# SUPPLEMENTARY MATERIALS

FOR

# Encapsulation of Citicoline within Solid Lipid Nanoparticles enhances its capability to counteract the 6-hydroxydopamine-induced cytotoxicity in human neuroblastoma SH-SY5Y cells

BY

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# Excel S1: RESULTS ANALYSIS OF THE TEM SIZE OF MORE THAN 100 PARTICLES BY IMAGE J SOFTWARE

| | Label | Length |
|---|---|---|
| 1 | Particle 1 | 869.300 |
| 2 | Particle 2 | 980.235 |
| 3 | Particle 3 | 757.701 |
| 4 | Particle 4 | 775.779 |
| 5 | Particle 5 | 813.907 |
| 6 | Particle 6 | 448.884 |
| 7 | Particle 7 | 155.482 |
| 8 | Particle 8 | 280.505 |
| 9 | Particle 9 | 272.350 |
| 10 | Particle 10 | 355.064 |
| 11 | Particle 11 | 541.527 |
| 12 | Particle 12 | 244.346 |
| 13 | Particle 13 | 231.271 |
| 14 | Particle 14 | 231.621 |
| 15 | Particle 15 | 389.596 |
| 16 | Particle 16 | 304.379 |
| 17 | Particle 17 | 855.767 |
| 18 | Particle 18 | 497.907 |
| 19 | Particle 19 | 295.291 |
| 20 | Particle 20 | 257.517 |

| | | |
|---|---|---|
| 21 | Particle 21 | 209.383 |
| 22 | Particle 22 | 309.063 |
| 23 | Particle 23 | 407.352 |
| 24 | Particle 24 | 185.432 |
| 25 | Particle 25 | 227.424 |
| 26 | Particle 26 | 294.065 |
| 27 | Particle 27 | 313.737 |
| 28 | Particle 28 | 347.375 |
| 29 | Particle 29 | 506.896 |
| 30 | Particle 30 | 363.849 |
| 31 | Particle 31 | 224.129 |
| 32 | Particle 32 | 214.291 |
| 33 | Particle 33 | 422.589 |
| 34 | Particle 34 | 408.508 |
| 35 | Particle 35 | 120.728 |
| 36 | Particle 36 | 141.858 |
| 37 | Particle 37 | 102.488 |
| 38 | Particle 38 | 94.409 |
| 39 | Particle 39 | 321.994 |
| 40 | Particle 40 | 91.454 |
| 41 | Particle 41 | 87.756 |
| 42 | Particle 42 | 187.963 |
| 43 | Particle 43 | 278.597 |
| 44 | Particle 44 | 102.549 |
| 45 | Particle 45 | 135.019 |
| 46 | Particle 46 | 81.024 |
| 47 | Particle 47 | 82.299 |
| 48 | Particle 48 | 92.005 |
| 49 | Particle 49 | 108.501 |
| 50 | Particle 50 | 224.773 |
| 51 | Particle 51 | 214.817 |

| | | |
|---|---|---|
| 52 | Particle 52 | 324.301 |
| 53 | Particle 53 | 114.989 |
| 54 | Particle 54 | 100.837 |
| 55 | Particle 55 | 236.777 |
| 56 | Particle 56 | 144.767 |
| 57 | Particle 57 | 152.767 |
| 58 | Particle 58 | 195.312 |
| 59 | Particle 59 | 395.284 |
| 60 | Particle 60 | 296.506 |
| 61 | Particle 61 | 254.019 |
| 62 | Particle 62 | 208.254 |
| 63 | Particle 63 | 289.123 |
| 64 | Particle 64 | 99.848 |
| 65 | Particle 65 | 93.205 |
| 66 | Particle 66 | 238.224 |
| 67 | Particle 67 | 184.126 |
| 68 | Particle 68 | 148.064 |
| 69 | Particle 69 | 68.993 |
| 70 | Particle 70 | 132.362 |
| 71 | Particle 71 | 169.155 |
| 72 | Particle 72 | 377.489 |
| 73 | Particle 73 | 205.859 |
| 74 | Particle 74 | 102.052 |
| 75 | Particle 75 | 630.662 |
| 76 | Particle 76 | 476.518 |
| 77 | Particle 77 | 105.302 |
| 78 | Particle 78 | 241.550 |
| 79 | Particle 79 | 84.735 |
| 80 | Particle 80 | 91.580 |
| 81 | Particle 81 | 110.275 |
| 82 | Particle 82 | 108.568 |

| | | |
|---|---|---|
| 83 | Particle 83 | 103.660 |
| 84 | Particle 84 | 130.620 |
| 85 | Particle 85 | 127.295 |
| 86 | Particle 86 | 142.896 |
| 87 | Particle 87 | 241.106 |
| 88 | Particle 88 | 295.541 |
| 89 | Particle 89 | 254.245 |
| 90 | Particle 90 | 308.342 |
| 91 | Particle 91 | 332.531 |
| 92 | Particle 92 | 250.473 |
| 93 | Particle 93 | 341.119 |
| 94 | Particle 94 | 406.560 |
| 95 | Particle 95 | 245.190 |
| 96 | Particle 96 | 307.897 |
| 97 | Particle 97 | 246.850 |
| 98 | Particle 98 | 232.027 |
| 99 | Particle 99 | 314.227 |
| 100 | Particle 100 | 228.042 |
| 101 | Particle 101 | 120.767 |
| 102 | Particle 102 | 117.932 |
| 103 | Particle 103 | 128.377 |
| 104 | Particle 104 | 121.562 |
| 105 | Particle 105 | 87.087 |
| 106 | Particle 106 | 85.770 |
| 107 | Particle 107 | 104.238 |

| | | |
|---|---|---|
| | Particle | |
| 108 | 108 | 87.120 |
| | Particle | |
| 109 | 109 | 84.122 |
| | Particle | |
| 110 | 110 | 377.066 |
| | Particle | |
| 111 | 111 | 189.108 |
| | Particle | |
| 112 | 112 | 780.238 |
| | Particle | |
| 113 | 113 | 427.928 |
| | Particle | |
| 114 | 114 | 818.428 |
| | Particle | |
| 115 | 115 | 807.828 |
| | Particle | |
| 116 | 116 | 511.949 |
| | Particle | |
| 117 | 117 | 366.276 |
| | Particle | |
| 118 | 118 | 244.255 |
| | Particle | |
| 119 | 119 | 134.592 |
| | Particle | |
| 120 | 120 | 272.707 |
| | Mean | 276.819 |
| | SD | 198.225 |
| | Min | 68.993 |
| | Max | 980.235 |

Excel S2: RESULTS OBTAINED FROM THE STATISTICAL APPROACH DESCRIBED IN SECTION 2.8

| | CTRL NT | 6OHDA 0.04 mM | 0.05 mM Cit | 0.05 mM SLN-Cit | 0.05 mM Plain-SLNs | 0.06 mM Cit | 0.06 mM SLN-Cit |
|---|---|---|---|---|---|---|---|
| Exp 1 replicates | 0,750 | 0,618 | 0,640 | 0,721 | 0,622 | 0,670 | 0,677 |
| Exp 1 replicates | 0,718 | 0,652 | 0,615 | 0,760 | 0,618 | 0,622 | 0,701 |
| Exp 1 replicates | 0,701 | 0,719 | 0,691 | 0,724 | 0,711 | 0,652 | 0,692 |
| Exp 2 replicates | 0,805 | 0,700 | 0,700 | 0,770 | 0,738 | 0,618 | 0,749 |
| Exp 2 replicates | 0,848 | 0,626 | 0,701 | 0,806 | 0,699 | 0,761 | 0,750 |
| Exp 2 replicates | 0,805 | 0,59 | 0,652 | 0,705 | 0,647 | 0,7 | 0,732 |
| Exp 3 replicates | 0,482 | 0,406 | 0,425 | 0,523 | 0,415 | 0,393 | 0,486 |
| Exp 3 replicates | 0,546 | 0,416 | 0,415 | 0,556 | 0,506 | 0,453 | 0,508 |
| Exp 3 replicates | 0,531 | 0,36 | 0,387 | 0,5 | 0,428 | 0,367 | 0,474 |
| Exp 4 replicates | 0,584 | 0,554 | 0,578 | 0,575 | 0,582 | 0,514 | 0,588 |
| Exp 4 replicates | 0,59 | 0,547 | 0,566 | 0,6 | 0,57 | 0,605 | 0,572 |
| Exp 4 replicates | 0,565 | 0,57 | 0,623 | 0,605 | 0,562 | 0,573 | 0,538 |
| Exp 5 replicates | 0,378 | 0,31 | 0,298 | 0,319 | 0,319 | 0,315 | 0,322 |
| Exp 5 replicates | 0,396 | 0,357 | 0,34 | 0,376 | 0,332 | 0,33 | 0,376 |
| Exp 5 replicates | 0,379 | 0,325 | 0,326 | 0,353 | 0,334 | 0,306 | 0,325 |

| | CTRL NT | 6OHDA 0.04 mM | 0.05 mM Cit | 0.05 mM SLN-Cit | 0.05 mM Plain-SLNs | 0.06 mM Cit | 0.06 mM SLN-Cit |
|---|---|---|---|---|---|---|---|
| Meane Exp 1 | 0,723 | 0,663 | 0,649 | 0,735 | 0,650 | 0,648 | 0,690 |
| Meane Exp 2 | 0,819 | 0,639 | 0,684 | 0,760 | 0,695 | 0,693 | 0,744 |
| Meane Exp 3 | 0,520 | 0,394 | 0,409 | 0,526 | 0,450 | 0,404 | 0,489 |
| Meane Exp 4 | 0,580 | 0,557 | 0,589 | 0,593 | 0,571 | 0,564 | 0,566 |
| Meane Exp 5 | 0,384 | 0,331 | 0,321 | 0,349 | 0,328 | 0,317 | 0,341 |

| | CTRL NT | 6OHDA 0.04 mM | 0.05 mM Cit | 0.05 mM SLN-Cit | 0.05 mM Plain-SLNs | 0.06 mM Cit | 0.06 mM SLN-Cit |
|---|---|---|---|---|---|---|---|
| Number of values | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Minimum | 0,3843 | 0,3307 | 0,3213 | 0,3493 | 0,3283 | 0,317 | 0,341 |
| 25% Percentile | 0,452 | 0,3623 | 0,3652 | 0,4378 | 0,389 | 0,3607 | 0,4152 |
| Median | 0,5797 | 0,557 | 0,589 | 0,5933 | 0,5713 | 0,564 | 0,566 |
| 75% Percentile | 0,7712 | 0,6508 | 0,6665 | 0,7477 | 0,6725 | 0,6705 | 0,7168 |
| Maximum | 0,8193 | 0,663 | 0,6843 | 0,7603 | 0,6947 | 0,693 | 0,7437 |
| | | | | | | | |
| Mean | 0,6052 | 0,5167 | 0,5305 | 0,5929 | 0,5389 | 0,5253 | 0,566 |
| Std. Deviation | 0,1706 | 0,148 | 0,1578 | 0,1673 | 0,15 | 0,1602 | 0,1608 |
| Std. Error | 0,07632 | 0,06617 | 0,07056 | 0,07483 | 0,06709 | 0,07166 | 0,07192 |
| | | | | | | | |
| Lower 95% CI | 0,3933 | 0,333 | 0,3346 | 0,3851 | 0,3526 | 0,3263 | 0,3663 |
| Upper 95% CI | 0,8171 | 0,7004 | 0,7264 | 0,8006 | 0,7252 | 0,7242 | 0,7657 |

| | |
|---|---|
| Table Analyzed | Data 6 |
| | |
| Repeated Measures ANOVA | |
| P value | < 0,0001 |
| P value summary | *** |
| Are means signif. different? (P < 0.05) | Yes |
| Number of groups | 7 |
| F | 8,996 |
| R square | 0,6922 |
| | |
| Was the pairing significantly effective? | |
| R square | 0,9297 |
| F | 258 |
| P value | < 0,0001 |
| P value summary | *** |
| Is there significant matching? (P < 0.05) | Yes |

| ANOVA Table | SS | df | MS |
|---|---|---|---|
| Treatment (between columns) | 0,03639 | 6 | 0,006064 |
| Individual (between rows) | 0,6956 | 4 | 0,1739 |
| Residual (random) | 0,01618 | 24 | 0,0006741 |
| Total | 0,7482 | 34 | |

| Bonferroni's Multiple Comparison Test | Mean Diff, | t | Significant? P < 0,05? | Summary | 95% CI of diff |
|---|---|---|---|---|---|
| CTRL NT vs 6OHDA 0.04 mM | 0,08853 | 5,392 | Yes | *** | 0,03277 to 0,1443 |
| CTRL NT vs 0.05 mM Cit | 0,07473 | 4,551 | Yes | ** | 0,01897 to 0,1305 |
| CTRL NT vs 0.05 mM SLN-Cit | 0,01233 | 0,7511 | No | ns | -0,04343 to 0,06810 |
| CTRL NT vs 0.05 mM Plain-SLNs | 0,06633 | 4,04 | Yes | * | 0,01057 to 0,1221 |
| CTRL NT vs 0.06 mM Cit | 0,07993 | 4,868 | Yes | ** | 0,02417 to 0,1357 |
| CTRL NT vs 0.06 mM SLN-Cit | 0,0392 | 2,387 | No | ns | -0,01656 to 0,09496 |
| 6OHDA 0.04 mM vs 0.05 mM Cit | -0,0138 | 0,8404 | No | ns | -0,06956 to 0,04196 |
| 6OHDA 0.04 mM vs 0.05 mM SLN-Cit | -0,0762 | 4,641 | Yes | ** | -0,1320 to -0,02044 |
| 6OHDA 0.04 mM vs 0.05 mM Plain-SLNs | -0,0222 | 1,352 | No | ns | -0,07796 to 0,03356 |
| 6OHDA 0.04 mM vs 0.06 mM Cit | -0,0086 | 0,5237 | No | ns | -0,06436 to 0,04716 |
| 6OHDA 0.04 mM vs 0.06 mM SLN-Cit | -0,04933 | 3,004 | No | ns | -0,1051 to 0,006430 |
| 0.05 mM Cit vs 0.05 mM SLN-Cit | -0,0624 | 3,8 | Yes | * | -0,1182 to -0,006636 |
| 0.05 mM Cit vs 0.05 mM Plain-SLNs | -0,0084 | 0,5116 | No | ns | -0,06416 to 0,04736 |
| 0.05 mM Cit vs 0.06 mM Cit | 0,0052 | 0,3167 | No | ns | -0,05056 to 0,06096 |
| 0.05 mM Cit vs 0.06 mM SLN-Cit | -0,03553 | 2,164 | No | ns | -0,09130 to 0,02023 |
| 0.05 mM SLN-Cit vs 0.05 mM Plain-SLNs | 0,054 | 3,289 | No | ns | -0,001764 to 0,1098 |
| 0.05 mM SLN-Cit vs 0.06 mM Cit | 0,0676 | 4,117 | Yes | ** | 0,01184 to 0,1234 |
| 0.05 mM SLN-Cit vs 0.06 mM SLN-Cit | 0,02687 | 1,636 | No | ns | -0,02890 to 0,08263 |
| 0.05 mM Plain-SLNs vs 0.06 mM Cit | 0,0136 | 0,8282 | No | ns | -0,04216 to 0,06936 |
| 0.05 mM Plain-SLNs vs 0.06 mM SLN-Cit | -0,02713 | 1,652 | No | ns | -0,08290 to 0,02863 |
| 0.06 mM Cit vs 0.06 mM SLN-Cit | -0,04073 | 2,481 | No | ns | -0,09650 to 0,01503 |

FURTHER TEM IMAGES THAT CONTAIN MANY SMALL PARTICLES AND DENOTE THE BROAD DISTRIBUTION OF THE SLNs

Figure S1. Additional TEM images of CIT-SLNs