Supplementary Information

Influence of Lyophilization and Cryoprotection on the Stability and Morphology of Drug-Loaded Poly(Ethylene Glycol-*b*-ε-caprolactone) Micelles

**Table S1.** Concentration and volume of stock solutions combined to prepare copolymer solutions in ultrapure water for the determination of the CAC of PEG-*b*-PCL block copolymer micelles.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Conc. of copolymer in PBS (mg/L)a | Volume of pyrene stock solution (µL)b | Conc. of copolymer stock solution (mg/L)c | Volume of copolymer stock solution (µL) | Volume of ultrapure water added (µL) |
| 500 | 20 | 2000 | 500 | 2000 |
|
|
| 100 | 20 | 2000 | 100 | 2000 |
|
|
| 50.0 | 20 | 1000 | 100 | 2000 |
|
|
| 10.0 | 20 | 1000 | 20 | 2000 |
|
|
| 5.00 | 20 | 1000 | 10 | 2000 |
|
|
| 2.50 | 20 | 100 | 50 | 2000 |
|
|
| 1.00 | 20 | 100 | 20 | 2000 |
|
|
| 0.75 | 20 | 100 | 15 | 2000 |
|
|
| 0.50 | 20 | 100 | 10 | 2000 |
|
|
| 0.25 | 20 | 10 | 50 | 2000 |
|
|

a Copolymer solutions were prepared in high purity water *via* the solvent evaporation approach with a constant pyrene concentration of 6 × 10-7 M. b A 6 × 10-5 M pyrene stock solution in acetone was used to prepare all solutions. c All stock solutions were prepared in acetone.

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**Figure S1.** Pyrene (6 x 10-7 M) fluorescent emission spectra (λex = 334 nm) for (a) PEG2PCL0.4,(b) PEG2PCL1.1,(c) PEG2PCL1.8 and(d) PEG2PCL4.0 copolymers at various copolymer concentrations.

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**Figure S2.** Pyrene (6 x 10-7 M) fluorescent emission spectra (λex = 334 nm) for (a) PEG5PCL0.6,(b) PEG5PCL1.3,(c) PEG5PCL2.4,(d) PEG5PCL4.2 and(e) PEG5PCL9.5 copolymers at various copolymer concentrations.

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**Figure S3.** Pyrene (6 x 10-7 M) fluorescent emission spectra (λex = 334 nm) for (a) PEG10PCL3.2,(b) PEG10PCL7.9,(c) PEG10PCL10.7 and(d) PEG10PCL14.9 copolymers at various copolymer concentrations.

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**Figure S4.** Illustration of preparation, lyophilization and characterisation of PEGX-*b*-PCLY micelles.

**Table S2.** Polydispersity index (PDI) of micelles before and after lyophilization/reconstitution in the absence and presence of β-CD (-/+CD) as cryoprotectant, as determined by dynamic light scattering DLS; values are expressed as mean ± standard deviation (n=3).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Copolymer | Blank | | Gossypol (GP) | Phloretin (PH) | Before  freeze-drying  (-CD)  (PDI ± SD) | Before  freeze-drying  (+CD)  (PDI ± SD) | After  freeze-drying  (-CD)  (PDI ± SD) | After  freeze-drying  (+CD)  (PDI ± SD) |
| PEG2PCL0.4 | √ | | - | - | 0.149 ± 0.010 | 0.156 ± 0.009 | 0.196 ± 0.010 | 0.185 ± 0.013 |
| PEG2PCL1.1 | √ | | - | - | 0.189 ± 0.011 | 0.245 ± 0.014 | 0.145 ± 0.048 | 0.149 ± 0.008 |
| PEG2PCL1.1 | - | | √ | - | 0.140 ± 0.004 | 0.179 ± 0.052 | 0.162 ± 0.025 | 0.145 ± 0.005 |
| PEG2PCL1.1 | | - | - | √ | 0.135 ± 0.023 | 0.158 ± 0.003 | 0.157 ± 0.009 | 0.126 ± 0.002 |
| PEG2PCL1.8 | | √ | - | - | 0.225 ± 0.006 | 0.252 ± 0.007 | 0.141 ± 0.001 | 0.228 ± 0.002 |
| PEG2PCL1.8 | | - | √ | - | 0.160 ± 0.025 | 0.187 ± 0.034 | 0.136 ± 0.008 | 0.132 ± 0.005 |
| PEG2PCL1.8 | | - | - | √ | 0.147 ± 0.011 | 0.178 ± 0.030 | 0.158 ± 0.008 | 0.184 ± 0.005 |
| PEG2PCL4.0 | | √ | - | - | 0.245 ± 0.030 | 0.195 ± 0.003 | 0.210 ± 0.011 | 0.186 ± 0.039 |
| PEG5PCL0.6 | | √ | - | - | 0.262 ± 0.007 | 0.242 ± 0.003 | 0.210 ± 0.016 | 0.177 ± 0.050 |
| PEG5PCL1.3 | | √ | - | - | 0.241 ± 0.008 | 0.245 ± 0.038 | 0.185 ± 0.016 | 0.188 ± 0.006 |
| PEG5PCL1.3 | | - | √ | - | 0.126 ± 0.010 | 0.185 ± 0.016 | 0.148 ± 0.006 | 0.147 ± 0.007 |
| PEG5PCL1.3 | | - | - | √ | 0.205 ± 0.003 | 0.257 ± 0.050 | 0.205 ± 0.003 | 0.246 ± 0.056 |
| PEG5PCL2.4 | | √ | - | - | 0.192 ± 0.008 | 0.145 ± 0.030 | 0.176 ± 0.035 | 0.163 ± 0.011 |
| PEG5PCL2.4 | | - | √ | - | 0.220 ± 0.060 | 0.201 ± 0.004 | 0.236 ± 00.17 | 0.158 ± 0.008 |
| PEG5PCL2.4 | | - | - | √ | 0.197 ± 0.002 | 0.139 ± 0.005 | 0.131 ± 0.002 | 0.159 ± 0.014 |
| PEG5PCL4.2 | | √ | - | - | 0.123 ± 0.006 | 0.132 ± 0.046 | 0.176 ± 0.006 | 0.138 ± 0.005 |
| PEG5PCL4.2 | | - | √ | - | 0.192 ± 0.004 | 0.175 ± 0.003 | 0.156 ± 0.019 | 0.158 ± 0.016 |
| PEG5PCL4.2 | | - | - | √ | 0.168 ± 0.028 | 0.085 ± 0.009 | 0.140 ± 0.040 | 0.137 ± 0.034 |
| PEG5PCL9.5 | | √ | - | - | 0.138 ± 0.004 | 0.145 ± 0.011 | 0.128 ± 0.011 | 0.116 ± 0.021 |
| PEG5PCL9.5 | | - | √ | - | 0.193 ± 0.002 | 0.197 ± 0.005 | 0.153 ± 0.011 | 0.156 ± 0.016 |
| PEG5PCL9.5 | | - | - | √ | 0.144 ± 0.013 | 0.127 ± 0.011 | 0.118 ± 0.019 | 0.168 ± 0.030 |
| PEG10PCL3.2 | | √ | - | - | 0.173 ± 0.004 | 0.186 ± 0.006 | 0.133 ± 0.002 | 0.186 ± 0.015 |
| PEG10PCL7.9 | | √ | - | - | 0.170 ± 0.009 | 0.176 ± 0.003 | 0.195 ± 0.020 | 0.172 ± 0.009 |
| PEG10PCL7.9 | | - | √ | - | 0.183 ± 0.013 | 0.172 ± 0.007 | 0.243 ± 0.011 | 0.232 ± 0.009 |
| PEG10PCL7.9 | | - | - | √ | 0.149 ± 0.014 | 0.164 ± 0.006 | 0.182 ± 0.006 | 0.216 ± 0.024 |
| PEG10PCL10.7 | | √ | - | - | 0.146 ± 0.017 | 0.149 ± 0.007 | 0.119 ±0.006 | 0.125 ± 0.002 |
| PEG10PCL10.7 | | - | √ | - | 0.138 ± 0.006 | 0.153 ± 0.008 | 0.186 ± 0.006 | 0.187 ± 0.013 |
| PEG10PCL10.7 | | - | - | √ | 0.116 ± 0.007 | 0.225 ± 0.042 | 0.230 ± 0.005 | 0.191 ± 0.017 |
| PEG10PCL14.9 | | √ | - | - | 0.160 ± 0.008 | 0.160 ± 0.011 | 0.128 ±0.013 | 0.187 ± 0.025 |

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**Figure S5.** Number, volume and intensity particle size distributions for (a) PEG2PCL0.4, (b) PEG2PCL1.1, (c) PEG2PCL1.8 and (d) PEG2PCL4.0 micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

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**Figure S6.** Number, volume and intensity particle size distributions for (a) PEG5PCL0.6,(b) PEG5PCL1.3,(c) PEG5PCL2.4,(d) PEG5PCL4.2 and(e) PEG5PCL9.5 micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

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**Figure S7.** Number, volume and intensity particle size distributions for (a) PEG10PCL3.2,(b) PEG10PCL7.9,(c) PEG10PCL10.7 and(d) PEG10PCL14.9 micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

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**Figure S8.** SAXS profiles for (a) PEG2PCL0.4,(b) PEG2PCL1.1,(c) PEG2PCL1.8 and(d) PEG2PCL4.0 micelles/aggregates as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

Graphical user interface, application

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**Figure S9.** SAXS profiles for (a) PEG5PCL0.6,(b) PEG5PCL1.3,(c) PEG5PCL2.4,(d) PEG5PCL4.2 and(e) PEG5PCL9.5 micelles/aggregates as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD). Background multiplication factor (BMF) for PEG5PCL1.3 LR+CD, PEG5PCL2.4 LR+CD, PEG5PCL4.2 LR+CD and PEG5PCL9.5 LR+CD was 0.72, 0.55, 0.55 and 0.53, respectively.

Graphical user interface, application

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**Figure S10.** SAXS profiles for (a) PEG10PCL3.2,(b) PEG10PCL7.9,(c) PEG10PCL10.7 and(d) PEG10PCL14.9 micelles/aggregates as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD) (BMF for PEG10PCL3.2 LR+CD, PEG10PCL7.9 LR+CD and PEG10PCL10.7 LR+CD was 0.45, 0.55 and 0.53, respectively).

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**Figure S11.** Number, volume and intensity particle size distributions for PEG5PCL1.3 (a) blank micelles and (b) gossypol (GP) and (c) phloretin (PH) loaded micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

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**Figure S12.** Number, volume and intensity particle size distributions for PEG5PCL2.4 (a) blank micelles and (b) gossypol (GP) and (c) phloretin (PH) loaded micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

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**Figure S13.** Number, volume and intensity particle size distributions for PEG5PCL4.2 (a) blank micelles and (b) gossypol (GP) and (c) phloretin (PH) loaded micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

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**Figure S14.** Number, volume and intensity particle size distributions for PEG5PCL9.5 (a) blank micelles and (b) gossypol (GP) and (c) phloretin (PH) loaded micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

Graphical user interface, chart

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**Figure S15.** SAXS profiles for (a) gossypol and (b) phloretin loaded PEG5PCL1.3 micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

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**Figure S16.** SAXS profiles for (a) gossypol and (b) phloretin loaded PEG5PCL2.4 micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

Graphical user interface, application

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**Figure S17.** SAXS profiles for (a) gossypol and (b) phloretin loaded PEG5PCL4.2 micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD) (BMF for PEG5PCL4.2 GP LR+CD and PEG5PCL4.2 PH LR+CD was 0.65 and 0.91, respectively).

Graphical user interface

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**Figure S18.** SAXS profiles for (a) gossypol and (b) phloretin loaded PEG5PCL9.5 micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD) (BMF for PEG5PCL9.5 PH LR+CD was 0.91).

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**Figure S19.** Number, volume and intensity particle size distributions for PEG2PCL1.8 (a) blank micelles and (b) gossypol (GP) and (c) phloretin (PH) loaded micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

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**Figure S20.** Number, volume and intensity particle size distributions for PEG10PCL10.7 (a) blank micelles and (b) gossypol (GP) and (c) phloretin (PH) loaded micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

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**Figure S21.** Hydrodynamic diameter (*Dh*) versus weight fraction of PCL (*fPCL*) as determined from number particle size distributions (PSDs) for (a) gossypol (GP) and (b) phloretin (PH) loaded PEG2PCL1.8 micelles as prepared (AP) and after lyophilization/reconstitution (LR) and in the absence and presence of β-CD (-/+CD). All values are reported as the mean + std. dev. (n = 3).

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**Figure S22.** *Dh* versus *fPCL* as determined from number PSDs for (a) gossypol (GP) and (b) phloretin (PH) loaded PEG10PCL10.7 micelles as prepared (AP) and after lyophilization/reconstitution (LR) and in the absence and presence of β-CD (-/+CD). All values are reported as the mean + std. dev. (n = 3).

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**Figure S23.** SAXS profiles for (a) gossypol and (b) phloretin loaded PEG2PCL1.8 micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).

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**Figure S24.** SAXS profiles for (a) gossypol and (b) phloretin loaded PEG10PCL10.7 micelles as prepared (AP) and after lyophilization/reconstitution (LR) in the absence and presence of β-CD (-/+CD).