x 0.9 x 15 = 17.74 mg/mL

* 18JAN18A

Polymer after the concentration step: 0.79 mg/mL x 0.9 x 0.9 x 15 = 9.60 mg/mL

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | sample | Polymer [mg/mL] | DiI [µM] | Absorbance |
| 1 | nps 18JAN18A conc.x10, dil 15x | 0.0640 | na | 0.63757 |
| 2 | nps 18JAN18A conc.x10, dil 10x | 0.0960 | na | 0.61231 |
| 3 | nps 18JAN18A conc.x10, dil 5x | 0.1920 | na | 0.61231\* |
| 4 | cal std dil.20x | 0.509 | 0.356 | 0.05457 |
| 5 | cal std dil.15x | 0.678 | 0.474 | 0.07438 |
| 6 | cal std dil.10x | 1.017 | 0.711 | 0.10254 |
| 7 | cal std dil.5x | 2.035 | 1.422 | 0.20927 |
| 8 | DiI nps stock dil. X15 | 0.118 | ? | 0.0129 |
| 9 | DiI nps stock dil. X10 | 0.177 | ? | 0.01959 |
| 10 | DiI nps stock dil. X5 | 0.355 | ? | 0.04195 |

\* baseline

Calibration curve equation: y=0.1477x; R^2 = 0.9979

Samples:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dilution | Absorbance | [DiI] measured [µM] | [DiI] expected w losses [µM] | TDL [µM] | EE% |
| 15x | 0.0129 | 0.0873 | 0.0833 | 0.1029 | 84.88 |
| 10x | 0.01959 | 0.13207 | 0.1250 | 0.1543 | 85.49 |
| 5x | 0.04195 | 0.2840 | 0.2500 | 0.3087 | 92.01 |
| 3x | 0.07092 | 0.4802 | 0.4167 | 0.5145 | 93.33 |

**Conclusion**

Linearity of the calibration curve and of three dilutions of the sample.

Measured average EE = 88.9% which is higher than expected (more losses in the process and nps recovery was assumed)

Calculated number of dye molecules per nanoparticle: