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# Polycaprolactone-*block*-poly(ethylene oxide) Micelles: A Nanodelivery System for 17$\beta$-Estradiol

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**Abstract:** Various hormone replacement regimens and delivery systems have been developed; however, there is still a need for additional, easily controllable and biocompatible systems. We have developed and characterized biocompatible polycaprolactone-*block*-poly(ethylene oxide) (PCL-*b*-PEO) micelles for the delivery of 17$\beta$-estradiol (E2) and investigated their loading and release properties using fluorescence spectroscopy. The micelles are spherical aggregates that range in size from 20 to 40 nm, as determined by both transmission electron microscopy and dynamic light scattering. A high loading efficiency for E2 of up to 96%, as well as a high drug loading capacity of up to 4000 molecules of E2 per micelle (equivalent to 190% (w/w)), is obtainable. In addition, the E2 loading and release can be controlled by modifying the block length of the polycaprolactone core and the initial estradiol concentration. The release of E2 from the micelles showed a biphasic profile under perfect sink conditions: there is an initial burst release, followed by a slow and prolonged release for up to 5 days, until complete release is achieved. The release of E2 from the micelles was shown to be diffusional, as shown by the linearity of the release as a function of the square root of time. Approximate diffusion coefficients of the order of $10^{-17}$ cm$^2$/s were obtained. In vitro and in vivo experiments confirmed that the biological activity of E2 was retained after preparation of the micelles. This micelle carrier could serve as a versatile and efficient nanodelivery system for steroids and other poorly water soluble drugs that require solubilizing agents for delivery.

**Keywords:** Drug delivery; estradiol; micelle; block copolymer; loading; release

## Introduction

Estrogen replacement therapy was originally proposed as a solution for estrogen deficiency;[1−5] however, hormone replacement therapies have also been the subject of controversy.[4] A variety of drug delivery systems for estradiol and contraceptives have been developed, among them transdermal, polymeric, and block copolymeric systems. We will outline each of them briefly. Transdermal delivery systems including Estraderm,[6] FemPatch,[7] and other FDA approved systems are reviewed by Ramachandran et al.[8] Liposomes containing phosphatidylcholine and surfactants, which serve

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to disturb the lipid bilayer, have also been used as transdermal delivery vehicles for E2.[9] Both proniosomes (liposomes that are formed from nonionic surfactants) and niosomes (proniosomes that have been hydrated in water) have also been used to increase the permeation of estradiol across the skin.[10,11] Among the advantages of the transdermal delivery system are avoidance of the hepatic first pass elimination, improved patient compliance, and reduction of some side effects.[6] However, variation in a patient's skin permeability results in insufficient or excess mean serum concentrations in the blood. In addition, adverse effects such as depression, breast tenderness, headaches, and nausea are associated with both oral and transdermal E2 formulations.[8]

Biocompatible polymers have also been used to deliver 17$\beta$-estradiol. Poly(lactide-*co*-glycolide) (PLGA) microspheres have been used to load and release E2.[12,13] A range of concentrations (0.15−15% (w/w)) can be loaded into the microspheres, and controlled release is achievable. However, the large sizes of microspheres are not ideal for avoiding the body's defense mechanisms, i.e., the reticuloendothelial system (RES), and there is a large burst release lasting up to 24 h due to the presence of E2 on the surface of the particles.[13,14] Block copolymers made from polycaprolactone and polylactide have been used to create microspheres.[15] Also, disk and cylinder-type laminate systems have been employed for the controlled release of E2.[16]

Block copolymers have also been used in the preparation of micellar drug delivery systems.[17−24] Amphiphilic self-assembled systems are attractive drug delivery vehicles, mostly due to their size, stability, versatility, and biocompatibility. Very few micellar systems exist for estradiol aside from Pluronic-*b*-poly(acrylic acid)[25] and various Carbopol (poly(acrylic acid))/surfactant systems.[26] However, a new biocompatible micelle system for the delivery of estradiol might prove advantageous. In our group, polycaprolactone-*block*-poly(ethylene oxide) (PCL-*b*-PEO) copolymer micelles have been explored as a drug delivery system.[27−29] PCL, the hydrophobic or core block, is a well-known biodegradable and biocompatible polymer that has been used in various biomedical applications because of its excellent biocompatibility and degradability. Poly(ethylene oxide) (PEO) serves as the hydrophilic block in the corona of the micelle. It is one of few water soluble polymers that have been widely used to improve the biocompatibilities of blood-contacting biomaterials, because it helps prevent uptake by the RES by prolonging the circulation time of the carrier in the blood.[30] It has been shown that PCL-*b*-PEO micelles are an effective carrier for hydrophobic probes,[31] and lipophilic drugs such as FK506,[32] L-685,818,[33] and dihydrotestosterone (DHT).[34]


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To investigate and assess polycaprolactone-*block*-poly(ethylene oxide) micelles as a delivery system for E2, we examined its loading and release parameters. E2 is fluorescent, and its presence can be quantified using fluorescence spectroscopy. The influence of the initial concentration of E2 and of the polycaprolactone block length on the loading and release parameters are also examined. The release of E2 from the micelles is studied using a perfect sink apparatus in order to obtain information about the profile of drug release, and diffusion coefficients are calculated. In vitro and in vivo experiments show that E2 incorporated into block copolymer micelles did not lose its biological effectivness. The results from these studies provide evidence for the possibility of controlling these relevant properties of the micelle self-assembly drug delivery system, and for its versatility in incorporating other sex hormones.

## Experimental Section

**Materials.** The block copolymer that was used in this study was polycaprolactone-*block*-poly(ethylene oxide). A description of the complete synthesis of $PCL_{23}$-*b*-$PEO_{45}$ block copolymers can be found in a previous publication by Luo et al. in connection with another project.[33] A series of block copolymers with the same number of units of ethylene oxide but different polycaprolactone units ($PCL_{12}$-*b*-$PEO_{44}$ to $PCL_{151}$-*b*-$PEO_{44}$) were synthesized by anionic polymerization by Yu et al. in connection with another project.[35] The subscripts after PCL or PEO refer to the number of repeat units in each block. 17β-Estradiol was purchased from Sigma Aldrich (Oakville, ON, Canada) and used as received. E2 has a molecular weight of 272.4 g/mol and a melting point of 173−179 °C (refer to Table 1 in the Supporting Information for the structure of E2 and its physical properties). Mini dialysis chambers (Slide-A-Lyzer mini dialysis unit) used for the release experiments were purchased from MJS BioLynx Inc. (Brockville, ON, Canada) and had a molecular weight cutoff (MWCO) of 3500 g/mol.

**Preparation of PCL-*b*-PEO Micelles with E2 for Loading and Release Studies.** An aliquot of E2 solution in acetone was placed into an empty vial in quantities such that, in the final solution, the concentration of the drug ranged from 10 to 72 mM. The acetone was allowed to evaporate. The block copolymer (5−10 mg) was then added to the vial, and 15 mg of dimethylformamide (DMF) was added to dissolve both the drug and the copolymer. The solution was stirred for 4 h. To induce micellization, MilliQ water was added slowly at a rate of approximately 2.5%/min until 35 mg of MilliQ water had been added. The total mass of this solution was ca. 500 mg, which yielded a 1−2% (w/w) polymer solution. The micelle solution was stirred overnight and dialyzed against MilliQ water in the dark to remove the DMF solvent and any excess E2 molecules. For the first 4 h, the water was changed twice every 2 h, then once every hour for the next 4 h, and then it was left overnight. After dialysis, the micelle solution was diluted to 0.4−1.0% (w/w) of polymer.

**Fluorescence Measurements for Loading and Release Experiments.** The loading efficiency of 17β-estradiol into the micelles was determined by fluorescence spectroscopy. A calibration curve of E2 in dimethyl sulfoxide (DMSO) was created to determine the linear range of fluorescence as a function of concentration of the drug. A small aliquot of micelles containing drug (i.e., 10 μL) was dissolved in 490 μL of DMSO and placed into a quartz microcuvette. This was performed for determinations of the concentration of E2 in both loading and release experiments. The micelles are completely dissolved in DMSO, releasing all of the E2, and the solution is then analyzed by fluorescence. The copolymer and the solvent do not contribute significantly to the fluorescence, and the background value obtained was subtracted. The fluorescence was measured using a SPEX FluoroMax 2 in the right-angle geometry (90° emission collection). The emission fluorescence spectra were obtained at an excitation wavelength ($\lambda_{ex}$ = 281 nm). The loading efficiency and the drug content were calculated using the experimental values from fluorescence spectroscopy and from the following equations:[36]

$$\text{loading efficiency (\%)} = \frac{\text{mass of drug in micelles (g)}}{\text{total mass of drug used (g)}} \times 100 \quad (1)$$

$$\text{drug content (\% w/w)} = \frac{\text{mass of drug in micelles (g)}}{\text{mass of micelles (g)}} \times 100 \quad (2)$$

**Release of E2 from PCL-*b*-PEO Micelles.** The solution of the micelles with the incorporated E2 used for the release experiment had a polymer concentration of 0.4−1.0% (w/w) and an initial concentration of 3−35 mM. Samples of micelles (10−20 μL) containing the E2 were placed into dialysis chambers (MWCO: 3500 g/mol). MilliQ water (380−390 μL) was then added to each dialysis chamber. The experiment was performed under perfect sink conditions; after being released from the micelles, all of the drug molecules were immediately washed away into the exterior solution, hence there was no drug that accumulates in the reservoir and steady state conditions were not reached. Briefly, a number of dialysis chambers (up to 20) were placed into a dialysis float device, which was placed into a large beaker filled with tap water. This beaker was placed into a crystallization dish (160 × 100) equipped with a side neck. Tap water was allowed to run into the beaker so that when the water overflowed from the beaker, the excess water would go into the crystallization dish and out the side neck

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attached with a tube. This allowed for constant stirring and also for perfect sink conditions to be observed. At specific time intervals, a dialysis chamber was removed and an aliquot was sampled, dissolved in DMSO, and analyzed by fluorescence.

**Transmission Electron Microscopy (TEM).** The micelles in the solutions were examined using a JEOL JEM-2000FX electron microscope operating at an accelerating voltage of 80 keV. Dilute solutions of the micelles containing the E2 in water (0.05% (w/w)) were deposited on 400 mesh copper grids (EMS Sciences, USA) that were precoated with a thin film of Formvar (poly(vinylformal)) and carbon. The samples were allowed to remain on the grids for a few seconds, and then a blotter was applied to remove the excess solution. The grids were then left overnight to air-dry. Digital images were taken with a Gatan 792 Bioscan 1k × 1k wide angle multiscan CCD camera (JEM-2000 FX).

**Dynamic Light Scattering (DLS).** The sizes and size distributions of the micelles containing the E2 were determined on a Brookhaven Instruments photon correlation spectrophotometer with a BI-9000AT digital correlator. The instrument was equipped with a compass 315M-150 laser (Coherent Technologies) that was used at a wavelength of 532 nm. Micelles containing the E2 were filtered through a 200 nm filter and used at a concentration of 0.05% (w/w). Dust free vials were used for the aqueous micelle solutions, and measurements were performed at an angle of 90° at room temperature. The CONTIN algorithm was used to analyze the DLS data.

**Assessment of Effectiveness of $PCL_{23}$-*b*-$PEO_{45}$ Micelles Containing the E2 in Vitro and in Vivo.** All animal work was performed according to guidelines approved by the local Animal Research Committee of the Institute, and all efforts were made to minimize animal suffering and to reduce the number of animals used. Dorsal root ganglia (DRG) explant cultures were prepared as described by Tam et al.[37] Explants obtained from 3−4 month old C57BL mice were treated with nonincorporated E2 (1 nM) and E2 incorporated into PCL-*b*-PEO micelles (1 nM), and empty micelles (equivalent amount of polymer as in micelles containing E2). The neurite outgrowth was determined after 7 days by assessing relative optical density (ROD) of the total neuritic area using MCID-M5+5.1 image analysis software (Imaging Research).

Twenty 4 day old female mice (C57BL) were fed ab libitum with mouse standard diet and kept in a 12 h light/dark cycle. Prior to injection, 20 $\mu$L of micelle stock solution (27 mM) was redispersed in saline to make a total volume of 100 $\mu$L (retaining the polymer concentration approximately 950 times above the critical micelle concentration (CMC):[27] $2.8 \times 10^{-7}$ M). The Supporting Information contains details of the calculation. At postnatal day 17, the suspension providing pharmacological plasma concentration (150 $\mu$g of E2 in olive oil) was injected into the backs of mice (i.e., subcutaneous). One week later, the animals were sacrificed by decapitation. Positive control animals were treated once a day (0.5 $\mu$g of E2) and were also sacrificed 1 week after treatment. Negative controls were injected with empty micelles or a vehicle for E2 (i.e., olive oil). There was no significant difference between animals treated with empty micelles or olive oil, so these two groups were merged into the control group. After the animals were sacrificed, wet uterine weight was determined in all experimental groups. Results are expressed as mean ± SEM. All statistics were performed using SYSTAT 9 (SPSS Inc., Chicago, IL). Statistical significance was determined using one-way ANOVA with a Bonfferoni post hoc test.

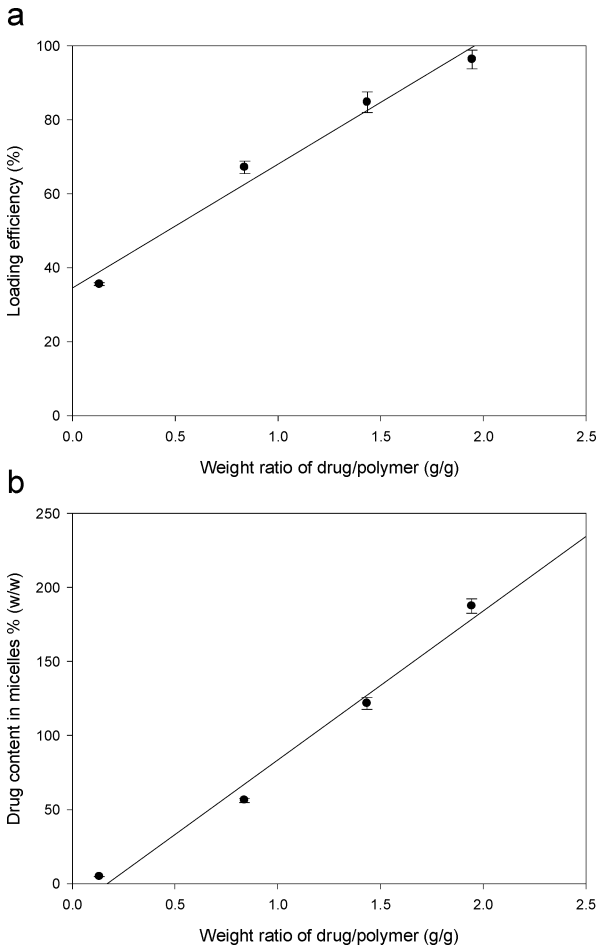


***Figure 1.*** Loading efficiency (a) and drug content (b) of E2 in $PCL_{23}$-*b*-$PEO_{45}$ micelles. The straight line is only meant to serve as a guide for the eye.

## Results and Discussion

**Loading Properties of E2 in PCL-*b*-PEO Micelles.** The loading of E2 into $PCL_{23}$-*b*-$PEO_{45}$ micelles was investigated for a series of samples ranging in initial (prior to loading) drug concentrations from 10 to 72 mM in solution. The loading efficiency is an indication of the percentage of the drug present in solution that can potentially be incorporated into the carrier. The loading efficiency of micelles for E2 can be determined from eq 1, and the results are shown in Figure 1a. The loading efficiency increases from 36% when

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***Table 1.*** Loading Properties of E2 in $PCL_{23}$-*b*-$PEO_{45}$ Micelles

| wt ratio of drug/polymer (w/w) | loading efficiency (%) | drug content % (w/w) | molecules of E2/micelle | wt of E2/ wt of PCL core % (w/w) |
|---|---|---|---|---|
| 0.1 | 36 | 5 | $9.9 \times 10^1$ | 10 |
| 0.8 | 67 | 55 | $1.2 \times 10^3$ | 100 |
| 1.4 | 85 | 120 | $2.6 \times 10^3$ | 215 |
| 2.0 | 96 | 190 | $4.0 \times 10^3$ | 330 |

the weight ratio of drug to polymer is 0.1 (w/w) to 67% as this value increases to 0.8 (w/w), and it reaches 96% (w/w) when the weight ratio of drug to polymer is 2 (w/w). An increase in the loading efficiency of E2 with initial drug concentration is generally seen for copolymer micelles. For example, Hagan et al.[38] reported this trend for both testosterone and Sudan black B in poly(D,L-lactide)-*block*-poly(ethylene glycol) (PLA-*b*-PEG) micelles. Similarly, Govender et al.[39] also observed this trend for procaine hydrochloride in PLA-*b*-PEG micelles. In all of these cases, as the drug concentration in the solution increases, one eventually reaches a point when the loading efficiency decreases because the micelles are unable to take up any more drug molecules.[31] Increases in loading efficiency with initial drug concentration were also seen for microspheres; for example, Birnbaum et al. showed that an increase in the initial concentration of E2 increased the loading efficiency of the drug.[13] They reported loading efficiencies ranging from 67% to 100% into PLGA microspheres.

The drug content (i.e., the actual amount of drug incorporated in the micelles) of E2 is determined from eq 2 and is shown in Figure 1b. There is a nearly linear relation between the drug content and the weight ratio of drug to polymer in the initial solution. A remarkably high amount of E2 can be incorporated into the $PCL_{23}$-*b*-$PEO_{45}$ micelles. The drug content of E2 ranges from approximately 5% to 190% (w/w) for a weight ratio of drug to polymer of 0.1 to 2 (w/w). By comparison with microspheres, 0.2−2.3% (w/w) and 5−15% (w/w) were reported in PLGA microspheres ranging in size from 30 to 150 $\mu$m[12] and from 60 to 75 $\mu$m,[13] respectively. Thus, the $PCL_{23}$-*b*-$PEO_{45}$ micelles can incorporate approximately 13 times the maximum drug content achieved in these PLGA microspheres (15% vs 190% (w/w)). However, from the microspheres studies, it is not clear whether the limits of incorporation had been reached, so it is conceivable that a greater amount of drug could have been incorporated. Additional insight into the loading capacity can be obtained from the data in Table 1.

The number of molecules per micelle is another indication of the loading properties of the delivery system. To calculate the amount of drug per micelle, the association number of $PCL_{20}$-*b*-$PEO_{44}$ which has been previously determined by our group[27] was used since the copolymer (i.e., $PCL_{23}$-*b*-$PEO_{45}$) did not greatly differ in terms of block lengths of either PCL or PEO. The $PCL_{23}$-*b*-$PEO_{45}$ micelles can incorporate up to a maximum of ca. 4000 molecules of E2 per micelle, representing a drug content of 190% (w/w). These numbers suggest that these block copolymer micelles can be suitable carriers for steroids and similar hydrophobic drugs, in contrast to what has been stated for small molecule surfactants.[40] In general, the great affinity of PCL for steroid molecules such as E2 is well documented.[41] It is also of interest to compare estradiol with DHT loaded into block copolymer micelles. Allen et al. incorporated DHT into $PCL_{21}$-*b*-$PEO_{44}$ micelles (ca. 50 nm) and showed that, at a weight ratio of drug to polymer of 2 (w/w), ca. 1300 DHT molecules were incorporated, representing 134% (w/w).[34] Hence, for any given weight ratio of drug to polymer, $PCL_{23}$-*b*-$PEO_{45}$ micelles can incorporate approximately 2−3 times more E2 than DHT molecules.

The weight ratio of the amount of E2 and the PCL core is also an important indication of the loading properties of the micelle, because most of the hydrophobic drug should be present in the hydrophobic core. The weight ratio of E2 and the PCL core ranges from approximately 10% to 330% (w/w) for a weight ratio of drug to polymer of 0.1−2 (w/w). In comparison, data for DHT ranges from approximately 6% to 130% for a weight ratio of drug to polymer of 0.1−2 (w/w).[34] For the same drug per polymer ratios in the initial solution, the incorporation of E2 is approximately 2−3-fold higher than for DHT in the PCL cores. This comparison is more meaningful, because we compare the amount of drug incorporated in the PCL core. Exact comparisons between micelle systems are not trivial, due to the differences in the total molecular weight, the size of the micelles, the size of the core, and the size of the corona chains; hence this was the reason why we compared the loading data using many different units.

In order to investigate the effect of polycaprolactone block length on the loading capacity of E2, a series of copolymers were synthesized with different polycaprolactone blocks (ranging from 12 to 151 units) with the same poly(ethylene oxide) blocks (e.g., 44 units). The influence of the PCL block length on the loading efficiency of E2 is shown in Figure 2. There is a linear increase ($r^2 = 0.998$) in the loading efficiency, from 10% to 90%, as the PCL block length increases. More information about the loading characteristics of the different $PCL_x$-*b*-$PEO_{44}$ copolymers is given in Table 2.

The drug content ranges from 15% to 130% (w/w) as the PCL block length increases. The number of molecules of E2 increases from approximately 230 to 11 000, which leads to a weight ratio of drug and PCL core of 36−144% (w/w)

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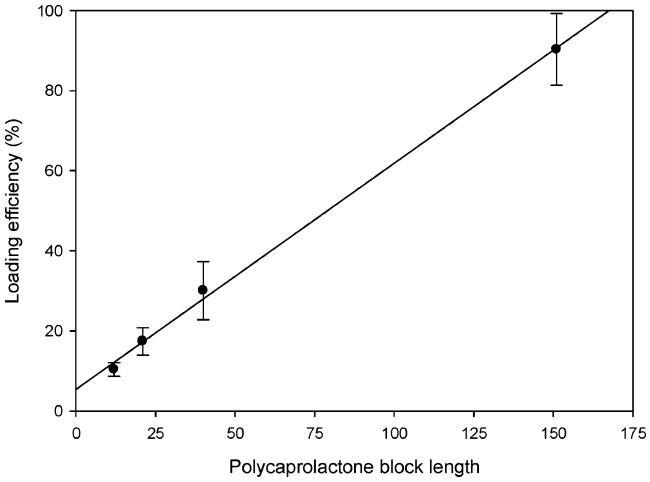


***Figure 2.*** Dependence of loading efficiency of E2 on the block length of polycaprolactone in $PCL_x$-*b*-$PEO_{44}$ micelles.

***Table 2.*** Loading Properties of E2 in Different $PCL_x$-*b*-$PEO_{44}$ Micelles

| PCL block length | loading efficiency (%) | drug content % (w/w) | molecules of E2/ micelle | wt of E2/ wt of PCL core % (w/w) |
|---|---|---|---|---|
| 12 | 10 | 15 | $2.3 \times 10^2$ | 36 |
| 21 | 17 | 22 | $5.1 \times 10^2$ | 40 |
| 40 | 30 | 45 | $1.3 \times 10^3$ | 64 |
| 151 | 90 | 130 | $1.1 \times 10^4$ | 144 |

as the PCL block length increases. The increase in the block length of PCL results in a larger core diameter, so more drug molecules can be incorporated. The reason for this is that as the PCL block length increases, the aggregation number of the micelle increases, resulting in a larger core, which allows for a higher loading efficiency. This has been reported in the literature by Gadelle et al., who showed that as the length of the hydrophobic block increases, the CMC decreases and a resulting increase in the diameter of the core facilitates the loading of more drug molecules into the core.[42] Similarly, Kozlov et al. showed that a decrease in the CMC was the direct result of increasing the length of the core-forming block.[43]

The increase in the PCL block length also influences the partition coefficient, which is a convenient way to express the affinity of the drug for the micelle core or for the external environment. The partition coefficient increases with increasing PCL block lengths. Allen et al. showed that the partition coefficient of pyrene increased from 240 to 1450 as the PCL block length increased from 14 to 40.[27] In the previous section, the high estradiol loading into the micelles would suggest that there is a great affinity of the drug molecules for the polycaprolactone core. 17$\beta$-Estradiol is highly lipophilic and prefers to partition into the hydrophobic PCL core of the micelle. In the cell membrane, E2 tends to associate with the hydrophobic domains.[44]

A value for the partition coefficient of E2 in the PCL-*b*-PEO micelles was not determined, but a comparison of the partition coefficients reported in the literature gives a good indication of the value. Lundberg et al. determined the log water−octanol partition of E2 to be 4.0.[45] The log apparent partition coefficient of DHT was determined to range from 2.9 to 4.3 in $PCL_{20}$-*b*-$PEO_{44}$ micelles.[34] On the basis of the water solubilities of dihydrotestosterone and estradiol (e.g., 42 mg/L and 1.7 mg/L, respectively),[45] we estimate that the partition coefficient for E2 would be larger than that for DHT, and E2 would partition into the PCL core more than DHT. In comparison with another system, Ye et al. calculated the log partition coefficient of E2 in block copolymers of varying ratios of caprolactone and lactide and found that increasing amount of caprolactone (from 60% to 90%) led to an increase of log *P* from 3.0 to 4.3.[46] Similarly, a high partition coefficient (log $P = 3.8$) was determined between a model fluorescent hydrophobic probe (CM-DiI) and PCL-*b*-PEO micelles and resulted in a high loading capacity for the probe.[31]

**PCL-*b*-PEO Morphology and Size.** The size and morphology of the $PCL_{23}$-*b*-$PEO_{45}$ micelles were determined previously,[33] and the findings are confirmed in the present study. Briefly, the micelle aggregates were spherical with sizes (determined by both DLS and TEM) of $25 \pm 2$ nm. A TEM image of micelles containing E2 (concentration = 2 mM) revealed spherical shapes and a diameter of $30 \pm 7$ nm (Figure 3a). Dynamic light scattering was also used to determine the size of the micelles containing E2 (concentration = 3 mM), and the results are shown in Figure 3b.

There should not be a significant change in the size and size distributions due to small differences in drug loading. The majority of the micelles are less than 50 nm and the average micelle diameter is 33 nm, as determined by Contin analysis using DLS. Although we have not studied the effect of drug loading of E2 on the micelle size, we speculate that, with higher amounts of E2 loaded, there may be a possible increase in the micelle size. Such an increase has been previously observed for the incorporation of estrone into PLA microspheres[47] and antiestrogen (RU 58668) into PLGA, PLA, and PCL nanospheres.[48]

**Release Kinetics of E2 from PCL-*b*-PEO Micelles.** E2 from $PCL_{23}$-*b*-$PEO_{45}$ micelles with different E2 contents (3 and 35 mM) is released at different rates (Figure 4).

In order to obtain more quantitative information on the release of estradiol from the micelles, the release data was plotted against the square root of time; the linearity of the

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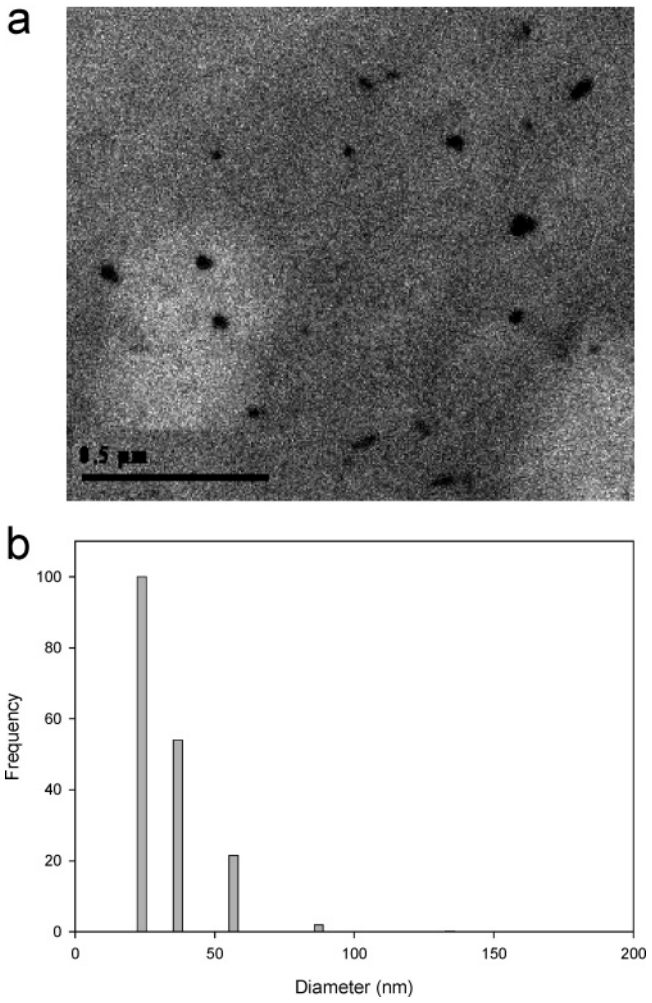


***Figure 3.*** (a) Transmission electron microscopy of E2 (concentration = 2 mM) in $PCL_{23}$-*b*-$PEO_{45}$ micelles. Size range is 30 ± 7 nm. (b) Dynamic light scattering of E2 (concentration = 3 mM) in $PCL_{23}$-*b*-$PEO_{45}$ micelles. Average diameter is 33 nm.

plot is indicative of a diffusional release. The diffusion coefficients, $D$ (expressed in $cm^2/s$), from these release plots were determined using the Higuchi equation,[49]

$$Q = 2C_0\left(\frac{Dt}{\pi}\right)^{1/2} \qquad (3)$$

where $Q$ is the amount of E2 released per unit area of the micelles (expressed in $mol/cm^2$), $C_0$ is the initial concentration of E2 per volume of PCL (expressed in $mol/cm^3$), and $t$ is the time (expressed in seconds). Previously, we have used this model to fit release data of model hydrophobic probes from micelles.[31] The release plotted against the square root of time is shown in Figure 5.

The release fits relatively well with the Higuchi model (i.e., correlation coefficients of 0.978 for 35 mM and 0.980 for 3 mM). Diffusion coefficients were determined assuming a micelle diameter of 25 nm, and omitting the data for the

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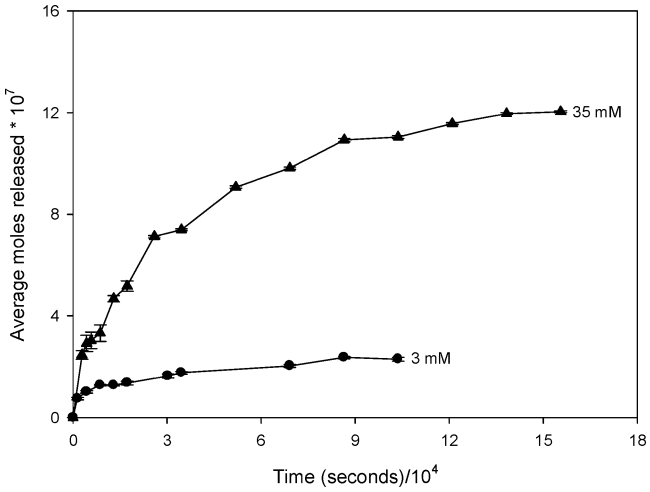


***Figure 4.*** Release of E2 (concentrations = 3 mM (●) and 35 mM (▲)) from $PCL_{23}$-*b*-$PEO_{45}$ micelles under perfect sink water conditions.

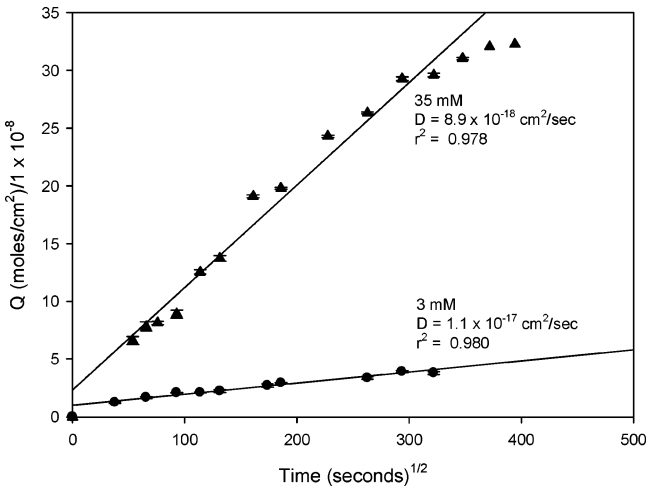


***Figure 5.*** The fit of the release data to the Higuchi model using two different estradiol concentrations: 3 mM (●) and 35 mM (▲) under perfect sink water conditions. The linearity of the line of best fit is indicative of a diffusional release mechanism. The assumption is that the average diameter of the micelle is 25 nm.

burst release phase.[49] For the more concentrated sample (35 mM), the diffusion coefficient was $8.9 \times 10^{-18}$ $cm^2/s$, while for the lower concentration sample (3 mM), the diffusion coefficient was $1.1 \times 10^{-17}$ $cm^2/s$. The reader is referred to the Supporting Information for sample calculations. Gref et al. showed that, at low loadings of lidocaine, the release of the drug occurred more quickly from PLGA-*b*-PEO micelles than at higher loadings.[17] The release of norfloxacin from poly(γ-benzyl L-glutamate)-*block*-poly(ethylene oxide) (PBLG-*b*-PEO) micelles was observed to be slower at higher drug contents due to increased hydrophobic interaction between the PBLG core and the hydrophobic drug.[50] Similarly, in a recent paper, we showed that the release of hydrophobic

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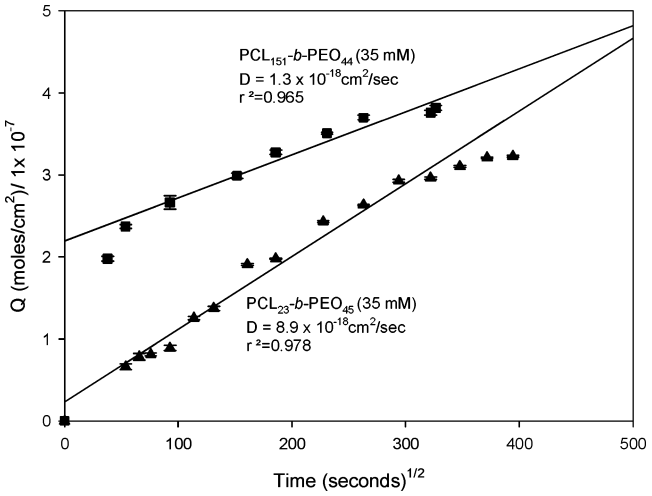


***Figure 6.*** Release of E2 (35 mM) from PCL-*b*-PEO micelles with different PCL lengths $PCL_{151}$ (●) and $PCL_{23}$ (▲) under perfect sink water conditions. The linearity of the line of best fit is indicative of a diffusional release mechanism. The average diameter of the micelle was taken as 25 nm.

probes (CM-DiI and benzo[*a*]pyrene) from samples with higher concentrations was slower than the release from samples with lower concentrations.[31] Diffusion controlled release was reported in the literature for E2 from another micelle system. Bromberg et al. observed a slow diffusional release from Pluronic-PAA micelles.[25]

The influence of the PCL block length on the release of estradiol (35 mM) was determined using different PCL-*b*-PEO micelles ($PCL_{23}$-*b*-$PEO_{45}$ and $PCL_{151}$-*b*-$PEO_{44}$) (Figure 6). The release of E2 from the $PCL_{23}$-*b*-$PEO_{45}$ micelles ($D = 8.9 \times 10^{-18}$ cm$^2$/s) occurred more quickly than the release from $PCL_{151}$-*b*-$PEO_{44}$ micelles ($D = 1.3 \times 10^{-18}$ cm$^2$/s). The drug is released more slowly from $PCL_{151}$-*b*-$PEO_{44}$ because it presumably has a larger core due to the longer hydrophobic block (151 units of PCL), compared to the shorter hydrophobic block (23 units of PCL). The drug has further to diffuse in a core with a longer hydrophobic block. A longer core block would also have a higher glass transition temperature, so that, closer to room temperature, the higher viscosity of the medium would result in a slower release. Finally, the larger core diameter could result in a higher crystallinity of the core in comparison to a smaller core diameter; the higher crystallinity would slow the release of the drug. Similar findings of increased hydrophobic block lengths resulting in a slower release have also been reported in the literature for other block copolymer micelles. Jeong et al. showed that for poly($\gamma$-benzyl L-glutamate)-*b*-poly(ethylene oxide) (PBLG-*b*-PEO) micelles with different PBLG contents, but similar loadings of adriamycin, the release was slower for the copolymer with higher PBLG content.[51] The increased hydrophobic interactions between the drug and the micelles with higher PBLG content were responsible for the slower release. Similarly, Nah et al. incorporated norfloxacin and clonazepam into the PBLG-*b*-PEO micelles and observed that release of the drugs was slower for longer PLBG chains.[50,52] Gorshokova et al. found that, over a 15 day period, the release rate of daunomycin is reduced from 16% to 4% due to the introduction of hydrophobic blocks (decylamine).[53]

During the release experiments, all the micelle samples showed a small initial burst release (i.e., 20−30% in the first hour) of 17$\beta$-estradiol. This is a result of the E2 being released from the interfacial regions of the micelles. Burst release has been observed for other micelle systems: e.g., Bromberg et al. observed a 5−11% initial rate of release of E2 from the expanded PAA corona of the Pluronic-PAA micelles.[25] Similarly, Teng et al., showed an initial burst release of pyrene from the inner corona of micelles from poly(*tert*-butyl acrylate)-*b*-poly(2-vinylpyridine) and a blend of PBA-*b*-P2VP with P2VP-*b*-PEO.[54] Burst release has also been observed in microspheres.[14] Studies showed that incorporating PLGA microparticles within a silicone matrix reduced the initial burst release of E2.[55] Makino et al. controlled the time interval between the initial burst and the subsequent release by mixing different types of PLGA microspheres.[56] Although a burst release is undesirable, in some cases, such a release can be beneficial.[47]

**Biological Effectiveness of $PCL_{23}$-*b*-$PEO_{45}$ Micelles Containing E2.** The objective was to test the effectiveness of E2 incorporated into $PCL_{23}$-*b*-$PEO_{45}$ micelles in biological systems. In vitro studies were conducted by employing dorsal root ganglia explant cultures. As seen from Figure 7, there was a significant increase in neurite outgrowth in DRG explants treated with E2 (nonincorporated into micelles) or micelle loaded with E2. Results from these studies show that E2 treatments of DRG induced significant neurite outgrowth regardless of the delivery system. In contrast, there was barely any detectable increase in neurite density from the explanted DRG without the sex hormone treatment.

To test the effectiveness of $PCL_{23}$-*b*-$PEO_{45}$ micelles containing E2 in vivo, we injected micelles into female C57BL mice ($n = 5$). The uterine weights were determined after 1 week, and the results (Table 3) clearly show that there is a significant increase in uterine weight following the treatment with E2 alone or with micelles containing the E2. There was an approximate 4-fold increase in uterine weight after the treatment with micelle-incorporated E2 and an approximate 3-fold increase following daily injections with

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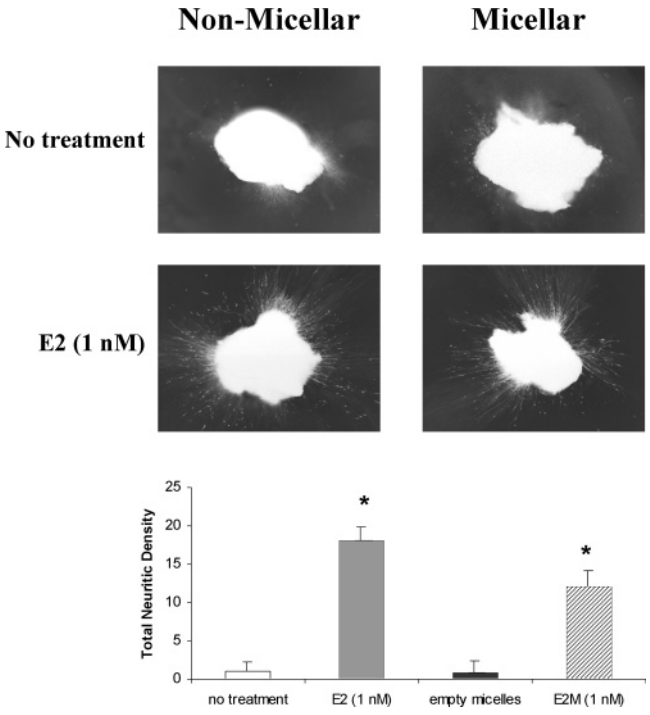


***Figure 7.*** Stimulation of neurite outgrowth from explanted DRG treated with E2 (nonincorporated into micelles) or micelles loaded with E2. Explants obtained from 3−4 month old C57BL mice were treated with E2 nonincorporated and incorporated into micelles, and empty micelles. The neurite outgrowth was determined after 7 days by assessing relative optical density (ROD) of total neuritic area. Each bar represents a mean value of three independent measurements, and differences are considered significant at $p < 0.05$.

E2 (non micelle incorporated). These results indicate that E2 did not lose its biological activity during the preparation of the micelles.

## Conclusions

The incorporation and release of 17$\beta$-estradiol from polycaprolactone-*block*-poly(ethylene oxide) micelles were investigated as a potential sex hormone nanodelivery system. A high percentage of E2 (loading efficiency up to 96% (w/w)) and a high drug content (up to 4000 molecules of E2 per micelle (equivalent to 190% (w/w)) can be incorporated. Micelles were spherical and ranged in size from 20 to 40 nm as determined by transmission electron microscopy and dynamic light scattering. Application of the Higuchi model showed that the E2 release followed a diffusional mechanism, and diffusion coefficients of the order of $10^{-17}$ $cm^2/s$ were obtained. The loading and release of E2 can be controlled by the initial concentration of E2 and by the length of the PCL block. The biological activity of E2 in vitro and in vivo was retained after the preparation of the micelles. This biocompatible, tailorable, self-assembled nanodelivery vehicle not only is suitable for 17$\beta$-estradiol but also could provide a versatile, noninvasive system for other sex hormones individually or in combination.

***Table 3.*** Uterine Weight in Mice after 1 Week Treatment with E2 and E2 Incorporated in Micelles[a]

| treatment | uterine wt (mg) |
|---|---|
| control ($n = 17$) | 13.7 ± 1.0 |
| estradiol alone ($n = 10$) | 43.8 ± 2.7* |
| micelles containing E2 ($n = 5$) | 52.2 ± 8.6* |

[a] The number of animals for which uterine weight was determined is indicated in parentheses. Differences were considered significant at $p < 0.05$. Asterisks refer to the bars in Figure 7 representing E2 alone and micelles containing E2.

**Acknowledgment.** The work was supported by the Natural Sciences and Engineering Research Council of Canada, the Partnership for Tomorrow Program, and the Juvenile Diabetes Research Foundation. The authors thank Professor Christine Allen for many useful discussions, Dr. Laibin Luo and Dr. Yisong Yu for synthesizing the PCL-*b*-PEO copolymers, Joseph Tam for performing the experiments with the DRG explants used in this study, and Professor Hanadi Sleiman for generous permission to use the fluorescence instrument.

**Supporting Information Available:** Physical properties of E2, details of the determination of the polymer concentration of the micelle stock injected into the C57BL female mice, and details of the determination of the diffusion coefficients for E2 released from $PCL_{23}$-*b*-$PEO_{45}$. This material is available free of charge via the Internet at http://pubs.acs.org.

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